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Systemic lupus erythematosus : from diagnosis to prognosis

Rijnink, E.C.

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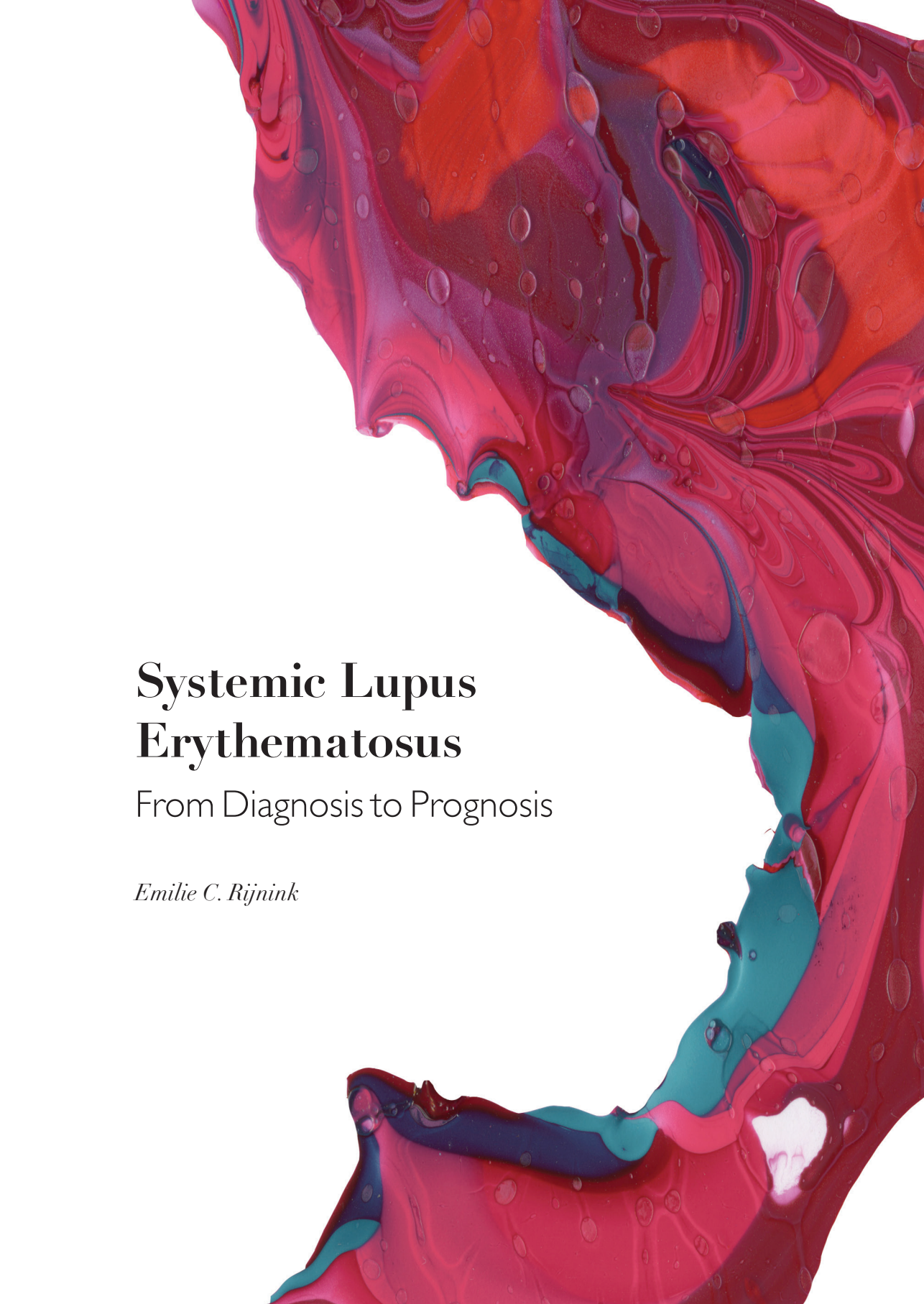


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Systemic Lupus Erythematosus

From Diagnosis to Prognosis

Emilie C. Rijnink

SYSTEMIC LUPUS ERYTHEMATOSUS FROM DIAGNOSIS TO PROGNOSIS

Emilie C. Rijnink

Colophon

The studies described in this thesis were performed at the Department of Pathology (Head: prof. dr. V.T.H.B.M. Smit) of the Leiden University Medical Center, the Netherlands

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Promotor

Prof. dr. J.A. Bruijn

Co-promotores

Dr. I.M. Bajema

Dr. Y.K.O. Teng

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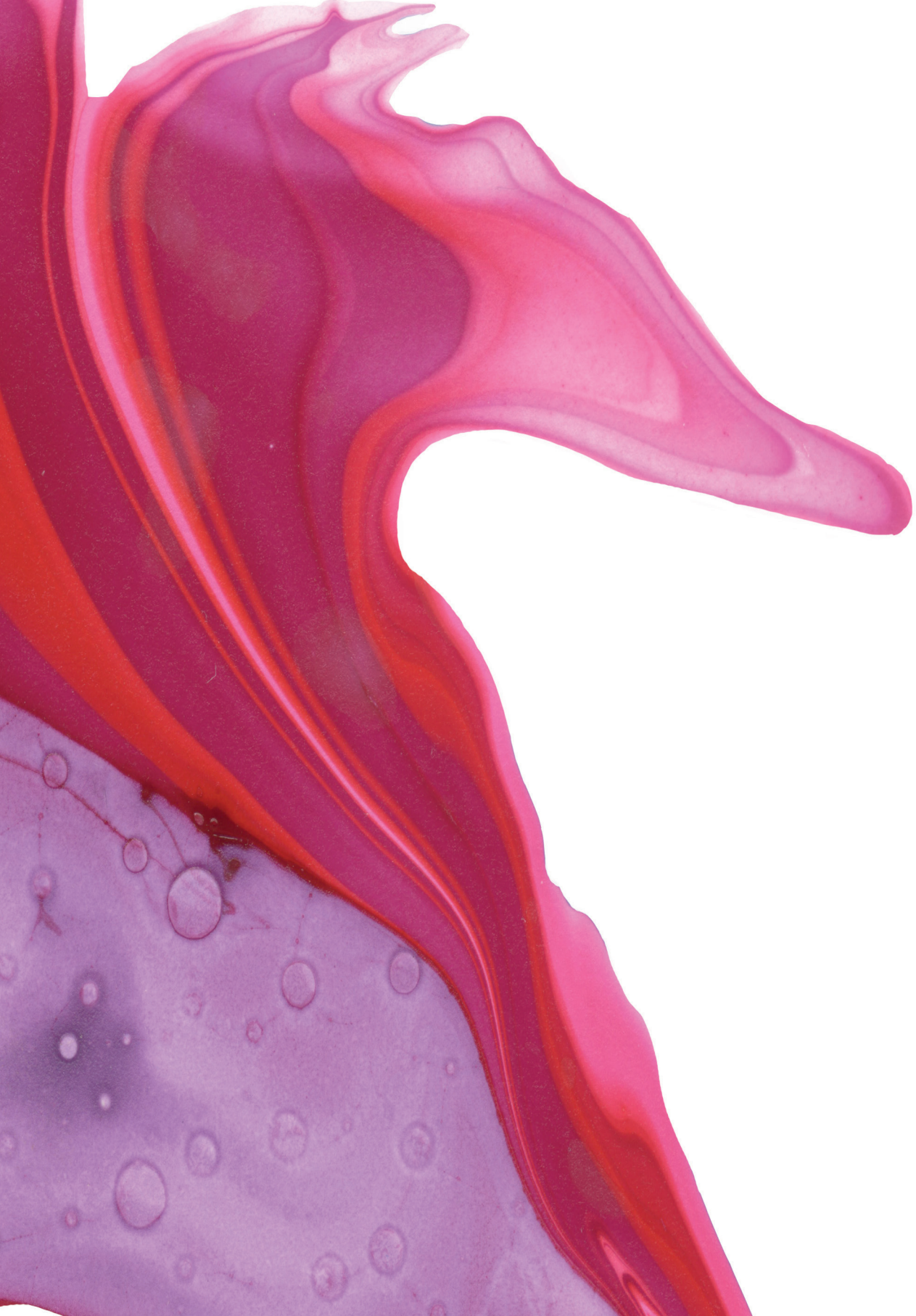
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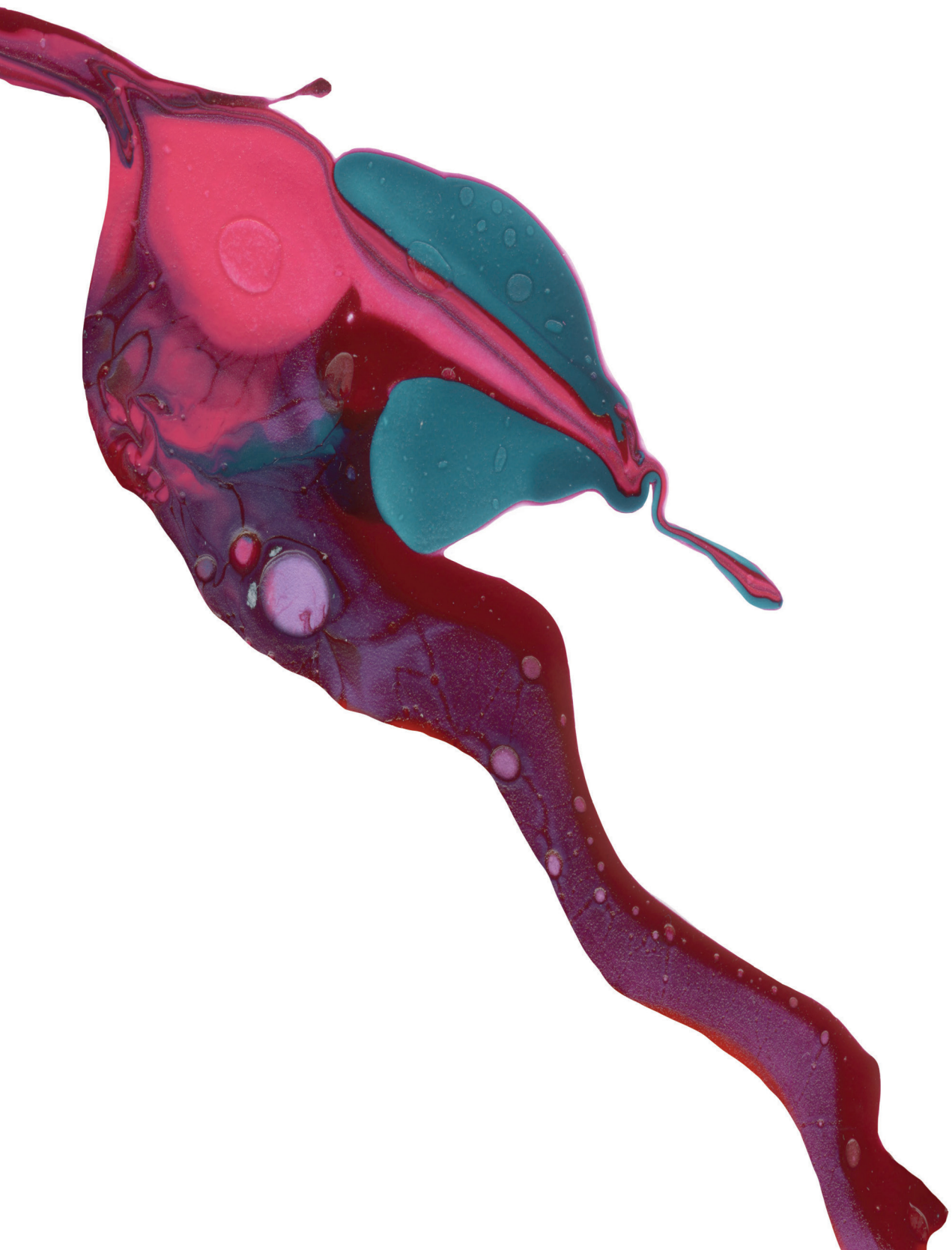
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Voor mijn ouders en grootouders



CONTENTS

Chapter 1	General Introduction and Outline of this Thesis	9
Chapter 2	Validation of the Systemic Lupus International Collaborating Clinics Classification Criteria in a Cohort of Patients with Full House Glomerular Deposits <i>Kidney Int. 2017, In Print.</i>	47
Chapter 3	Idiopathic Non-lupus Full House Nephropathy is Associated With Poor Renal Outcome <i>Nephrol Dial Transplant. 2017; 32: 654-662.</i>	63
Chapter 4	Long-term Preserved Renal Function in Patients with Class III or IV Lupus Nephritis Undertreated According to Guidelines: Recognition of a Subgroup with a Favourable Natural History	85
Chapter 5	Clinical and Histopathologic Characteristics Associated with Renal Outcomes in Lupus Nephritis <i>Clin J Am Soc Nephrol. 2017; 12: 734-743.</i>	99
Chapter 6	Brain Histopathology in Patients with Systemic Lupus Erythematosus: Identification of Lesions associated with clinical Neuropsychiatric Lupus Syndromes and the Role of Complement <i>Rheumatology (Oxford). 2017; 56: 77-86.</i>	119
Chapter 7	Tissue Microchimerism is Increased During Pregnancy: A Human Autopsy Study <i>Mol Hum Reprod. 2015; 21: 857-64.</i>	139
Chapter 8	Increased Microchimerism in Peripheral Blood of Women with Systemic Lupus Erythematosus	157
Chapter 9	Summary and General discussion	173
Chapter 10	Nederlandse samenvatting	197
	Appendix	209
	Affiliations	237
	Curriculum vitae	239
	List of publications	241
	Dankwoord	242



Chapter 1

GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

PROLOGUE: HIPPOCRATES' LEGACY	11
Diagnosis	11
Prognosis	12
PART 1: SYSTEMIC LUPUS ERYTHEMATOSUS	13
Historic background	13
Epidemiology and symptomatology	14
Classification of SLE	16
Aetiology	18
Pathogenesis	23
Treatment and Prognosis	24
PART 2: FOCUS ON MAJOR ORGAN MANIFESTATIONS OF SLE	25
Lupus nephritis	25
The kidney as a site of injury in SLE	25
Treatment and prognosis	28
Neuropsychiatric lupus	31
The brain as a site of injury in SLE	32
Treatment and prognosis	34
PART 3: THIS THESIS	35

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.

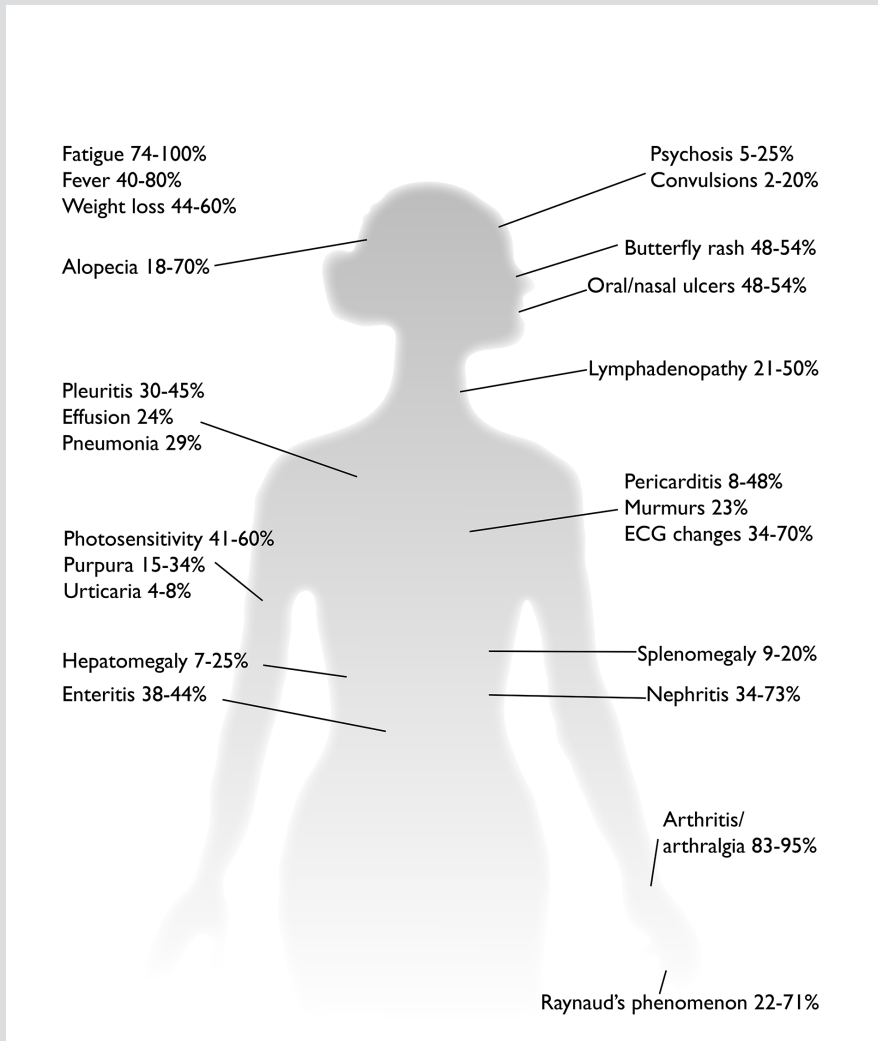
Epidemiology and symptomatology

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 (<i>in the absence of other causes</i>) or Psychosis (<i>in the absence of other causes</i>)
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 NETosis²⁹) 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 twins³⁴ and the increased risk of SLE in first-degree relatives³⁵ 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 (<i>not if discoid</i>) or b. Bullous lupus or c. Toxic epidermal necrolysis variant of SLE or d. Maculopapular lupus rash or e. Photosensitive lupus rash (<i>in the absence of dermatomyositis</i>) 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 (<i>In the absence of vasculitis, Behçet's disease, infection (herpes), inflammatory bowel disease, or acidic foods</i>)
4. Non-scarring alopecia	Diffuse thinning or hair fragility with visible broken hairs (<i>In the absence of other causes such as alopecia areata, drugs, iron deficiency, and androgenic alopecia</i>)
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 <i>(In the absence of other causes, such as infection, uraemia, or Dressler's pericarditis)</i>
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 (<i>in the absence of other known causes such as primary vasculitis</i>) or d. Myelitis or e. Peripheral or cranial neuropathy (<i>in the absence of other known causes such as primary vasculitis, infection, and diabetes mellitus</i>) or f. Acute confusional state (<i>in the absence of other causes, including toxic/metabolic, uraemia, drugs</i>)
9. Haemolytic anaemia	
10. Leukocytopenia/lymphocytopenia	a. Leukocytopenia: at least once ($<4000/\text{mm}^3$) (<i>In the absence of other known causes such as Felty's syndrome, drugs, and portal hypertension</i>) or b. Lymphocytopenia: at least once ($<1000/\text{mm}^3$) (<i>In the absence of other known causes such as corticosteroids, drugs, and infection</i>)
11. Thrombocytopenia	At least once ($<100,000/\text{mm}^3$) (<i>In the absence of other known causes such as drugs, portal hypertension, and thrombotic thrombocytopenic purpura</i>)

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	<i>In the absence of haemolytic anaemia</i>

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 aetiological 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 aetiological 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 1955⁷⁶ 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

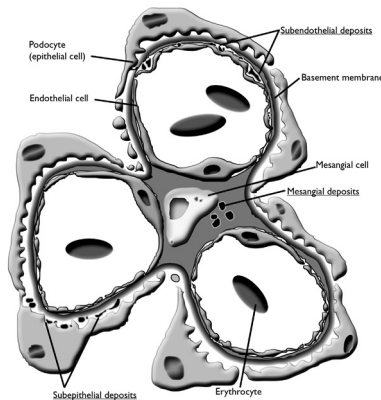
The anatomy and physiology of the kidney make it highly susceptible to inflammatory insults caused by autoantibodies. A number of factors contribute to the nonspecific trapping of immune complexes in the kidney.⁸⁷ First, immune complexes in the circulation are delivered at a high rate to the kidney because 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/m² 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 cytotoxic 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

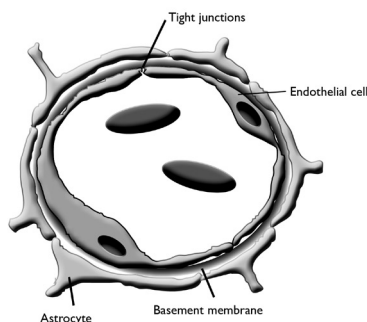
In 1999 “The ACR Nomenclature and Case Definitions for Neuropsychiatric Lupus Syndromes” was published, serving as a guide for clinicians and researchers to identify individual NP-SLE disorders (**Table 4**).¹²⁵ Because NP-SLE remains a condition that is diagnosed *per exclusionem*, the ACR nomenclature 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.

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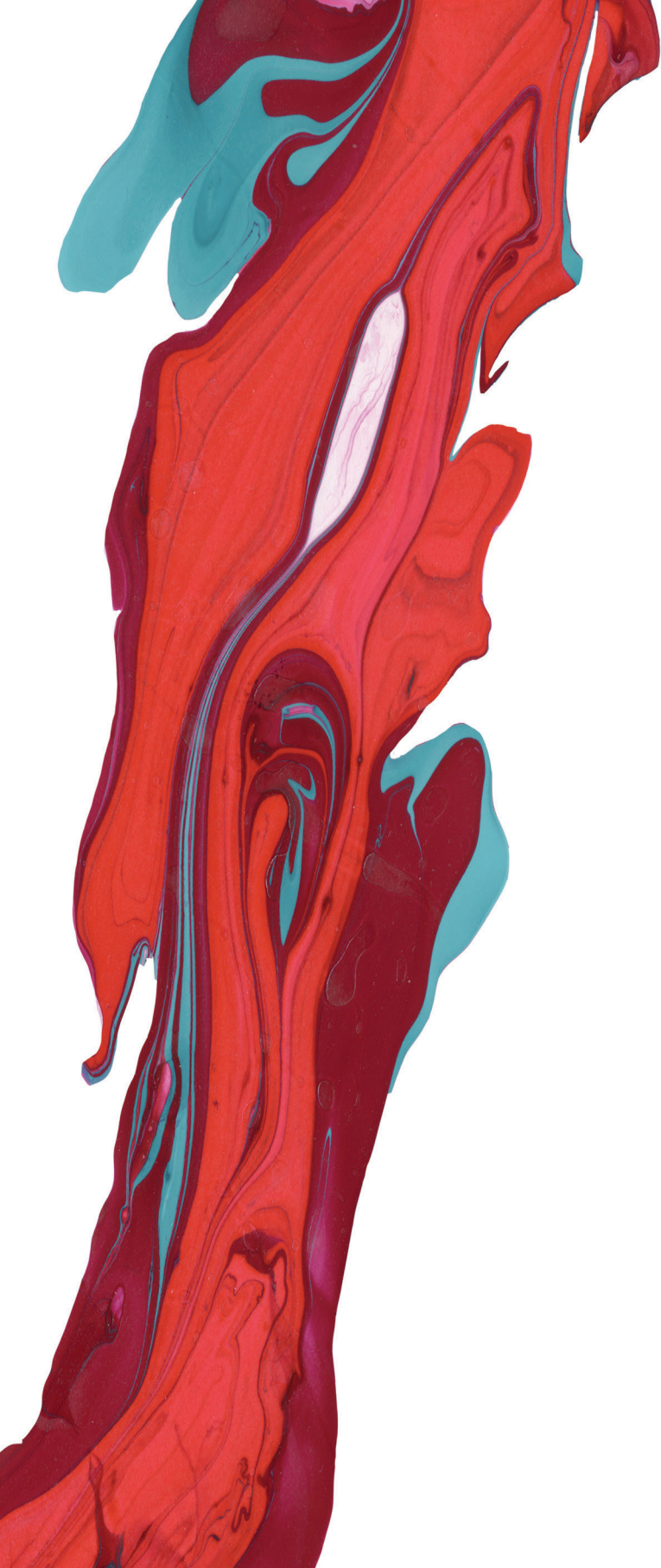
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Chapter 2

Validation of the Systemic Lupus International Collaborating Clinics' Classification Criteria in a Cohort of Patients with Full House Glomerular Deposits

Emilie C. Rijnink, Y.K. Onno Teng, Tineke Kraaij, Olaf M. Dekkers, Jan A. Bruijn, Ingeborg M. Bajema

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ABSTRACT

Background

In 2012, the Systemic Lupus International Collaborating Clinics (SLICC) presented a new classification for systemic lupus erythematosus (SLE). In this classification, biopsy-confirmed lupus nephritis (LN) with positive antinuclear or anti-double stranded DNA antibodies became a stand-alone criterion. Because of the unknown diagnostic performance among patients from nephrology clinics, we aimed to test the validity of the SLICC classification, compared with the American College of Rheumatology (ACR) classification, in a cohort of patients whose renal biopsies would raise the clinicopathologic suspicion of LN.

Methods

All patients with a renal biopsy showing full house glomerular deposits between 1968–2014 and clinical follow-up in our centre were included and re-evaluated after which clinicians and a pathologist reached a consensus on the reference-standard clinical diagnosis of SLE. The diagnostic performance, and net reclassification improvement (NRI) were assessed. We included 149 patients, 117 of whom had clinical SLE.

Results

Compared with the ACR classification, the SLICC classification had better sensitivity (100 vs. 94%); although, this was at the expense of specificity (91 vs. 100%; NRI -0.03 , $P=0.56$). Excluding the stand-alone renal criterion, the specificity of the SLICC classification reached 100%, with an NRI of 0.06 ($P<0.01$) compared with the ACR classification.

Conclusions

The SLICC classification performed well in terms of diagnostic sensitivity among patients with full house glomerular deposits; whereas, the stand-alone renal criterion had no additional value and compromised the specificity. Clearly, putative LN patients in nephrology clinics reflect a distinct SLE disease spectrum warranting caution when applying SLE classification criteria.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with diverse clinical manifestations and presenting symptoms that have considerable overlap with other diseases.¹ SLE classification criteria have been designed to create homogeneous groups of SLE patients to conduct collaborative and reproducible research. Although SLE classification criteria have been designed for research purposes, they are often used for the purpose of diagnosis in clinical practice. The focus of SLE classification criteria has traditionally been on patients encountered in rheumatology clinics, although a need for input from non-rheumatology specialists who frequently see lupus patients was recognised.² This may be particularly relevant to nephrologists, since patients with renal biopsy findings reminiscent of lupus nephritis (LN) would readily be evaluated in light of these criteria to confirm the diagnosis. The recent descriptions of entities including “renal-limited lupus-like nephritis”,³ and idiopathic^{4,5} and secondary “non-lupus full house nephropathy”⁴⁻¹⁰ stress the importance of valid SLE classification criteria in the nephrology clinic: to help distinguish LN patients based on clinical and laboratory findings.

The importance of valid SLE classification criteria in the nephrology clinic recently gained attention by the increased weight that was attributed to renal lupus in the Systemic Lupus International Collaborating Clinics (SLICC) 2012 classification.¹¹ In the SLICC classification,¹¹ biopsy-confirmed LN in the presence of antinuclear antibodies (ANA) or anti-double stranded DNA (anti-dsDNA) antibodies was introduced as an exception to the conventional requirement of four or more criteria employed in the original¹² and updated¹³ American College of Rheumatology (ACR) classifications. Remarkably, the definition of biopsy-confirmed LN was unspecified in both the ACR and the SLICC classification, and firm criteria for biopsy-confirmed LN in the histopathologic classification referred to are missing.¹⁴

In light of the increased weight of renal lupus in the SLICC classification and the unknown diagnostic performance of the classification in patients from nephrology clinics, we aimed to test the validity of the SLICC classification in a cohort of patients whose renal biopsies would raise the clinicopathologic suspicion of LN. Since the definition of “biopsy-confirmed” LN is left open to interpretation, we selected our cohort based on a biopsy feature characteristic of LN – so as to raise the clinicopathologic suspicion – but concise enough to identify a consistent cohort. From a nephropathologic perspective, the finding of a so-called “full house” pattern of immunofluorescence, with concurrent positive glomerular staining for IgA, IgG, IgM, C3, and C1q, would certainly raise the possibility of SLE as a differential diagnostic consideration warranting the evaluation of clinical criteria. Here, we tested the validity of the SLICC compared with the ACR classification criteria to distinguish SLE patients with full house glomerular deposits. Moreover, we studied additional biopsy findings that may distinguish SLE patients in this setting. This is the first validation study of

the SLICC classification in a cohort selected on the basis of renal biopsy findings raising the clinicopathologic suspicion LN, reflecting a diagnostic problem area encountered in the nephrology clinic.

METHODS

The pathology archives of the Leiden University Medical Center were searched to identify all native renal biopsies between 1968–2014 showing full house immunofluorescence, defined as concurrent positive staining for IgA, IgG, IgM, C3, and C1q with $\geq 1+$ intensity on a 0–3+ scale. Only biopsies showing granular fluorescent staining along capillary walls and/or in the mesangium were included. Patients from our centre accordingly identified entered the study. In accordance with the ethics committee of the Leiden University Medical Center, all patient data were coded and kept anonymously throughout the study. All biopsies were processed for light and immunofluorescence microscopy according to the standard techniques at our centre. Sections for light microscopy were stained with haematoxylin and eosin, periodic acid-Schiff, and methenamine-silver. All biopsies were (re)classified by an experienced pathologist (IMB) according to the ISN/RPS classification of LN,¹⁴ regardless of clinicopathologic diagnosis. For immunofluorescence microscopy, sections were frozen in liquid nitrogen, and cryostat sections were stained with FITC-labelled antisera to human IgA, IgG, IgM, C3, and C1q. Immunofluorescence reports were originally prepared by four experienced nephropathologists who routinely scored the immunofluorescence intensity on a 0–3+ scale. Not all biopsies were sent in for analysis by electron microscopy; but if at hand, tissue was fixed in 1.5% glutaraldehyde and 1% paraformaldehyde and embedded in Epon. Electron microscopy was reviewed if available, and findings were compared to the original report.

Following the methodology of previous validation studies, the reference standard for the clinical diagnosis of SLE was based on clinician's and pathologist's expert opinion.^{11, 15–17} Three investigators (ECR, YKOT, TK) independently reviewed medical records in consultation with an experienced nephropathologist (IMB), assessing whether patients had a reference-standard clinicopathologic diagnosis of SLE at the time of renal biopsy by considering biopsy findings and the constellation of presenting clinical features, supportive laboratory studies, and demographics, and by exclusion of alternative diagnoses. Because of the evolving manifestations of SLE, we also confirmed the diagnosis of SLE patients at the time of renal biopsy by considering follow-up (including follow-up biopsies and the post-transplantation course and biopsies) as a separate examination in addition to the diagnosis at the time of biopsy. Patients who were not diagnosed with SLE at the time of biopsy were also studied during follow-up to see if they could be diagnosed with SLE at a later time. Consensus on the clinical diagnosis of SLE at the time of biopsy or during follow-up was achieved by conference.

Furthermore, clinical records were reviewed by the same investigators individually to assess the presence of ACR^{12,13} and SLICC¹¹ criteria for SLE at any time up to and including the moment of renal biopsy. Qualitative ANA testing was performed routinely at our centre using incubation of HEp 2000 cells (Biomedical Diagnostics, Belgium) with 1:40 diluted serum samples. A positive test result was reported if a clear and distinct immunofluorescence pattern was observed that was more intense than the negative control. Similarly, qualitative anti-dsDNA testing was performed using C. Luciliae kit (Aesku.Diagnostics, Germany) using 1:10 diluted samples. Fulfilment of sufficient criteria according to either classification was assessed. Patients who did not fulfil sufficient ACR or SLICC criteria were studied for the presentation of any of the criteria during available follow-up by the same investigators. Fulfilment of classification criteria was agreed on by conference.

Statistical analyses

Normally distributed data were compared using *t*-tests. Categorical data were compared using Fisher's exact tests, chi-square tests, or linear-by-linear analysis. The number of classification criteria was compared between patients with and without a reference standard diagnosis of SLE by Mann Whitney U test. The sensitivity and specificity of ACR and SLICC classifications were assessed and reported including 95% confidence intervals. Information retrieved from follow-up was not considered when assessing diagnostic performance. Improvement of the SLICC classification compared with the ACR classification was assessed by calculating the net reclassification improvement (NRI) at the time of biopsy.¹⁸ The NRI is based on reclassification tables constructed separately for cases with and without SLE, and quantifies the correct movement in categories, upwards for cases with SLE and downwards for cases without SLE. The null hypothesis $NRI=0$ was tested using the Z-statistic following McNemar's asymptotic test for correlated proportions. All *P*-values are two-sided and $P<0.05$ was considered significant. All analyses were performed using SPSS 23.0 (IBM, Armonk, New York).

RESULTS

A total of 149 patients with renal biopsies fulfilling our inclusion criteria were identified from the pathology archives between August 1968 and July 2014. Fourteen patients were biopsied before 1980, 32 from 1980–1989, 47 from 1990–1999, 42 from 2000–2009, and 14 from 2010–2014.

Reference standard clinical diagnosis of SLE

According to clinicians' and pathologist's expert opinion, 117/149 patients fulfilled the diagnosis SLE at the time of biopsy. These diagnoses composed the reference standard; these patients will be referred to hereafter as patients with "clinical SLE". Of the patients with clinical SLE, renal involvement first appearing at the time of renal biopsy was the decisive factor establishing the clinical diagnosis in 40 patients. In addition, 75 patients had a clinical

diagnosis of SLE prior to renal biopsy. For two patients, the time since onset of SLE could not be retrieved from the records. The median time between SLE diagnosis and renal biopsy was 1.4 years (interquartile range 0–5.3). For all patients with clinical SLE at the time of renal biopsy, the diagnosis was confirmed by the clinical course during follow-up (median 10.6 years [interquartile range 4.9–18.4]). None of the 32/149 patients without clinical SLE at the time of renal biopsy were clinically diagnosed with SLE during median follow-up of 20.0 years (interquartile range 8.3–33.8). The consensus clinicopathologic diagnoses of these 32 patients were: membranous nephropathy (anti-PLA2R-positive, $n=1$; cancer-associated, $n=3$), IgA nephropathy ($n=4$), infection-related glomerulonephritis ($n=2$), ANCA-associated glomerulonephritis ($n=2$), and idiopathic non-lupus full house nephropathy ($n=20$).⁵ Details on the clinical presentation, biopsy findings, and clinical follow-up of these patients are provided elsewhere.⁵

General characteristics of patients with and without clinical SLE

General characteristics of patients in our cohort and the prevalence of individual ACR and SLICC classification criteria are shown in **Table 1**. Patients with clinical SLE were significantly younger and more often female than patients without clinical SLE. For some patients, the absence or presence of cutaneous and/or immunologic criteria was unconvincing, in which cases these criteria were excluded from the comparisons. The 32 patients without clinical SLE less frequently fulfilled individual ACR and SLICC classification criteria than the 117 patients with clinical SLE, except for the criteria oral/nasal ulcers, discoid rash, anti-Sm, and antiphospholipid antibodies.

Biopsy findings in patients with and without clinical SLE

Comparisons of biopsy findings between patients with and without clinical SLE are shown in **Table 2** (see also Rijnink *et al.*⁵ for a comparison excluding patients without SLE due to secondary causes). Briefly, the pattern of histopathologic injury by light microscopy was different between patients with and without clinical SLE ($P=0.003$); with absent lesions or a purely mesangial pattern of injury and a membranous pattern being more prevalent in patients without clinical SLE, and with endocapillary and/or extracapillary lesions being more prevalent in patients with clinical SLE. C1q and IgM immunofluorescence staining was significantly more intense in patients with clinical SLE compared to patients without (both $P<0.01$). By electron microscopy, patients with clinical SLE more often had subendothelial deposits ($P=0.008$).

Fulfilment of ACR and SLICC classification criteria

At the time of renal biopsy, 110 patients in our cohort with clinical SLE fulfilled ≥ 4 ACR and SLICC criteria for the classification of SLE and seven patients fulfilled ≥ 4 SLICC criteria only (**Figure 1**). Of the 32 patients without clinical SLE, three fulfilled the stand-alone renal criterion of the SLICC classification because of their renal biopsy findings in combination with ANA but had <4 ACR and SLICC criteria at the time of renal biopsy. Twenty-nine patients without clinical SLE did not meet the classification requirements at the time of renal biopsy.

Table 1 General characteristics and prevalence of individual 1997 ACR^a and 2012 SLICC^b criteria in patients with and without clinical SLE in the full house cohort.

Characteristic	Clinical SLE (n=117)	No clinical SLE (n=32)	P
Age, $\bar{y} \pm \text{SD}$	32.6 $\pm$ 14.6	38.7 $\pm$ 16.2	0.041
Sex, male:female	30:87	21:11	<0.001
ACR criteria, median (range)	5 (3–9)	1 (1–3)	<0.001
SLICC criteria, median (range)	7 (4–14)	1 (1–3)	<0.001
Clinical criteria	n/total (%)	n/total (%)	
Acute/subacute cutaneous lupus ^b	66/117 (56.4)	0/32 (0)	<0.001
Malar rash ^a	48/112 (42.9)	0/32 (0)	<0.001
Photosensitivity ^a	25/112 (22.3)	0/32 (0)	0.001
Chronic cutaneous lupus ^b	14/117 (12.0)	0/32 (0)	0.041
Discoid rash ^a	11/106 (9.4)	0/32 (0)	0.122
Non-scarring alopecia ^b	21/117 (17.9)	0/32 (0)	0.008
Oral/nasal ulcers ^{a,b}	25/117 (21.4)	3/32 (9.4)	0.200
Arthritis ^{a,b}	86/117 (73.5)	1/32 (3.1)	<0.001
Serositis ^{a,b}	44/117 (37.6)	1/32 (3.1)	<0.001
Neurologic disorder ^{a,b}	18/117 (15.4)	0/32 (0)	0.013
Haemolytic anaemia ^{a,b}	20/117 (17.1)	0/32 (0)	0.008
Lympho-/leukopenia ^{a,b}	36/117 (30.8)	0/32 (0)	<0.001
Thrombocytopenia ^{a,b}	28/117 (23.9)	0/32 (0)	0.001
Immunologic criteria			
Antinuclear antibody ^{a,b}	116/117 (99.1)	3/26 (11.5)	<0.001
Anti-dsDNA ^{a,b}	80/109 (73.4)	0/28 (0)	<0.001
Anti-Smith ^{a,b}	18/56 (32.1)	0/8 (0)	0.093
Antiphospholipid antibody ^{a,b}	41/81 (50.6)	0/5 (0)	0.057
Hypocomplementaemia ^b	94/109 (86.2)	2/23 (8.7)	<0.001
Direct Coombs' test ^b	25/75 (33.3)	0/13 (0)	0.016

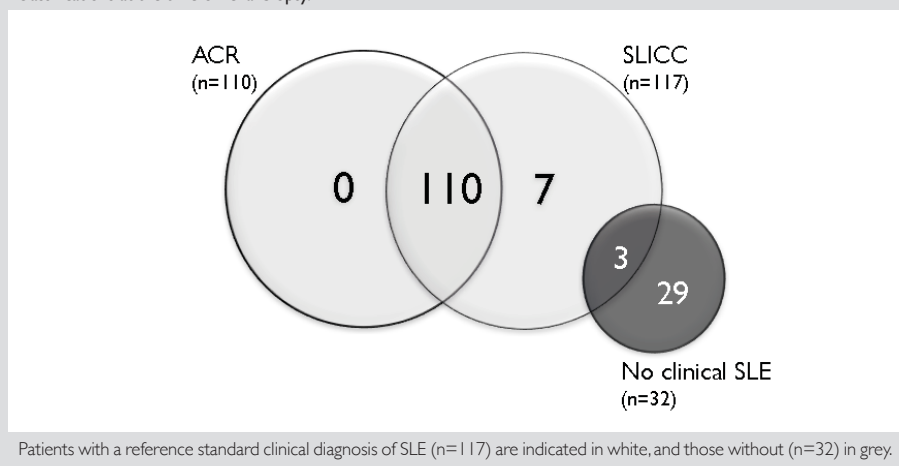
Classification criteria were registered up to and including the time of renal biopsy. Fractions indicate the number of patients with a particular criterion divided by the total number of patients for whom the presence or absence of a criterion could be retrieved. The total number of SLE classification criteria was compared using Mann Whitney U test. The prevalence of individual SLE criteria were compared using Fisher's exact tests. Anti-dsDNA, anti-double stranded DNA antibody; Anti-Smith, anti-Smith antibody.

Table 2 Histopathologic findings in patients with and without clinical SLE.

	Clinical SLE (n=117)	No clinical SLE (n=32)	P
Light microscopy			
ISN/RPS 2003 class, n (%)			
I	1 (1)	3 (9)	
II	2 (2)	1 (3)	
III	24 (21)	11 (34)	
IV	79 (68)	8 (25)	
III/IV + V	2 (2)	3 (9)	
V	9 (8)	6 (19)	<0.001
III/IV (+V) A	45 (43)	8 (35)	
III/IV (+V) A/C	59 (56)	11 (48)	
III/IV (+V) C	1 (1)	4 (17)	0.058*
No lesions/ purely mesangial lesions, n (%)	3 (3)	4 (13)	
Endo- and/or extracapillary lesions, n (%)	105 (90)	23 (72)	
Membranous lesions, n (%)	11 (9)	9 (28)	0.003
Immunofluorescence microscopy			
IgA, n (%)			
+	26 (22)	11 (34)	
++	60 (51)	15 (47)	
+++	31 (26)	6 (19)	0.158*
IgM, n (%)			
+	27 (23)	16 (50)	
++	64 (55)	13 (41)	
+++	26 (22)	3 (9)	0.004*
IgG, n (%)			
+	21 (18)	9 (28)	
++	67 (57)	10 (31)	
+++	29 (25)	13 (41)	0.682*
C3, n (%)			
+	8 (7)	7 (22)	
++	64 (55)	14 (44)	
+++	45 (39)	11 (34)	0.131*
C1q, n (%)			
+	9 (8)	11 (34)	
++	67 (57)	14 (44)	
+++	41 (35)	7 (22)	0.002*
Electron microscopy			
	(n=29)	(n=14)	
Mesangial deposits, n (%)	10 (35)	5 (36)	1.000
Subendothelial deposits, n (%)	23 (80)	5 (36)	0.008
Subepithelial deposits, n (%)	24 (83)	11 (79)	1.000
Food process effacement, n (%)	24 (83)	11 (79)	1.000
Tubuloreticular inclusions, n (%)	5 (17)	2 (14)	1.000
Number of locations with deposits†, n (%)			
<2	5 (17)	9 (64)	
2	20 (69)	2 (14)	
>2	4 (14)	3 (21)	0.078*

* Linear-by-linear analysis. † Locations include: mesangial, subendothelial and subepithelial.

Figure 1 Venn diagram illustrating the distribution of patients with and without clinical SLE identified by the ACR and SLICC classifications at the time of renal biopsy.



ACR classification: false negatives

Classification according to the ACR criteria resulted in seven false-negative classifications. These patients had, in addition to renal involvement, hypocomplementaemia and two other criteria (synovitis, neuropsychiatric lupus, lupus anticoagulant, leukopenia, positive ANA, and/or anti-dsDNA). Thus, they were classified according to exactly 4 SLICC criteria. Classification of SLE according to the ACR criteria was not possible, as hypocomplementaemia is not included. During median follow-up of 9.0 years (interquartile range 3.2–19.0), 2 of these 7 patients could also be classified as SLE according to ≥ 4 ACR criteria after 5 and 6 years. Thus, the sensitivity of the ACR classification increased from 94 to 96% after 6 years of follow-up.

SLICC classification: false positives

Classification according to the SLICC criteria resulted in three false positive classifications. These patients had a renal biopsy with a full house immunofluorescence pattern in combination with lesions by light and electron microscopy consistent with LN as detailed below. Because of a positive ANA, they were classified as SLE based on the stand-alone renal SLICC criterion assuming they had “biopsy-confirmed” LN, but had < 4 ACR and SLICC criteria at the time of biopsy and during follow-up (median 7.7 [range 2.9–9.0] years). One patient had crescentic and endocapillary glomerulonephritis with 2+ intensity for IgG, IgA, IgM, C3, and C1q by immunofluorescence, and no material for electron microscopy available. This patient was clinically diagnosed with PR3-ANCA-associated glomerulonephritis and had a positive ANA 4 years prior to renal biopsy, but negative ANA during follow-up. Two other patients had cancer-associated membranous nephropathy; one of them with membranous nephropathy with glomerular sclerosis by light microscopy, IgG 3+, IgA 1+, IgM 1+, C3 1+ and C1q 3+ by immunofluorescence, and subepithelial deposits by electron microscopy; the other with focal endocapillary glomerulonephritis with spikes, IgG 1+, IgA

I+, IgM I+, C3 3+ and C1q 2+ by immunofluorescence, and subepithelial deposits and tubuloreticular inclusions by electron microscopy. Both had a positive ANA at the time of renal biopsy, and the first also after 4 years of follow-up. A fourth patient without clinical SLE at the time of biopsy and during follow-up fulfilled no additional SLE classification criteria at the time of renal biopsy, but had a persistently positive ANA after 12 years of follow-up. The renal biopsy at baseline showed focal endocapillary glomerulonephritis, IgG 3+, IgA I+, IgM I+, C3 3+, C1q 3+ by immunofluorescence, and subepithelial deposits by electron microscopy. Therefore, the specificity of the SLICC classification decreased from 91 to 88% after 12 years follow-up.

Comparing the diagnostic performance of the ACR and SLICC classifications

The performance of the ACR and SLICC classifications in our cohort is shown in **Table 3**. Compared with the ACR classification, the SLICC classification was more sensitive (100 vs. 94%) but less specific (91 vs. 100%). The sensitivity and specificity of the SLICC classification were 100% when excluding the stand-alone renal criterion, based on the finding that three patients were incorrectly classified as SLE based on the stand-alone renal criterion. Compared with the ACR classification, there was no reclassification improvement of the SLICC classification (NRI -0.034, $P=0.563$). Exclusion of the stand-alone renal criterion resulted in 6% of SLE patients being appropriately reclassified in this cohort as compared with the ACR classification, resulting in significant reclassification improvement (NRI 0.060; $P=0.014$). Reclassification tables are shown in **Appendix 2.1**.

Table 3 Performance of the SLICC (2012) and ACR (1997) classifications in the full house cohort.								
Classification	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	NRI _{SLE(+)} , %	NRI _{SLE(-)} , %	NRI	P
ACR 1997	94.0 (87.6; 97.4)	100 (86.7; 100)	100 (95.8; 100)	82.1 (65.9; 91.9)	-	-	-	-
SLICC 2012	100 (96.0; 100)	90.6 (73.8; 97.5)	97.5 (92.3; 99.3)	100 (85.4; 100)	6.0	-9.4	-0.034	0.563
SLICC 2012 without stand-alone renal criterion*	100 (96.0; 100)	100 (86.7; 100)	100 (96.0; 100)	100 (86.7; 100)	6.0	0	0.060	0.008

NRI (net reclassification improvement) was calculated relative to the ACR 1997 classification (reference) and was computed separately for clinical SLE cases (SLE(+)) and non-SLE cases (SLE(-)). P-values were calculated for the null hypothesis NRI = 0. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value. * Stand-alone renal criterion is defined as a full house renal biopsy in combination with antinuclear or anti-double stranded DNA antibodies.

Comparing our cohort with the SLICC derivation cohort

The prevalence of individual SLICC classification criteria in our cohort was compared to the reported prevalence of these criteria in the SLICC derivation cohort (**Appendix 2.2**). Compared with SLE patients from the SLICC derivation cohort, SLE patients in our cohort had a lower frequency of non-scarring alopecia, oral/nasal ulcers, and leukopenia. Conver-

sely, they had a significantly higher frequency of renal disorder (by selection), neurologic disorder, haemolytic anaemia, thrombocytopenia, anti-dsDNA, and hypocomplementaemia. Compared with control patients without SLE from the SLICC derivation cohort, control patients without SLE in our cohort had significantly less frequent acute/subacute cutaneous lupus and arthritis, while the occurrences of other SLICC classification criteria were similar.

DISCUSSION

We performed the first study to test the validity of the SLICC classification criteria for SLE in a cohort of patients with a clinicopathologic suspicion of SLE based on the finding of full house glomerular deposits in their renal biopsies, a group reflecting a diagnostic problem area in the nephrology clinic. Our findings show that, overall, the SLICC criteria were more sensitive (100 vs. 94%) and enabled earlier classification than the ACR criteria. However, this was at the expense of specificity (91 vs. 100%). We identified three patients with biopsy findings consistent with LN and a positive ANA, who were classified as SLE according to the stand-alone renal SLICC criterion, but who had no further signs or symptoms of SLE. Conversely, no patients with clinical SLE were found who only fulfilled the stand-alone renal criterion. Exclusion of the stand-alone renal criterion from the SLICC classification resulted in significant reclassification improvement compared with the ACR classification in this cohort. We conclude that overall the SLICC classification performed well in our cohort, but the stand-alone renal criterion compromised the specificity. False-positive diagnoses emanating from the adaptation of the stand-alone renal SLICC criterion in clinical practice may have major consequences for patients, given the implications of the diagnoses LN and SLE.

Bayes' theorem states that the odds of disease equal the disease frequency (pretest odds in Bayesian terms) multiplied by the likelihood ratio. In this equation, the likelihood ratio stems from the sensitivity and specificity of the classification criteria set. Both sensitivity and specificity are dependent on the population that is studied. First, the sensitivity of a criterion may vary when studying a different disease spectrum. Patients with SLE in our cohort seemed to represent a different phenotype of SLE than the SLE patients in the SLICC derivation cohort who were selected from rheumatology clinics. Compared with patients in the SLICC derivation cohort, SLE patients with renal involvement in our cohort had a significantly lower frequency of alopecia, oral/nasal ulcers, and leukopenia, and a higher frequency of neurologic disorders, haemolytic anaemia, thrombocytopenia, anti-dsDNA antibodies, and hypocomplementaemia. These results are in agreement with those from a recently published large inception cohort comparing SLE patients with and without nephritis¹⁹ and support the notion that SLE is a disease with heterogeneous phenotypes.²⁰ Second, the specificity of a criterion is fully dependent on the control population studied. In designing a classification criteria set, the control population is chosen to represent the diagnostic problem area.² By design, renal involvement was less specific in our cohort than

in the SLICC derivation cohort, as the latter did not select patients with renal diseases that would appear in the differential diagnosis of LN. Moreover, the control population used for derivation of the SLICC criteria had more cutaneous and joint manifestations, reflecting typical findings in patients from rheumatology clinics. Thus, the sensitivities, specificities, and emanating likelihood ratios of criteria used in the development of the SLICC classification cannot be unequivocally applied to potential SLE patients in the nephrology clinic.

From our results, the combination of ANA and biopsy-confirmed LN as stand-alone criterion is questionable, as both had suboptimal specificity. Three patients were false-positively classified as SLE according to the stand-alone renal criterion by the SLICC. Although the specificity of ANA in our cohort was 88.5%, ANAs are associated with various other rheumatic and non-rheumatic diseases and can be detected in up to 27% of the general population.²¹ Most telling, two patients in our cohort had membranous nephropathy and concurrent malignancy, the latter known for its association with ANA-positivity.²² A positive ANA itself is very sensitive for SLE, but clearly ANAs are more accurate in ruling out SLE than confirming the diagnosis. Since ANA testing was performed qualitatively using 1:40 diluted serum and titres were unavailable for the patients in our study, it is unknown whether higher cut-offs would have resulted in more patients being appropriately classified using the stand-alone renal criterion. The classification of SLE according to the combination of a renal biopsy consistent with LN and positive anti-dsDNA antibodies in the absence of other criteria for SLE was not observed in our cohort. Possibly, anti-dsDNA antibodies would be more suitable than ANA as part of the stand-alone criterion given their higher specificity for SLE.

In addition to the problems emanating from the autoantibodies included in the stand-alone criterion, there is no consensus among pathologists and clinicians of what defines biopsy-confirmed LN. In our opinion, the finding of a full house pattern of immunofluorescence would certainly raise SLE as a differential diagnostic consideration. However, 32 patients in our cohort without SLE had full house immunofluorescence, affirming that full house immunofluorescence *per se* is a far from optimal indicator for LN and must be interpreted in light of clinical and additional biopsy findings. Clearly, the SLICC criteria excluding the stand-alone renal criterion appeared to be useful to identify SLE cases clinically. Concerning the additional biopsy findings, we found endocapillary and/or extracapillary hypercellularity, relative intensity of IgM and C1q, and subendothelial deposits by electron microscopy to support the diagnosis of SLE. Other biopsy findings, including tubuloreticular inclusions and coexistent mesangial, subendothelial and subepithelial deposits, that have previously been found to be suggestive of LN³ were not significantly different between patients with and without clinical SLE in our cohort. It may be anticipated that in some cases, these other renal biopsy findings characteristic of LN would lead to the designation of biopsy-confirmed LN.³ This emphasises that the extent to which the stand-alone criterion by the SLICC may result in false-positive classifications of SLE may be underestimated by this study.

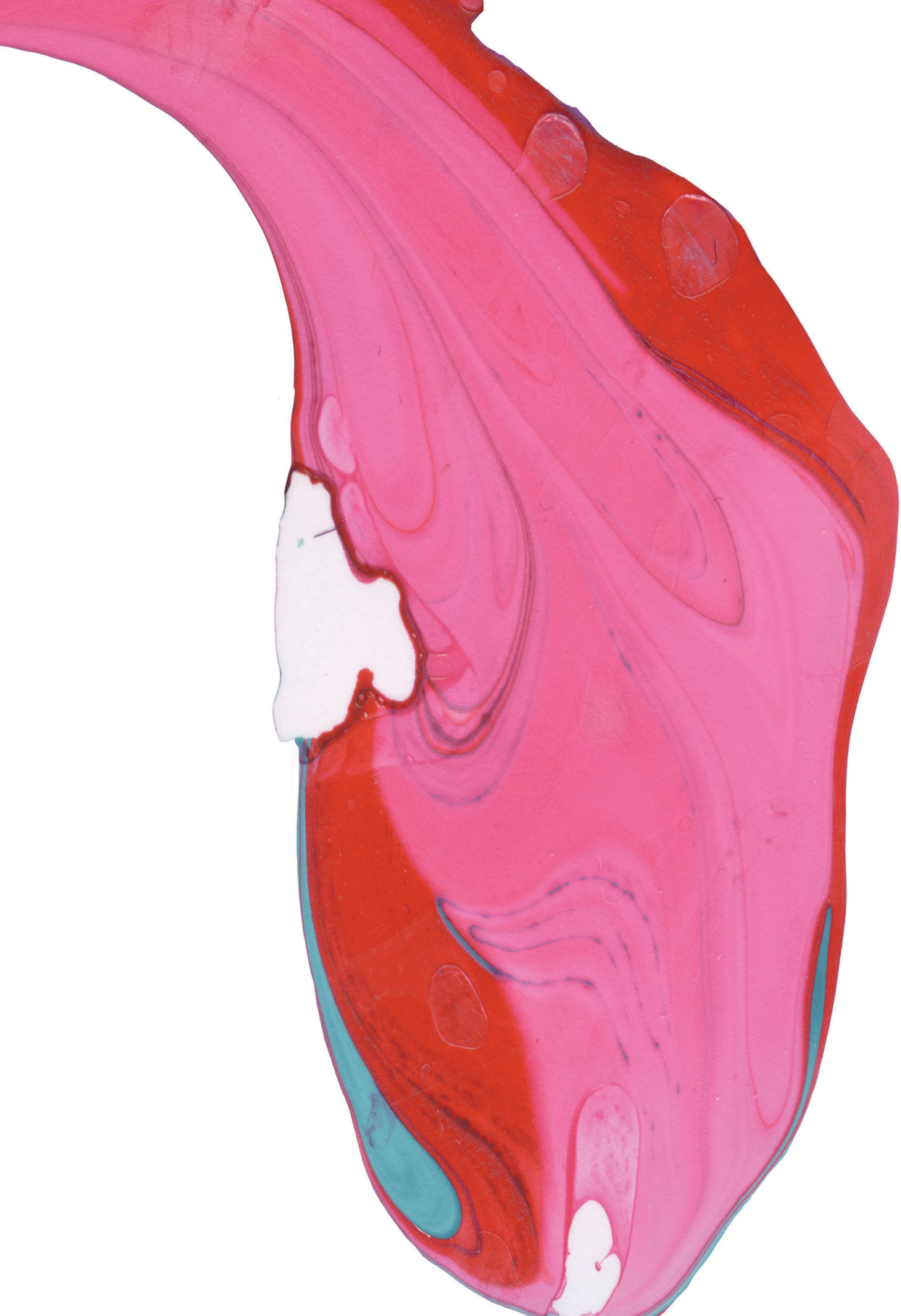
Conversely, the number of false positives may also be overestimated by our study. In the literature, anecdotal reports of patients with “non-lupus full house nephropathy” – similar to the 32 patients without clinical SLE in our study – have shown that the minority of these patients become seropositive and/or develop extrarenal symptoms of SLE during up to 10 years follow-up.²³⁻²⁷ However, during a median follow-up of 20.0 years (interquartile range 8.3–33.8), none of the 32 patients without clinical SLE in our study were clinically diagnosed with SLE. It cannot be excluded that some patients had latent SLE that would be diagnosed if more long-term follow-up data would be available. However, other studies have shown that non-lupus full house nephropathy can also be associated with atypical presentations of other well-established renal diseases or may occur idiopathically.^{3, 5, 7-10, 24, 28-31}

We have shown that the SLICC classification proved to have great sensitivity among patients with a renal biopsy with full house glomerular deposits. This superior sensitivity of the SLICC relative to the ACR classification in our cohort was entirely attributed to the criterion hypocomplementaemia, which is absent in the ACR classification. Indeed, our results underline that complement consumption in the classical pathway is an essential finding in active severe SLE with renal involvement.^{32, 33} In our cohort, the specificity of the SLICC classification was lower than the ACR classification, and this was attributed to the introduction of the stand-alone renal criterion to the SLICC classification. Other validation studies similarly showed a higher sensitivity and lower specificity of the SLICC compared with the ACR classification, although none of them elaborated on the stand-alone renal criterion.^{11, 15-17, 34, 35} Only Ungprasert *et al.* identified three patients with SLE among 55 who met SLICC criteria who were classified based upon the stand-alone criterion without commenting on their biopsy findings.³⁵ We conclude that the SLICC classification may perform well as classification and conceivably also as diagnostic criteria for patients with renal biopsy findings consistent with LN, but we suggest re-evaluation of the stand-alone renal criterion for clinical and research purposes.

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Chapter 3

Idiopathic Non-lupus Full House Nephropathy is Associated With Poor Renal Outcome

Emilie C. Rijnink, Y.K. Onno Teng, Tineke Kraaij, Ron Wolterbeek, Jan A. Bruijn, Ingeborg M. Bajema

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ABSTRACT

Background

Full house immunofluorescence in combination with various histopathologic lesions in the renal biopsies of patients without overt systemic lupus erythematosus (SLE) poses a diagnostic challenge. In this setting, the biopsy findings are sometimes termed non-lupus “full house nephropathy” (FHN). It is presently unknown whether idiopathic non-lupus FHN is clinicopathologically and prognostically distinct from lupus FHN.

Methods

We included non-lupus FHN patients and lupus FHN controls (four or more ACR or SLICC criteria) who were biopsied between 1968–2014 at the Leiden University Medical Center. Non-lupus FHN patients were studied for progression to SLE and/or the presence of other conditions with FHN. The clinicopathologic characteristics and prognosis of idiopathic non-lupus FHN patients were compared to those of lupus FHN patients.

Results

Of 149 included patients, 32 had non-lupus FHN. During the median follow-up of 20 years, no non-lupus FHN patients developed SLE. Twenty non-lupus FHN patients had idiopathic non-lupus FHN, and in 12 patients secondary non-lupus FHN was considered due to membranous nephropathy (anti-PLA2R-positive, $n=1$; cancer-associated, $n=3$), IgA nephropathy ($n=4$), infection-related glomerulonephritis ($n=2$), or anti-neutrophil cytoplasmic antibody-associated glomerulonephritis ($n=2$). Idiopathic non-lupus FHN patients were more often male ($P<0.001$) than lupus FHN patients, and their renal biopsies more often showed a mesangial ($P=0.04$) or membranous pattern of injury ($P=0.02$), and less intense C1q staining ($P=0.002$). Clinically, they presented with lower-range erythrocyturia ($P=0.04$), more proteinuria ($P<0.01$), and less complement consumption in the classical pathway ($P<0.001$) than lupus FHN patients. By multivariable Cox regression analysis of patients with a lupus nephritis class III/IV pattern of injury, idiopathic non-lupus FHN compared to lupus FHN was an independent risk factor for end-stage renal disease (HR 5.31; 95% CI 1.47 to 19.24).

Conclusion

Our results show that the clinical recognition of idiopathic non-lupus FHN as a diagnostic category is critical.

INTRODUCTION

A typical finding in renal biopsies of patients with lupus nephritis (LN) is a full house pattern by immunofluorescence, which is defined as concurrent positive staining for IgA, IgG, IgM, C3, and C1q.¹ In a patient with extrarenal signs and symptoms of systemic lupus erythematosus (SLE), identification of nephritis with a full house immunofluorescence pattern on renal biopsy is consistent with LN. However, a diagnostic challenge ensues when patients present with various histopathologic lesions – either or not resembling LN – in combination with a full house pattern by immunofluorescence in the absence of autoantibodies and other classifying signs or symptoms of SLE. In this setting, the biopsy findings are sometimes termed non-lupus “full house nephropathy” (FHN).² Non-lupus FHN was first systematically described in a group of 24 patients by Wen and Chen, who defined the clinicopathologic spectrum of this entity and indicated that in future studies, the emergence of overt SLE remained to be elucidated.² Anecdotal reports have shown that only a minority of these patients eventually develop the autoantibodies characteristic of SLE and/or extrarenal symptoms of SLE during follow-up,²⁻⁷ whereas other reports have shown that non-lupus FHN can be associated with atypical manifestations of other well-established pathologies.^{2, 8-15} It remains unclear how to classify and treat non-lupus FHN patients for whom the origin of FHN is idiopathic.

In the present study, patients with idiopathic non-lupus FHN and non-lupus FHN due to other disease processes were identified from the largest cohort of patients with renal biopsies showing full house immunofluorescence to date. The clinicopathologic characteristics and prognosis of idiopathic non-lupus FHN patients were compared to those of lupus FHN patients according to four or more American College of Rheumatology (ACR)¹⁶ or Systemic Lupus International Collaborating Clinics (SLICC)¹⁷ criteria.

METHODS

The pathology archives of the Leiden University Medical Center were searched from 1968 to 2014 to identify all patients with native renal biopsies showing full house immunofluorescence, which was defined as concurrent positive staining for IgA, IgG, IgM, C3, and C1q with $\geq 1+$ intensity on a 0–3+ scale. Only biopsies showing granular fluorescent staining along the capillary walls, in the mesangium, or both were included. Patients meeting these criteria and who were followed in our centre entered the study. In accordance with the ethics committee of the Leiden University Medical Center, all patient data were coded and kept anonymous throughout the study. Renal biopsies were re-evaluated by an experienced nephropathologist (IMB). In cases where renal biopsies were unavailable, the histopathologic diagnosis was deduced from the original biopsy report if possible; but they were excluded from analyses involving categorical pathology scoring. All biopsies were (re)

classified according to the ISN/RPS classification of LN,¹⁸ regardless of the clinicopathologic diagnosis. Global glomerulosclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis were scored semi-quantitatively as (0) <5%; (1) 5–25%; (2) 26–50%; (3) >50% of glomeruli, tubules, or cortical area. The scores were summed to generate a total chronicity score. Finally, the pathologist made a differential diagnosis based on light microscopy findings knowing that the patients had a full house pattern by immunofluorescence.

All biopsies were processed for light and immunofluorescence microscopy according to the standard techniques at our centre. For immunofluorescence microscopy, sections were frozen in liquid nitrogen, and cryostat sections were stained with FITC-labelled antisera to human IgA, IgG, IgM, C3, and C1q. Immunofluorescence reports were originally prepared by four experienced nephropathologists who consistently scored the immunofluorescence intensity on a 0–3+ scale. Not all biopsies were sent in for analysis by electron microscopy; but if at hand, tissue was fixed in 1.5% glutaraldehyde and 1% paraformaldehyde and embedded in Epon.

The medical records of the patients were reviewed independently by investigators (ECR, YKOT, and TK) for the presence of four or more cumulative ACR¹⁶ and/or SLICC¹⁷ criteria for SLE at the time of renal biopsy. After independent review, a consensus was reached for fulfilment of the classification criteria. Cases that did not fulfil four or more ACR¹⁶ or SLICC¹⁷ criteria at the time of biopsy were considered representative of non-lupus FHN and were evaluated in a clinicopathologic conference by investigators (ECR, YKOT, TK, and IMB) reaching consensus on the clinicopathologic differential diagnosis at the time of biopsy. Additional serologic parameters were recorded for non-lupus FHN patients if available. Follow-up data of non-lupus FHN patients were meticulously studied to investigate whether they fulfilled ACR¹⁶ or SLICC¹⁷ classification criteria at any time after renal biopsy.

For lupus FHN and non-lupus FHN patients, serum creatinine, estimated glomerular filtration rate (eGFR),^{19,20} 24h proteinuria, erythrocyturia, and grade of hypertension²¹ were recorded at the time of renal biopsy. Induction immunosuppression was recorded for all patients, as was immunosuppression initiated after renal transplantation in non-lupus FHN. End-stage renal disease (ESRD) was defined as dialysis-dependence for >3 months or renal transplantation. Patients lost to follow-up were checked in the population register to see if they were alive at the end of this study (5 October 2015); if not, the date of death was recorded.

Statistical analyses

Normally distributed data were compared with *t*-tests, and non-normally distributed data with Mann-Whitney U tests. Categorical data were compared with Fisher's exact tests, chi-square tests, or linear-by-linear analysis.

The outcomes time to ESRD, death, and ESRD/death were analysed using Kaplan-Meier

curves and log-rank tests. The observation period considered for the outcome analyses started at the time of renal biopsy demonstrating FHN. Multivariable analyses included idiopathic non-lupus FHN and lupus FHN patients. The following subgroups classified according to the ISN/RPS classification were analysed separately: (i) class I/II; (ii) class III/IV ($\pm$ V); and (iii) class V. The following candidate predictors of ESRD and/or death were entered in multivariable proportional hazards models: idiopathic non-lupus FHN; sex; age, eGFR, and proteinuria at the time of biopsy; and induction immunosuppression (model 1). A second model was designed including non-lupus FHN, induction immunosuppression and histologic chronicity score (model 2). All *P*-values are two-sided and were considered significant at *P*<0.05. Analyses were performed using SPSS 23.0 (IBM, Armonk, New York).

RESULTS

Of the 149 patients included, 117 patients had lupus FHN, while 32 patients had non-lupus FHN. A total of 26 non-lupus FHN and 99 lupus FHN patients had a renal biopsy available for re-evaluation. The median number of scorable glomeruli was 15 (range 6–65) in non-lupus FHN patients, and 14 (range 6–56) in lupus FHN patients.

Clinicopathologic diagnoses of non-lupus FHN patients

The clinicopathologic characteristics of individual non-lupus FHN patients are shown in **Table 1** (see **Appendix 3.1** for detailed descriptions). The 32 non-lupus FHN patients were neither originally diagnosed with SLE at the time of biopsy, nor after revision of the medical files and evaluation of SLE classification criteria. The following cumulative SLE criteria were registered at the time of biopsy in non-lupus FHN patients: oral/nasal ulcers (cases 5, 27, and 28), synovitis (case 6), serositis (case 6), hypocomplementaemia (cases 5 and 27), and anti-nuclear antibody (ANA) (cases 28, 31, and 32). During median follow-up of 20 years (interquartile range [IQR] 8.3–33.8), two cases developed additional ACR/SLICC criteria: case 12 developed arthritis 9 years after renal biopsy (likely attributable to rheumatoid arthritis), and case 19 developed serositis and arthritis 2 years after renal biopsy. However, none of the 32 patients were clinically diagnosed with SLE during follow-up or met four or more ACR or SLICC criteria.

In view of the presence of FHN in the absence of SLE in these 32 patients, we re-evaluated their clinical presentation including serologic and histopathologic findings, and long-term clinical follow-up (including follow-up biopsies) to investigate whether other differential diagnostic considerations than SLE would apply to some of them. In 12 patients, we considered atypical variants of the following entities (**Table 1** and **Appendix 3.1**): membranous nephropathy (anti-PLA2R-positive, *n*=1; cancer-associated, *n*=3), IgA nephropathy (*n*=4), infection-related glomerulonephritis (*n*=2), and anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (AAGN, *n*=2). We will henceforth refer to these 12 patients as “secondary non-lupus FHN”. Importantly, 20 non-lupus FHN patients had idiopathic FHN.

Table 1 Clinical presentation and histopathologic findings in patients with non-lupus full house nephropathy (FHN).

Patient number	Sex	Age, y	Presentation	Clinicopathologic differential diagnosis
Idiopathic non-lupus FHN				
1	F	16	NS	Idiopathic
2	M	58	NS	Idiopathic
3	M	27	Hematuria, subnephrotic proteinuria	Idiopathic
4	F	16	Acute renal insufficiency	Idiopathic
5	M	58	RPGN	Idiopathic
6	M	56	NS	Idiopathic
7	M	31	NS	Idiopathic
8	M	29	Haematuria	Idiopathic
9	M	58	NS, haematuria	Idiopathic
10	M	45	NS	Idiopathic
11	F	28	NS, renal insufficiency, malignant hypertension	Idiopathic
12	M	52	RPGN	Idiopathic
13*	F	29	NS	Idiopathic
14	M	31	NS	Idiopathic
15	M	31	NS	Idiopathic
16*	M	67	Acute renal insufficiency	Idiopathic
17*	F	22	NS	Idiopathic
18	M	21	RPGN	Idiopathic
19	M	52	NS	Idiopathic
20	F	19	Hypertension	Idiopathic
Secondary non-lupus FHN				
21*	M	34	Macroscopic haematuria, subnephrotic proteinuria	IgA-like nephropathy
22	M	31	Haematuria, subnephrotic proteinuria	Crescentic IgA-like nephropathy
23	F	32	Malignant hypertension, acute renal insufficiency	Crescentic IgA-like nephropathy
24	F	18	Haematuria, subnephrotic proteinuria	Crescentic IgA-like nephropathy
25	M	55	Haematuria, microalbuminuria	Infection-related glomerulonephritis
26*	M	50	RPGN	Infection-related glomerulonephritis
27	F	21	RPGN	ANCA-associated glomerulonephritis
28	F	47	Haematuria, subnephrotic proteinuria	ANCA-associated glomerulonephritis
29	M	30	NS	MN (anti-PLA2R)
30	M	55	Haematuria, subnephrotic proteinuria	MN (cancer-associated) + FSGS
31	M	71	NS	MN (cancer-associated) + secondary glomerular sclerosis
32	F	50	NS	MN (cancer-associated) + minimal endocapillary proliferation

ANCA, anti-neutrophil cytoplasmic antibody; F, female; FSGS, focal segmental glomerulosclerosis; M, male; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; NS, nephrotic syndrome; RPGN, rapidly progressive glomerulonephritis; TMA, thrombotic microangiopathy. *The histopathologic diagnosis was deduced from the original biopsy report. †ISN/RPS 2003 classification of LN.¹⁸

Light microscopy pattern	Immunofluorescence					Classification as in SLE†
	IgA, +	IgG, +	IgM, +	C3, +	C1q, +	
Minimal lesions	1	1	2	2	2	I
Minimal lesions	2	2	2	2	2	I
Mesangioproliferative	2	2	1	2	1	II
Diffuse proliferative, crescents	2	2	3	3	3	IV-G (A/C)
Diffuse proliferative	2	3	3	2	2	IV-G (A/C)
Segmental chronic lesions, spikes	3	2	2	3	3	III (C) + V
Focal proliferative	2	3	2	2	2	III (A/C)
Focal proliferative	1	1	1	1	1	III (A/C)
Focal proliferative	1	3	2	1	1	III (A)
Focal proliferative	1	3	1	3	3	III (A/C)
Diffuse proliferative	2	2	1	1	1	IV-G (A)
Diffuse proliferative, crescents, fibrinoid necrosis	2	1	2	3	2	IV-G (A/C)
MPGN, spikes	2	2	2	2	2	IV-G (A/C) + V
Focal proliferative	1	3	2	2	2	III (A/C)
Membranous	2	2	1	2	1	V
Membranous	3	3	1	1	1	V
Membranous	1	3	2	2	3	V
Membranous, interstitial nephritis, minimal vasculopathy	3	3	1	3	1	V
Segmental chronic lesions, occasional spikes	1	3	2	2	2	III (C)
FSGS, segmental chronic lesions, TMA	1	1	1	2	1	III (A/C)
Segmental chronic lesions	2	1	1	2	2	III (C)
Focal proliferative, crescents	2	1	1	1	2	III (A/C)
Diffuse proliferative, crescents	3	2	2	3	2	IV-G (A/C)
Focal proliferative, crescents, fibrinoid necrosis	2	1	1	3	1	III (A/C)
Minimal lesions	2	2	1	2	2	I
Diffuse proliferative	2	3	2	3	3	IV-G (A)
Diffuse proliferative, crescents	1	1	1	1	1	IV-G (A/C)
Diffuse proliferative, crescents	2	2	2	2	2	IV-S (A)
Membranous	3	3	3	3	3	V
Focal proliferative, FSGS, spikes	3	3	1	3	1	III (A/C) + V
Membranous	1	3	1	1	3	V
Focal proliferative, occasional spikes	1	1	1	3	2	III (A)

Clinical presentation of idiopathic non-lupus FHN compared to lupus FHN patients

Of the 20 idiopathic non-lupus FHN patients, 12 (60%) presented with a nephrotic syndrome, 2 (10%) with abnormal urinary sediment, 2 (10%) with acute renal insufficiency, 3 (15%) with rapidly progressive glomerulonephritis, and 1 (5%) with hypertension and raised serum creatinine in the absence of erythrocyturia/proteinuria (**Table 1**).

The clinical characteristics of idiopathic non-lupus FHN compared to lupus FHN patients at the time of biopsy are shown in **Table 2**. Briefly, compared to lupus FHN patients, idiopathic non-lupus FHN patients were more often male ($P<0.001$); they had significantly more proteinuria ($P<0.01$), less erythrocyturia ($P=0.04$), and less complement consumption in the classical pathway ($P<0.001$ for C3, C4, and CH50). Up to and including the time of renal biopsy, oral/nasal ulcers ($n=1$), synovitis ($n=1$), serositis ($n=1$), and hypocomplementaemia ($n=1$) were observed in idiopathic non-lupus FHN patients.

Table 2 Clinical characteristics of patients with lupus full house nephropathy (FHN) compared to idiopathic non-lupus FHN at the time of renal biopsy.

Characteristic	Lupus FHN (n=117)	Idiopathic non-lupus FHN (n=20)	P
Sex, male/female	30/87	14/6	<0.001#
Age, y	32.6 ± 14.6	37.3 ± 16.6	0.195§
Hypertension stage, no hypertension/stage 1/ stage 2/stage 3	74/29/11/3	15/4/1/0	0.702†
Serum creatinine, µmol/L	97 (76–150)	114 (94–222)	0.074¶
eGFR, mL/min	75 ± 36	78 ± 51	0.760§
ESR, mm/h	68 ± 33	83 ± 47	0.296§
Leukocytes, *10 ⁹ /L	6.6 ± 3.3	6.2 ± 1.0	0.783§
Thrombocytes, *10 ³ /mm ³	250 ± 103	225 ± 40	0.629§
Proteinuria, g/24h	3.9 ± 4.1	6.9 ± 5.5	0.006§
Erythrocyturia, missing/-/+ /++	28/16/28/45	13/4/2/1	0.037†
CH50, u/mL (ref. 207–467)	152 ± 118	433 ± 87	<0.001§
C3, mg% (ref. 47–80)	39 ± 20	81 ± 23	<0.001§
C4, mg% (ref. 13–39)	13 ± 11	40 ± 11	<0.001§
C1q, mg% (ref. 9–14)	11 ± 14	17 ± 2	0.463§
Acute cutaneous lupus, n/total (%)	66/117 (56)	0/20 (0)	<0.001‡
Malar rash, n/total (%)	48/112 (43)	0/20 (0)	<0.001‡
Photosensitivity, n/total (%)	25/112 (22)	0/20 (0)	0.014‡
Chronic cutaneous lupus, n/total (%)	14/117 (12)	0/20 (0)	0.222‡
Discoid rash, n/total (%)	11/106 (9)	0/20 (0)	0.367‡
Oral/nasal ulcers, n/total (%)	25/117 (21)	1/20 (5)	0.122‡
Non-scarring alopecia, n/total (%)	21/117 (18)	0/20 (0)	0.043‡
Synovitis, n/total (%)	86/117 (74)	1/20 (5)	<0.001‡

Table 2 Continued.

Characteristic	Lupus FHN (n=117)	Idiopathic non-lupus FHN (n=20)	P
Serositis, n/total (%)	44/117 (38)	1/20 (5)	0.004‡
Neurologic disorder, n/total (%)	18/117 (15)	0/20 (0)	0.074‡
Haemolytic anaemia, n/total (%)	20/117 (17)	0/20 (0)	0.044‡
Lympho-/leukopenia, n/total (%)	36/117 (31)	0/20 (0)	0.002‡
Thrombocytopenia, n/total (%)	28/117 (24)	0/20 (0)	0.013‡
Hypocomplementaemia, n/total (%)	94/109 (86)	1/13 (8)	<0.001‡
ANA, n/total (%)	116/117 (99)	0/16 (0)	<0.001‡
Anti-DNA, n/total (%)	80/109 (73)	0/19 (0)	<0.001‡
Anti-ENA, n/total (%)	50/66 (76)	0/3 (0)	1.000‡
Anti-SS-A, n/total (%)	26/57 (46)	0/3 (0)	1.000‡
Anti-SS-B, n/total (%)	14/57 (25)	0/3 (0)	1.000‡
Anti-RNP, n/total (%)	24/53 (45)	0/3 (0)	1.000‡
Anti-Sm, n/total (%)	18/56 (32)	0/3 (0)	0.546‡
Anti-Scl70, n/total (%)	8/53 (15)	0/3 (0)	1.000‡
Anti-Jo1, n/total (%)	12/53 (23)	0/3 (0)	1.000‡
Antiphospholipid antibody, n/total (%)	41/81 (51)	0/1 (0)	1.000‡
Direct Coombs', n/total (%)	25/75 (33)	0/7 (0)	0.095‡
PR3-ANCA, n/total (%)	2/39 (5)	0/4 (0)	1.000‡
MPO-ANCA, n/total (%)	30/39 (77)	0/4 (0)	0.006‡
Anti-C1q, n/total (%)	15/22 (68)	0/2 (0)	0.130‡

Normally distributed data are as expressed mean $\pm$ standard deviation. Non-normally distributed data are expressed as median (interquartile range). Cumulative SLE classification criteria were registered at the time of renal biopsy. Erythrocyturia was scored as: (–) 0–18 erythrocytes/ μ L; (+) 19–25 erythrocytes/ μ L; (++) >26 erythrocytes/ μ L. † Linear-by-linear analysis. ‡ Fisher's exact test. # Pearson chi-Square. § t-test. ¶ Mann-Whitney U test. ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; anti-ENA, anti-extractable nuclear antigen antibody; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate.

Histopathologic features of idiopathic non-lupus FHN compared to lupus FHN patients

The biopsies of idiopathic non-lupus FHN patients were histopathologically described as: no lesions (10%), focal segmental glomerulosclerosis (5%), mesangioproliferative (5%), focal proliferative (25%), segmental chronic lesions (10%), diffuse proliferative (10%), active crescentic (10%), membranoproliferative (5%), and membranous lesions (20%; see **Table 1**). By electron microscopy, immune deposits were consistent with findings in LN; showing the combination of mesangial, subendothelial and subepithelial deposits. Furthermore, in case 6 tubuloreticular inclusions were identified by electron microscopy (**Appendix 3.2**).

The histopathologic findings in lupus FHN compared to idiopathic non-lupus FHN patients are shown in **Table 3**. After classification of non-lupus FHN according to the ISN/RPS

classification, the distribution across LN classes was significantly different between lupus FHN and idiopathic non-lupus FHN ($P<0.001$). Absence of glomerular lesions or a purely mesangial pattern of injury by light microscopy (akin class I/II) was significantly more prevalent in idiopathic non-lupus FHN compared to lupus FHN ($P=0.04$), as was a membranous pattern (akin class V, $P=0.02$). Proliferative lesions (akin class III/IV) were significantly less prevalent in non-lupus FHN ($P<0.01$). Idiopathic non-lupus FHN patients had a different distribution across activity/chronicity subclasses (A, A/C, and C) associated with a class III/IV pattern of injury than lupus FHN patients – with chronic subclasses being present more frequently. However, individual chronic lesions were similar between lupus and idiopathic non-lupus FHN patients (global glomerulosclerosis, fibrous crescents, interstitial fibrosis and tubular atrophy, see **Table 3**). C I q immunofluorescence staining was significantly more intense in lupus FHN compared to idiopathic non-lupus FHN ($P<0.01$).

Table 3 Histopathologic findings in patients with lupus full house nephropathy (FHN) compared to idiopathic non-lupus FHN.

Histopathologic finding	Lupus FHN (n=117)	Idiopathic non-lupus FHN (n=20)	P
<i>Light microscopy</i>			
ISN/RPS 2003 class, n (%)			
I	1 (1)	2 (10)	
II	2 (2)	1 (5)	
III	24 (21)	7 (35)	
IV	79 (68)	4 (20)	
III/IV + V	2 (2)	2 (10)	
V	9 (8)	4 (20)	0.001#
III/IV (+V) A	45 (43)	2 (15)	
III/IV (+V) A/C	59 (56)	9 (69)	
III/IV (+V) C	1 (1)	2 (15)	0.001#
No lesions/ purely mesangial lesions, n (%)	3 (3)	3 (15)	0.040‡
Proliferative lesions, n (%)	105 (90)	13 (65)	0.008‡
Membranous lesions, n (%)	11 (9)	6 (30)	0.020‡
Glomeruli with global glomerulosclerosis, n (%)			
0%	70 (71)	9 (56)	
1–25%	22 (22)	5 (31)	
26–50%	4 (4)	1 (6)	
≥50%	3 (3)	1 (6)	0.245†
Glomeruli with fibrous crescents, n (%)			
0%	75 (76)	12 (75)	
1–25%	23 (23)	4 (25)	
26–50%	1 (1)	0	0.984†

Table 3 Continued.

Histopathologic finding	Lupus FHN (n=117)	Idiopathic non-lupus FHN (n=20)	P
Interstitial fibrosis, n (%)			
Absent	57 (58)	6 (38)	
Mild	33 (33)	7 (44)	
Moderate	6 (6)	3 (19)	
Severe	3 (3)	0 (0)	0.186†
Tubular atrophy, n (%)			
Absent	60 (61)	6 (38)	
Mild	29 (29)	8 (50)	
Moderate	7 (7)	1 (6)	
Severe	3 (3)	1 (6)	0.168†
<i>Immunofluorescence microscopy</i>			
IgA, n (%)			
+	26 (22)	8 (40)	
++	60 (51)	9 (45)	
+++	31 (26)	3 (15)	0.087†
IgM, n (%)			
+	27 (23)	8 (40)	
++	64 (55)	10 (50)	
+++	26 (22)	2 (10)	0.076†
IgG, n (%)			
+	21 (18)	4 (20)	
++	67 (57)	7 (35)	
+++	29 (25)	9 (45)	0.265†
C3, n (%)			
+	8 (7)	4 (20)	
++	64 (55)	11 (55)	
+++	45 (39)	5 (25)	0.074†
C1q, n (%)			
+	9 (8)	8 (40)	
++	67 (57)	8 (40)	
+++	41 (35)	4 (20)	0.002†

All biopsies were rescored according to the ISN/RPS 2003 classification of LN regardless of clinicopathologic diagnosis.

† Linear-by-linear analysis. ‡ Fisher's exact test. # Pearson chi-Square.

Treatment and outcomes of non-lupus FHN patients

Half of the non-lupus FHN patients received immunosuppressive therapy after renal biopsy (Table 4). Four patients presented with acute renal insufficiency and required dialysis by the time of renal biopsy. Overall, ESRD developed in 8 of 15 non-lupus FHN patients who received immunosuppression (including corticosteroids), and in 9 of 16 who did not ($P=0.39$). In the idiopathic group, renal survival similarly was not different between

patients who did ($n=9$) and did not ($n=11$) receive immunosuppression ($P=0.42$). ESRD developed in 12 (60%) idiopathic and 6 (50%) secondary non-lupus FHN patients and death occurred in 8 (40%) idiopathic and 5 (42%) secondary non-lupus FHN patients (see **Appendix 3.1**). By Kaplan-Meier analysis, progression to ESRD and/or death did not differ between idiopathic non-lupus FHN patients and non-lupus FHN patients with other differential diagnoses ($P=0.55$). Considering both patients with idiopathic and secondary non-lupus FHN, the overall group of non-lupus FHN patients progressed significantly more rapidly to ESRD and ESRD/death than lupus FHN patients (both $P<0.01$).

Table 4 Induction and maintenance immunosuppressive therapy for patients with lupus and idiopathic non-lupus full house nephropathy (FHN) after renal biopsy.

Treatment regimen	Lupus FHN ($n=117$)	Idiopathic non-lupus FHN ($n=20$)
Induction		
No immunosuppression, n (%)	2 (2)	11 (55)
Corticosteroids, n (%)	4 (3)	5 (25)
Cyclophosphamide $\pm$ corticosteroids, n (%)	44 (38)	0 (0)
Azathioprine $\pm$ corticosteroids, n (%)	45 (38)	3 (15)
Mycophenolate mofetil $\pm$ corticosteroids, n (%)	10 (9)	0 (0)
Other, n (%)	1 (1)	1 (5)
Unknown, n (%)	11 (9)	0 (0)
Maintenance		
No specific maintenance immunosuppression *, n (%)	11 (9)	17 (85)
Azathioprine $\pm$ corticosteroids, n (%)	48 (41)	3 (15)
Mycophenolate mofetil $\pm$ corticosteroids, n (%)	37 (32)	0 (0)
Other, n (%)	3 (3)	0 (0)
Unknown, n (%)	18 (15)	0 (0)

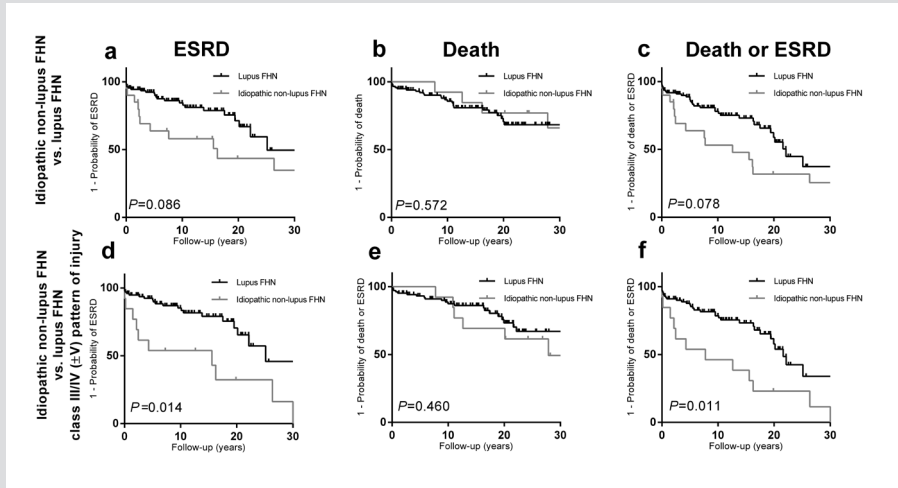
* "No specific maintenance immunosuppression" may include corticosteroids.

Outcomes of idiopathic non-lupus FHN compared to lupus FHN patients

Considering idiopathic non-lupus FHN patients only (excluding patients with secondary non-lupus FHN), outcomes were not significantly different between idiopathic non-lupus and lupus FHN patients (**Figure 1a-c**). No differences in outcome were noted between idiopathic non-lupus FHN and lupus FHN patients in the subgroups with classes I/II and class V (data not shown). In contrast, in the class III/IV ($\pm$ V) subgroup, idiopathic non-lupus FHN patients ($n=13$) progressed significantly more rapidly to ESRD ($P=0.01$) and ESRD/death ($P=0.01$) than lupus FHN patients ($n=105$; **Figure 1d,f**).

In the class III/IV ($\pm$ V) subgroup, idiopathic non-lupus FHN was an independent risk factor for ESRD adjusted for possible clinical confounders in models 1 (see overview of confounders in **Table 5**, HR 5.31; 95% CI 1.47 to 19.24) and 2 (HR 3.64; 95% CI 1.04 to 3.64).

Figure 1 Renal and overall survival of patient with lupus and idiopathic non-lupus full house nephropathy (FHN).



a-c: Kaplan-Meier analyses comparing lupus FHN (n=117) and idiopathic non-lupus FHN (n=20). a: Survival without end-stage renal disease (ESRD, median follow-up idiopathic non-lupus FHN: 10.2 years [interquartile range [IQR] 2.2–23.1]; lupus FHN: 9.3 years [IQR 3.8–16.0]). b: Overall survival (median follow-up idiopathic non-lupus FHN: 24.4 years [IQR 11.8–35.1]; lupus FHN: 14.1 years [IQR 7.2–20.8]). c: Survival without the combined outcome of death or ESRD (median follow-up idiopathic non-lupus FHN: 10.2 years [IQR 2.2–23.1]; lupus FHN: 9.5 years [IQR 4.4–16.5]). d-f: Kaplan-Meier analyses of the subset of patients with a class III/IV ($\pm$ V) pattern of injury comparing patients with lupus FHN (n=105) and idiopathic non-lupus FHN (n=13). d: Survival without ESRD. e: Overall survival. f: Survival without the combined outcome of death or ESRD. Vertical bars represent censored data.

Table 5 Predicting renal and/or patient survival in patients with a lupus (n=105) and idiopathic non-lupus (n=13) full house nephropathy (FHN) and a lupus nephritis class III/IV ($\pm$ V) pattern of injury.

Model	Outcome: ESRD		Outcome: ESRD/death	
	Hazard ratio	95% CI	Hazard ratio	95% CI
Model 1				
Idiopathic non-lupus FHN vs. lupus FHN	5.31	1.47; 19.24	3.23	1.01; 10.32
eGFR (mL/min)	0.99	0.98; 1.01	0.99	0.98; 1.00
Proteinuria (g/24h)	1.05	0.98; 1.12	1.02	0.96; 1.09
Female vs. male	1.05	0.40; 2.72	0.89	0.39; 2.02
Age (years)	1.00	0.97; 1.03	1.02	0.99; 1.04
Immunosuppressive therapy* vs. no immunosuppressive therapy	2.72	0.63; 11.84	1.93	0.51; 7.28
Model 2				
Idiopathic non-lupus FHN vs. lupus FHN	3.64	1.04; 3.64	1.32	0.76; 1.32
Immunosuppressive therapy* vs. no immunosuppressive therapy	1.96	0.43; 8.95	2.06	0.54; 7.79
Chronicity score	1.36	1.14; 1.63	1.41	1.22; 1.64

* Immunosuppressive therapy with at least corticosteroids. CI, confidence interval.

DISCUSSION

In this study, we investigated non-lupus FHN in the largest cohort of FHN patients to date. Of the 149 FHN patients, 32 (21%) patients had non-lupus FHN. None of these non-lupus FHN patients developed SLE during median follow-up of 20 years. Patients with non-lupus FHN represented a heterogeneous group with an overall poor outcome with some patients showing atypical presentations of other well-established renal diseases and 63% having idiopathic non-lupus FHN. Importantly, idiopathic non-lupus FHN was clinically and histopathologically distinct from lupus FHN, and idiopathic non-lupus FHN patients with a proliferative pattern of injury progressed significantly more rapidly to ESRD than lupus FHN patients with class III/IV ($\pm$ V) LN. Our results indicate that FHN is pattern of renal injury most often encountered in SLE, but also rarely occurring in a number of other diseases, as well as being representative of an idiopathic variant urging careful consideration by the nephrologist.

To confine the group of patients with idiopathic non-lupus FHN, we considered a number of atypical presentations of other diagnoses in non-lupus FHN patients: membranous nephropathy, IgA nephropathy, infection-related glomerulonephritis, and anti-neutrophil cytoplasmic antibody-associated glomerulonephritis (AAGN). The combination of severe

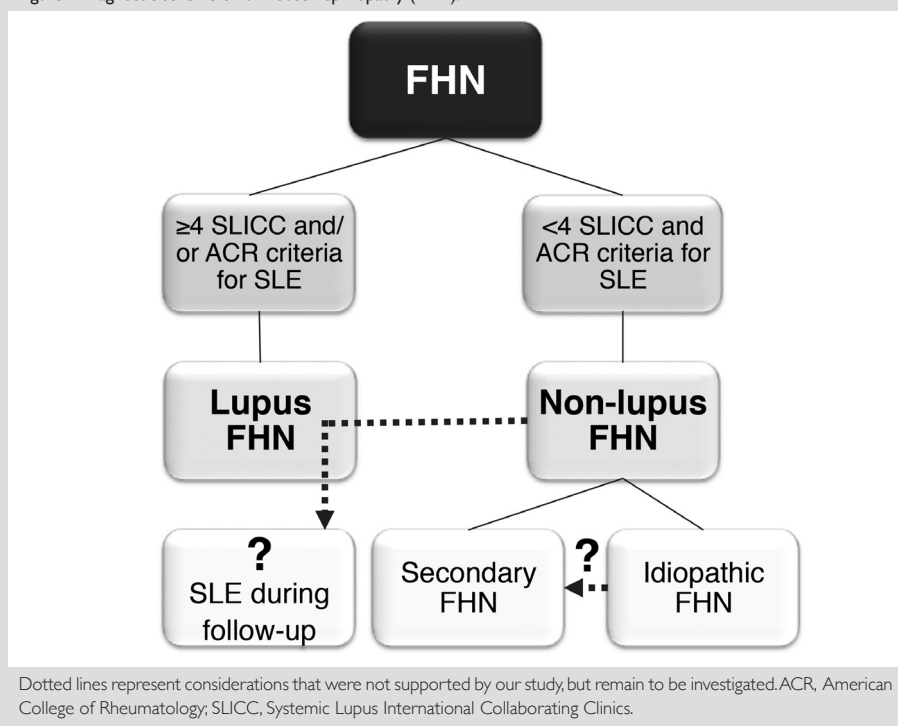
clinicopathologic features at presentation and poor renal survival of more than half of these patients raises the possibility that in some instances, non-lupus FHN may represent severe forms of other renal diseases that are accompanied by strong activation of the immune system, as has also been suggested in previous case series.^{2,8-15,22,23} Notably, three patients had recurrent IgA nephropathy with crescentic glomerulonephritis in their renal allograft, similar to patients reported by Kowalewska *et al.* showing an uncommon and aggressive form of IgA nephropathy.²⁴ Furthermore, we identified two patients with FHN and AAGN, one of whom required dialysis at the time of biopsy. Previous reports have demonstrated convincingly that glomerular immune complexes may be present in AAGN.²⁵ It has been suggested that immune complexes may act synergistically with ANCA to produce more severe glomerulonephritis than seen with either immune complexes or ANCA alone.²⁶ The possibility that secondary causes for which we could not test may underlie FHN in the remaining 20 idiopathic non-lupus FHN patients cannot be fully excluded, which is a limitation inherent to our retrospective study design. Nevertheless, it is crucial to realise that ESRD was associated with non-lupus FHN, irrespective of its cause. Importantly, although we did not observe such patients, patients have been reported in the literature with non-lupus FHN who developed SLE during follow-up,²⁻⁵ adding the possibility that some non-lupus FHN patients may have latent SLE. Taken these considerations together, we compiled a diagnostic scheme for FHN (**Figure 2**).

Idiopathic non-lupus FHN patients show similarities with an entity previously described as “renal-limited lupus-like nephritis”.²⁷ However, this entity was originally defined by a lupus-like constellation of immunofluorescence and electron microscopic evaluations, and not by a full house pattern *per se*.²⁷ In the study by Huerta and colleagues,²⁷ two of four “renal-limited lupus-like nephritis” patients had FHN, and both progressed to ESRD despite aggressive therapy. Importantly, our study indicates that idiopathic non-lupus FHN, as defined by the absence of sufficient SLE criteria and exclusion of other diagnoses, should be recognised as a category with poor outcome by itself, without the requirement of additional lupus-like pathologic features. Therefore, the term “lupus-like nephritis” should not be used for idiopathic non-lupus FHN – emphasising the clinical distinction from lupus.

The prevalence of non-lupus FHN in our cohort is in accordance with a previous study, in which non-lupus FHN was described in 28/94 (30%) cases with FHN identified over a 9-year period.² In our study, idiopathic non-lupus compared to lupus FHN patients were more often male. Moreover, laboratory results revealed lower-range erythrocyturia, predominantly nephrotic-range proteinuria, and less complement consumption in the classical pathway. We identified a number of immunohistochemical and histopathologic features that predominated in idiopathic non-lupus FHN compared to lupus FHN: weaker C1q staining by immunofluorescence; less frequent proliferative lesions and more frequent mesangial and membranous lesions by light microscopy; and an increased frequency of a chronic subclass associated with proliferative nephritis. Thus, in a patient with a renal biopsy showing FHN in the absence of sufficient SLE classification criteria, the features

above would support a diagnosis of idiopathic non-lupus FHN. Further study is warranted to identify additional indicators that could help to distinguish idiopathic non-lupus FHN from lupus FHN. Tubuloreticular inclusions would appear promising based on the study by Wen and Chen,² although we did find them in one patient with idiopathic non-lupus FHN. Other promising indicators, such as IgG subclass staining,²⁸ remain to be investigated.

Figure 2 Diagnostic scheme of full house nephropathy (FHN).



Since we performed the first study of idiopathic non-lupus FHN in which control cases with lupus FHN were included, we were able to compare outcomes between these groups to substantiate their clinical distinction. Given the heterogeneity of histopathologic lesions, we analysed renal and patient survival in subsets with relatively homogeneous injury patterns, and found that idiopathic non-lupus compared to lupus FHN was a strong independent risk factor for ESRD in the subset of patients with proliferative lesions (akin class III/IV [$\pm$ V] LN). Possibly because the number of patients with others histopathologic patterns of injury was small, we could not demonstrate a survival difference in these subgroups. Our results are in agreement with previous reports that hinted that the clinical course and outcome of idiopathic non-lupus FHN patients are worse than those of patients with similar patterns of glomerulonephritis with SLE.^{4, 6, 15, 27}

It is reasonable to speculate that a number of factors relating to idiopathic non-lupus FHN patients contributed to their poor outcome. The higher levels of proteinuria and the tendency towards a higher chronicity score in idiopathic non-lupus FHN patients are remarkable in this aspect, although these factors did not explain the renal survival difference between idiopathic non-lupus and lupus FHN in our statistical models. A major conundrum of this study is that 11 of 20 idiopathic non-lupus FHN patients did not receive any type of immunosuppression; and of the 9 patients who did, 5 received corticosteroids alone and none received cyclophosphamide. This contrasts to the 38% of lupus FHN patients who did receive cyclophosphamide. Whereas we did not find that immunosuppression including corticosteroids affected renal survival within the group of idiopathic non-lupus FHN patients, the poor outcome of idiopathic non-lupus FHN patients may well be related to their lack of cytotoxic therapy. In a recent study of paediatric non-lupus patients with complement (C1q, C3) and immunoglobulin (IgG, IgM and/or IgA) glomerular staining, patients who received intensive cytotoxic immunosuppression had a favourable renal outcome.⁷ Although FHN was defined differently, this study raises the possibility that idiopathic non-lupus FHN patients may also benefit from cytotoxic immunosuppression. On the other hand, the relative lack of immunosuppression in idiopathic non-lupus FHN patients in our study eliminates the possibility that treatment induced sustained remission and prevented latent lupus from becoming active systemically for the majority of these patients. This accentuates the clinical distinction from lupus, as none of the (idiopathic) non-lupus FHN patients developed SLE during long-term follow-up. It remains speculative as to whether the uncertainty of a definite diagnosis of SLE due to the lack of systemic signs and symptoms and/or the imminent progression to ESRD discouraged physicians from initiating intensive immunosuppression after risk-benefit assessment.

The aetiology and pathogenesis of idiopathic non-lupus FHN remain to be elucidated. Given its strong similarity to LN, it may be considered that the two entities share similar pathogenic mechanisms. In LN, it has been suggested that immune complexes may occur in the glomerulus both due to the deposition of preformed immune complexes and in situ formation. The formation of autoantibodies and the resulting immune complexes in LN may therefore originate from a combination of pathogenic mechanisms: (i) an increased antigenic load due to dysregulated apoptosis and defective clearance of apoptotic debris; (ii) a subsequent aberrant immune response directed to these endogenous antigens; and (iii) a defective clearance of immune complexes. Quantity, size, and type of the immune complexes determine the microscopic pattern of injury in LN related to the site of deposition.²⁹ The occurrence of full house glomerular deposits in LN may then be seen as the result of a pronounced immune response with polyclonal B cell activation in the setting of these mechanisms. The occurrence of full house glomerular deposits in the absence of a clinical diagnosis of SLE may similarly be seen as the expression of a more pronounced type of defective immune complex clearance following abnormal immune complex overload or immune complex handling.³⁰ The previously mentioned secondary forms of non-lupus FHN^{2, 8-15, 22, 23} are likely associated with these mechanisms. Unidentified endogenous and/

or exogenous antigens involved in immune complex formation may underlie idiopathic non-lupus FHN. Moreover, genetic factors resulting in defective clearance of immune complexes that have been implicated in LN, such as Fcγ-receptor deficiency³¹ and deficiency of the erythrocyte C3b-receptor,³² may also be involved in idiopathic non-lupus FHN.

In conclusion, our study indicates that idiopathic non-lupus FHN is associated with poor renal survival and therefore, deserves careful consideration by the nephrologist. We pointed out what should prompt the recognition of idiopathic non-lupus FHN, and hopefully initiated efforts to determine possible underlying cause(s) and potential treatment.

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Chapter 4

Long-term Preserved Renal Function in Patients with Class III or IV Lupus Nephritis Undertreated According to Guidelines: Recognition of a Subgroup with a Favourable Natural History

Emilie C. Rijnink, Marlies E.J. Reinders, Leendert A. van Es, Y.K. Onno Teng, Ron Wolterbeek, Jan A. Bruijn, Ingeborg M. Bajema

Submitted



ABSTRACT

Background

Present guidelines for lupus nephritis (LN) recommend that all patients with class III or IV LN receive cytotoxic immunosuppression. Scarce data from the past suggest that some patients with class III or IV LN maintained renal function without such therapy in clinical trials. In this study, we aimed to study the natural history of class III or IV LN and explore a subgroup of patients for whom treatment without cytotoxic drugs could suffice.

Methods

All patients were included with a renal biopsy showing class III or IV LN between 1983–2011 and with clinical follow-up at the Leiden University Medical Center. Evolution of eGFR during 10 years follow-up was compared between all consecutive patients who did and did not receive guideline-recommended cytotoxic immunosuppression.

Results

Three patients were identified with class III or IV LN presenting with relatively preserved renal function who did not receive cytotoxic immunosuppression, in defiance of current guidelines. Follow-up of 9–24 years revealed that these patients achieved remission with stable renal function and without LN flares, despite mild non-nephrotic proteinuria in the absence of erythrocyturia. Their eGFR evolution over 10 years did not differ from that observed in 98 patients from the same cohort who received cytotoxic immunosuppression ($P=0.56$).

Conclusions

The three cases undertreated according to guidelines represent a subgroup of patients with class III or IV LN for whom milder therapy regimens without cytotoxic immunosuppression appear sufficient. These findings signal a need to study similar patients in future studies.

INTRODUCTION

For patients with class III or IV lupus nephritis (LN), standard care includes intravenous cyclophosphamide or mycophenolate mofetil (MMF) in combination with corticosteroids.¹⁻⁵ However, such treatment is associated with severe side effects, including sepsis, avascular bone necrosis, gonadal dysfunction, and malignancy.⁶ Even with these therapies, LN carries considerable risks of end-stage renal disease (ESRD) and death.⁷⁻¹³ Randomised controlled trials (RCTs) have focused on treatment strategies to improve prognosis focusing on patients at high risk of these adverse outcomes. As a consequence, a subgroup of patients with class III or IV LN who might have a lower risk of adverse outcomes has been overlooked in these trials. Yet, it has always been clear from the landmark trials performed at the National Institute of Health (NIH), that about 10–40% of patients who received prednisolone therapy alone did not reach ESRD during 10 years follow-up.⁸ Unfortunately, the clinical course of these patients has never been detailed; thus, little is known about the natural history of class III or IV LN. Treating patients with prednisolone therapy alone is no longer recommended.¹⁻⁵ Therefore, it is presently unknown whether a conceivably lower-risk subgroup of patients with class III or IV LN could be treated with milder regimens omitting cytotoxic immunosuppression.

In this study, we aimed to explore the existence of a subgroup of patients with class III or IV LN for whom milder immunosuppressive regimens could suffice. We report the results of a retrospective study in which we identified patients with class III or IV LN as registered in the pathology archives of the Leiden University Medical Center between 1983–2011 who did and did not receive guideline-recommended cytotoxic immunosuppression. We present the clinical characteristics, therapeutic strategies, and clinical outcomes in all consecutive patients with class III or IV LN who did not receive guideline-recommended cytotoxic immunosuppression in this cohort. The evolution of eGFR during 10 years follow-up was compared between these patients and patients from the same cohort who did receive cytotoxic immunosuppression in accordance with the guidelines.

METHODS

Identification of cases

The pathology archives at the Leiden University Medical Center were searched for all patients biopsied between 1983–2011 with class III or IV LN. Patients were included if they had a renal biopsy available for re-evaluation, fulfilled ≥ 4 American College of Rheumatology (ACR) or ≥ 4 Systemic Lupus International Collaborating Clinics (SLICC) criteria for systemic lupus erythematosus (SLE),¹⁴ and had clinical follow-up at our centre.

Biopsies were reclassified according to the ISN/RPS 2003 classification.¹⁵ Medical records were evaluated for history of immunosuppressive medication prior to biopsy and during follow-up. The glomerular filtration rate was estimated using the Cockcroft-Gault formula (eGFR). eGFR was recorded at the time of renal biopsy, as well as at 1, 2, 3, 4, 5, and 10 years of follow-up. In accordance with the ethics committee of Leiden University Medical Center, all patient data were coded and kept anonymously throughout the study.

Statistical analysis

Normally distributed data are expressed as means $\pm$ standard deviation and non-normally distributed data are presented as median values and interquartile range. Normally distributed data were compared with *t*-tests and categorical data with Fisher's exact tests or chi-square tests. To compare non-normally distributed data, Mann-Whitney U tests were used.

A random intercept/random slope mixed model was used to compare the course of eGFR between patients. In the mixed model, "treatment according to guidelines" was analysed as fixed effect. Visual inspection of residuals was performed to assess normality assumptions. All analyses were performed using SPSS Statistics 23.0 (IBM, Armonk, New York).

RESULTS

We included 101 patients who had biopsy-confirmed class III or IV LN between 1983–2011. Of these, 98 received cytotoxic immunosuppression according to guidelines: corticosteroids in combination with intravenous cyclophosphamide (NIH protocol¹² [*n*=26]; Euro-Lupus protocol¹³ [*n*=30]), azathioprine (*n*=33), or MMF (*n*=9). We identified three consecutive patients who – in defiance of treatment guidelines – did not receive any of the aforementioned regimens prior to biopsy and during follow-up for LN. Clinical and histopathologic characteristics at the time of diagnostic renal biopsy of the latter three patients, as well as of the other 98 patients in the cohort, are shown in **Table I**. No differences in biopsy findings and clinical presentation were noted between the two groups. The case descriptions of the three patients undertreated according to the guidelines are detailed below.

Table 1 Clinical and histopathologic characteristics of patients at time of diagnostic renal biopsy.

Characteristic	Case 1	Case 2	Case 3	Patients with guideline-recommended immunosuppression (n=98)	P†
Age, y	23	33	32	30 ± 13	0.2
Sex	Female	Female	Male	20 Male 78 Female	0.6
ISN/RPS class of LN	III (A/C)	IV-G (A)	III (A/C)	23 III 24 IV S 45 IV G 6 III/IV + V	0.7
Time since diagnosis of SLE, y	0.3	0	0	3.7 ± 5.0	0.1
Glomeruli without abnormalities	14% (5/36)	0% (0/20)	30% (3/10)	10%	0.9
Glomeruli with proliferative lesions	33% (12/36)	95% (19/20)	10% (1/10)	55%	0.8
Glomeruli with global sclerosis	0% (0/36)	0% (0/20)	10% (1/10)	7%	0.4
NIH Activity Index ¹⁶	7	6	4	10 (6–12)	0.1
NIH Chronicity Index ¹⁶	I	0	4	I (0–3)	0.8
Serum creatinine, µmol/L	88	74	90	144 ± 135	0.3
eGFR, mL/min	72	86	92	73 ± 35	0.1
Proteinuria, g/24h	3.9	3.1	1.6	4.4 ± 3.8	0.7
ANA	+	+	+	97% (n=98)	1.00
Anti-dsDNA	+	not tested	-	80% (n=96)	0.5

Data are expressed as mean ± standard deviation or median (interquartile range). ANA, antinuclear antibody; anti-dsDNA, anti-double stranded DNA antibodies. † Patients with guideline-recommended immunosuppression (n=98) versus cases 1–3.

Case descriptions

Case 1

In 1993, a 22-year-old Caucasian woman was diagnosed with SLE based on a malar rash, pleuropericarditis, Libman-Sacks endocarditis, photosensitivity, pancytopenia, hypocomplementaemia, positive antinuclear, anti-dsDNA anti-(double stranded DNA), and antiphospholipid antibodies. Shortly after diagnosis, she experienced an epileptic insult and MRI showed multiple white matter lesions consistent with cerebral SLE involvement. Simultaneously, proteinuria increased (**Figure 1A**) and renal biopsy indicated LN class III (A/C) with endocapillary hypercellularity, fibrinoid necrosis, and cellular crescents. Virtually no chronic damage was present, except for one fibrous crescent.

The patient was started on 40 mg prednisolone and anti-proteinuric treatment with 5 mg enalapril. Due to the patient's stable renal function with class III LN (**Figure 1A**), conservative therapy was maintained and no additional immunosuppressive therapy was initiated. Two years later, the patient experienced two exacerbations of cutaneous SLE with secondary impetiginisation, for which she received 200 mg hydroxychloroquine. Four years after renal biopsy, SLE was in remission, and prednisolone treatment was discontinued.

The patient was followed in our centre for nine years, with her last follow-up showing no symptoms of active LN, despite persisting mild proteinuria of 1–2 g/24h (**Figure 1A**).

Case 2

In 1994, a 33-year-old Caucasian woman with a history of HLA-B27-positive oligoarthritis and preeclampsia presented with fluid retention in her fingers and macroscopic haematuria, anaemia, leukocytopenia, and proteinuria. One year later, she was diagnosed with post-streptococcal glomerulonephritis based on hypocomplementaemia, elevated anti-streptolysin O, erythrocyturia, nephrotic-range proteinuria (4–5 g/24h), hypertension (RR 150/90 mmHg), and a history of pharyngitis. Her renal function was normal (eGFR $\pm$ 90 mL/min). She was started on 20 mg enalapril with close follow-up, during which hypocomplementaemia, proteinuria (3–4 g/24h), and erythrocyturia persisted.

In 1996, renal biopsy revealed a membranoproliferative pattern with full house immunofluorescence, which was retrospectively classified as LN class IV-G (A). She had no other systemic symptoms of SLE, but additional testing showed a positive antinuclear antibody (ANA). At the time, the patient fulfilled 4 SLICC criteria: leukocytopenia, hypocomplementaemia, nephritis, and ANA. Due to the patient's wish for pregnancy, she declined immunosuppression and enalapril was switched to methyldopa. Her subsequent pregnancy 3 months later was complicated by preeclampsia (i.e. hypertension with proteinuria of 10 g/24h) in the first trimester, and was therefore terminated. Enalapril treatment was restarted, with the addition of 12.5 mg hydrochlorothiazide. In the following years, she showed stable normal renal function, but persisting proteinuria and erythrocyturia. Four years after renal biopsy, the patient was formally diagnosed with SLE based on the serial occurrence of haematologic disturbances, hypocomplementaemia, positive ANA, and LN.

Eleven years after renal biopsy, while LN was in remission, the patient presented with psychosis, which was diagnosed as cerebral SLE based on cerebellar atrophy on MRI. She was started on 60 mg prednisolone, antipsychotics, and intravenous cyclophosphamide following the NIH scheme. Her condition quickly improved. Within eight months, cerebral SLE was in remission and prednisolone treatment was discontinued. At her last follow-up in our centre, 19 years after renal biopsy, SLE was in complete remission with minimal proteinuria, absent erythrocyturia, and normal eGFR (**Figure 1B**).

Case 3

In 1990, a 31-year-old Caucasian man presented with erythema and arthralgia of his lower legs, for which he received a non-steroidal anti-inflammatory drug. Two months later, laboratory analyses revealed a strongly positive ANA, together with proteinuria and erythrocyturia, with a normal eGFR (**Figure 1C**). Renal biopsy revealed LN class III (A/C) with concomitant thrombotic microangiopathy. The diagnosis of SLE was confirmed based on LN, arthritis, positive ANA, and antiphospholipid antibodies. Therapy was initiated with hydroxychloroquine. For unknown reasons, no additional immunosuppressive therapy was

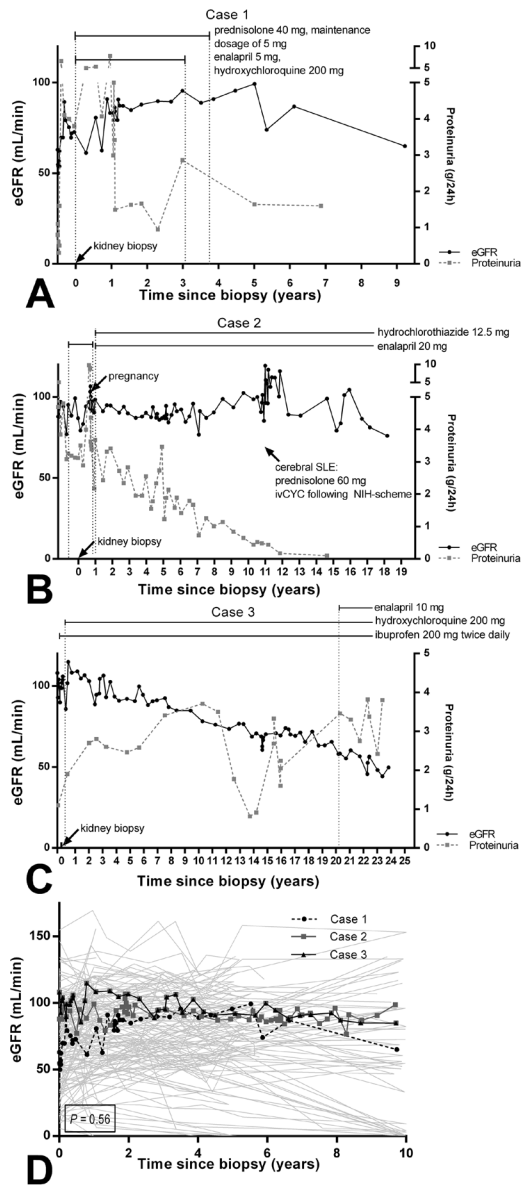
started. Over the following years, SLE was in remission, although mild sediment disturbances and proteinuria persisted (**Figure 1C**).

Fifteen years after renal biopsy, SLE activity increased, with myalgias, arthralgias, and maculous skin lesions of the buttocks and legs. These symptoms resolved after increasing the hydroxychloroquine dosage. Over the following years, eGFR was 55–75 mL/min, proteinuria 2–3.5 g/24h, and erythrocyturia was absent. In 2011, the patient was started on 10 mg enalapril due to persisting proteinuria. In 2013, a second renal biopsy was performed because of a temporarily raised serum creatinine (eGFR $\pm$ 45 mL/min) together with longstanding proteinuria without erythrocyturia. The biopsy showed one glomerulus with perihilar focal segmental glomerulosclerosis, but no active lesions. There were no chronic lesions, apart from one glomerulus showing global sclerosis. Immunofluorescence was negative, but electron microscopy revealed a limited number of transmembrane and subepithelial deposits. At the last follow-up in our centre, 24 years after initial renal biopsy, the patient showed a stable renal function (**Figure 1C**) without erythrocyturia and with adequate blood pressure (RR 130/80 mmHg).

Renal function in patients with and without guideline-recommended immunosuppression compared

The evolution of eGFR was compared between the 98 patients who did and the three patients who did not receive guideline-recommended cytotoxic immunosuppression during 10 years follow-up. A random intercept/random slope mixed model revealed that the evolution of the eGFR did not significantly differ between the two groups ($P=0.56$). **Figure 1D** shows the course of eGFR among the patients treated with and without cytotoxic immunosuppression, demonstrating the lack of notable differences in trend for the three patients without cytotoxic immunosuppression compared to the other 98 patients in the cohort.

Figure I Clinical course of patients undertreated according to current guidelines.



A: case 1. B: case 2. C: case 3. D: course of eGFR during 10 years follow-up of patients who were undertreated according to current guidelines (cases 1–3; highlighted) in relation to patients from the same cohort who received cyclophosphamide, MMF, or azathioprine (n=98; in grey).

DISCUSSION

We studied consecutive patients from the Leiden University Medical Center with class III or IV LN diagnosed between 1983 and 2011 who did not receive guideline-recommended cytotoxic immunosuppression. We found three patients with class III or IV LN who were undertreated according to current guidelines, having received prednisolone alone or no immunosuppressive therapy. During long-term follow-up, these patients maintained a relatively preserved renal function without LN flares. Their eGFR evolution over 10 years follow-up did not differ from that observed in patients from the same cohort who received guideline-recommended cytotoxic immunosuppression. To our knowledge, this is the first detailed analysis of patients belonging to the understudied subgroup of class III or IV LN who could benefit from a therapeutic approach omitting cytotoxic drugs for their renal manifestations, in disagreement with current treatment guidelines. The three patients described herein may signal a need to more closely investigate similar patients in future prospective studies.

Large RCTs have clearly demonstrated the benefits of various cytotoxic regimens for the pooled group of patients with class III or IV LN, upon which the current LN treatment guidelines are based. Attempts to identify prognostic subgroups of patients with class III or IV LN have focused on patients in high-risk groups. In the trial by Austin *et al.* performed at the NIH,⁸ high-risk patients identified by the presence of a chronicity index ≥ 1 had a significantly increased risk of ESRD as compared to the patients who had no chronic lesions. In the high-risk group, little over 10% of patients in the prednisolone arm did not reach the end-point ESRD after 10 years follow-up, whereas this percentage increased to nearly 40% in the total group of patients receiving prednisolone including low-risk patients with absent chronic lesions. Although there were only 28 patients in the prednisolone arm, these data suggest that improvement in the qualification of the subgroup of patients for whom milder therapy could suffice is attainable. Focusing on high-risk subgroups in RCTs is profitable, since a treatment effect – if present – is anticipated to be greater among these patients. However, separate analyses of lower-risk patients may reveal under which conditions milder therapies with less harmful side effects may suffice.

Interestingly, the same focus on high-risk patients is observed in the way eligibility criteria for RCTs are defined. Inspection of the inclusion criteria of landmark RCTs, upon which treatment guidelines are based, reveals that patients with a mildly disturbed eGFR and/or mild proteinuria were frequently excluded, as their smaller deviation from normal renal function is reasonably anticipated to be accompanied by a smaller treatment effect.⁷⁻¹² Consequently, results of these RCTs cannot be unequivocally applied to all patients with LN in a clinical setting, creating a gap in the guidelines for patients with relatively preserved renal function and a possibly favourable prognosis. Currently, more and more patients with class III or IV LN with preserved renal function and mild proteinuria are

encountered, since current guidelines recommend a renal biopsy for patients with SLE and any sign of renal involvement, particularly reproducible proteinuria of >0.5 g/24h.¹⁻⁵ Without supporting evidence, the EULAR/ERA-EDTA guideline² attempts to close this gap, proposing that patients with preserved renal function and without adverse histopathologic findings could be considered candidates for milder therapy regimens (e.g. azathioprine). The three patients described here showed relatively preserved renal function at the time of renal biopsy, notwithstanding that they are not typically mild cases unfulfilling the eligibility criteria of some large RCTs. Moreover, these patients' histopathologic findings would probably not be designated as "non-adverse," although this is uncertain since there is no specific definition for this entity. There remains a need for a clear definition of the specific histopathologic lesions and clinical parameters that characterise patients eligible for milder therapy regimens.

Further specification of this subgroup will be challenging. Previous studies showed that neither histopathologic nor clinical parameters are optimal prognosticators in LN. Patients with the same LN class may experience heterogeneous clinical courses, and clinical features alone are poor predictors of histopathologic findings. Most telling, in a recent study by Wakasugi *et al.*, protocolised biopsies of SLE patients without clinical renal involvement revealed class III or IV LN in 13 of 86 cases.¹⁷ Those 13 patients were followed for a median duration of 30 months, and only two patients with class III LN experienced exacerbated LN associated with pregnancy and malignancy. Remarkably, the remaining 11 patients showed no LN flares and had a good prognosis with prednisolone therapy alone. These findings underline three important points: the lack of coherence between clinical findings and histology, the difficulty of determining treatment purely based on LN class, and the challenge of predicting clinical course.

In the present guidelines, no prognostic subgroups of patients with class III or IV LN are recognised: treatment decisions are based solely on the histopathologic classification with no regard of clinical parameters. Here we have shown that these guidelines are at risk of overtreatment, as we demonstrate the existence of a subgroup of patients with class III or IV LN for whom therapy without cytotoxic drugs could suffice. Since we found only three consecutive patients who retrospectively appeared to have been unexposed to cytotoxic immunosuppression, a limitation of our study is that we were hampered by a lack of power to identify features characterising these patients. We speculate that the three patients had (combinations of) the following features in common which may give clues as to why they had a favourable prognosis in the absence of cytotoxic immunosuppression: newly or very recently diagnosed SLE at the time of renal biopsy, a relatively preserved and stable renal function at presentation (only gradual decline in eGFR during the month preceding the renal biopsy of 0–3 mL/min), and a relatively low percentage of proliferative lesions (cases 1 and 3). Awaiting the results of further studies investigating the specific subgroup of patients with yet undefined combinations of clinical and histopathologic manifestations, the following could be deliberated. For selected patients with class III or IV LN, it may be

advisable to institute a window of “watchful waiting” following renal biopsy during which patients are closely monitored, paralleling the strategy in other renal diseases like idiopathic membranous nephropathy.³ Tailored treatment may be optimal, with a tentative approach involving watchful waiting, and possible introduction of anti-inflammatory treatment with corticosteroids followed by suppression of autoimmunity using cytotoxic immunosuppressive drugs only if deemed necessary. Indeed, older studies have demonstrated that anti-inflammatory doses of corticosteroids alone were as effective as corticosteroids plus cyclophosphamide in the early phase of LN treatment.^{8, 18} Obviously, appropriate cases must be selected with extreme caution, as patients with class III or IV LN who are undertreated due to non-adherence to treatment may show a detrimental disease course.¹⁹ Also, SLE is a systemic disease and successful conservative treatment for LN does not imply that this approach can be extrapolated to all extrarenal SLE manifestations. Nevertheless, an approach involving watchful waiting was successful in the three presently described cases and could possibly decrease the burden of cytotoxic regimens in more patients.

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Chapter 5

Clinical and Histopathologic Characteristics Associated with Renal Outcomes in Lupus Nephritis

Emilie C. Rijnink, Y.K. Onno Teng, Suzanne Wilhelmus, Mathilde Almekinders,
Ron Wolterbeek, Karlien Cransberg, Jan A. Bruijn, Ingeborg M. Bajema

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ABSTRACT

Background

The prognostic significance of histopathologic (sub)classes in the current classification of lupus nephritis (LN) is controversial. We analysed clinical and histopathologic predictors of renal outcomes in LN outside the framework of the classification.

Methods

Variables (50 histopathologic and 10 clinical) were tested in mixed, linear, and Cox regression models for their association with renal flare, end-stage renal disease (ESRD), and estimated glomerular filtration rate (eGFR) during follow-up (1, 5, and 10 years) in 105 LN patients biopsied from 1987–2011.

Results

During median follow-up of 9.9 years (25th–75th percentile, 5.9–13.8), 47 patients experienced a renal flare and 21 progressed to ESRD. Renal flare was predicted by fibrinoid necrosis (hazard ratio [HR] 1.04 per % [95% confidence interval [CI], 1.00 to 1.07]) and non-Caucasian race (HR 2.23 [95% CI, 1.23 to 4.04]). ESRD was predicted by fibrinoid necrosis (HR 1.08 per % [95% CI, 1.02 to 1.13]), fibrous crescents (HR 1.09 per % [95% CI, 1.02 to 1.17]), interstitial fibrosis/tubular atrophy (IF/TA) $\geq 25\%$ (HR 3.89 [95% CI, 1.25 to 12.14]), eGFR at baseline (HR 0.98 per mL/min/1.73 m² [95% CI, 0.97 to 1.00]), and non-Caucasian race (HR 7.16 [95% CI, 2.34 to 21.91]). A higher mean eGFR during follow-up was associated with normal glomeruli (+0.2 mL/min/1.73 m²/% [95% CI, 0.1 to 0.4]). Like ESRD, a lower eGFR during follow-up was associated with fibrous crescents, IF/TA $\geq 25\%$, and non-Caucasian race, as well as with cellular/fibrocellular crescents (−0.4 mL/min/1.73 m²/% [95% CI, −0.6 to −0.2]) and age (−0.8 mL/min/1.73 m²/year [95% CI, −1.2 to −0.4]).

Conclusions

The LN classification should include an index of evidence-based prognosticators. Awaiting validation of a formal index, we suggest that at least fibrinoid necrosis, fibrous crescents, and IF/TA warrant explicit independent scoring to assess the risk of progressive renal dysfunction in conjunction with clinical findings.

INTRODUCTION

The disease manifestations and outcomes in lupus nephritis (LN) are heterogeneous, but 10–30% of patients progress to end-stage renal disease (ESRD) within 15 years.^{1,2} The prognosis can usually be improved by immunosuppression; although, there are severe and sometimes lethal adverse effects.³ There is a constant need for refined and novel indicators to help clinicians predict outcomes and determine when intensive immunosuppression should be initiated for individual LN patients.

Currently, clinical guidelines for LN^{4–6} reserve intensive immunosuppression primarily for patients with class III or IV ($\pm$ V) LN according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification.⁷ Although the ISN/RPS classification is useful in terms of standardisation and reproducibility of diagnosis,^{8–10} studies concerning the power of this classification in predicting disease outcome reported conflicting results.^{9,11–18} Conflicting results may be due to the grouping of a wide variety of prognostically, pathogenically, and chronically different glomerular lesions in broad classes, thereby assuming that the prognosis of individuals within classes is equal regardless of the type of lesions.

A revision of the ISN/RPS classification is called for.^{19,20} An evidence-based approach, similar to the Oxford classification of IgA nephropathy would be the resolution for a future classification.^{21,22} In the current study, we aimed to identify evidence-based clinical and histopathologic predictors of renal outcome in LN outside the framework of the ISN/RPS classification.

METHODS

We collected a cohort of patients, biopsied from 1987–2011, with biopsy-confirmed LN from the pathology archives at the Leiden University Medical Center (LUMC). Inclusion criteria were: patients with a first renal biopsy available for re-evaluation with ≥ 5 scorable glomeruli, fulfilling ≥ 4 of the revised American College of Rheumatology^{23,24} or Systemic Lupus International Collaborating Clinics²⁵ classification criteria for SLE, and with clinical follow-up at the LUMC, Bronovo hospital (The Hague, the Netherlands), or Erasmus Medical Center (Rotterdam, The Netherlands). In accordance with the ethics committee guidelines at the LUMC, all patient data were coded and kept anonymous. Biopsies were processed for light and immunofluorescence microscopy according to standard techniques in our centre, including haematoxylin and eosin, periodic acid-Schiff, and methenamine-silver staining. Immunofluorescence reports were originally prepared by four experienced nephropathologists who consistently scored immunofluorescence intensity on a 0–3+ scale.

Histopathology definitions and scoring

Definitions of histopathologic lesions are shown in **Appendix 5.1**. Slides were scored by an experienced nephropathologist (IMB). All glomerular variables were determined for each scorable glomerulus separately and tubulointerstitial parameters were scored categorically (**Appendix 5.1**). We treated glomerular parameters as continuous variables and expressed them as the percentage involved of all scorable glomeruli. We considered 50 histopathologic variables (**Figure 1**). To reduce the number of candidate variables, we excluded lesions with a low prevalence (occurring in ≤ 5 patients) and included only one of two strongly correlated variables ($r/p > 0.8$). The decision on which of the correlated variables to be included was based on relevance and ease of scoring.

Figure 1 Histopathologic variables that were assessed.

Glomerular variables

Global sclerosis	% glomeruli with global sclerosis
Normal glomeruli	% glomeruli noted as normal
Normal glomeruli/minimal leukocyte influx	% glomeruli noted as normal/containing 1–3 leukocytes
Minimal leukocyte influx	% glomeruli with 1–3 leukocytes
Ischemic glomeruli	% glomeruli noted as ischemic
Mesangial 1†	Mean score for mesangial hypercellularity
Mesangial 2	% glomeruli with mesangial hypercellularity
Mesangial 3	% glomeruli with mesangial matrix expansion
Segmental sclerosis	% glomeruli with segmental sclerosis
Endocapillary 1	% glomeruli with segmental endocapillary hypercellularity
Endocapillary 2	% glomeruli with segmental/global endocapillary hypercellularity
Endocapillary 3†	% glomeruli with segmental/global endocapillary hypercellularity/minimal leukocyte influx
Endocapillary 4	% glomeruli with global endocapillary hypercellularity
Endocapillary inflammatory infiltrate†	% glomeruli with influx of ≥ 4 inflammatory cells
Endocapillary granulocytes	% glomeruli with ≥ 4 endocapillary granulocytes
Endocapillary lymphocytes	% glomeruli with ≥ 4 endocapillary lymphocytes
Endocapillary monocytes†	% glomeruli with ≥ 4 endocapillary monocytes
Endothelial swelling†	% glomeruli with endothelial cell swelling
Wire loops	% glomeruli with wire loops
Adhesions	% glomeruli with adhesions
Fibrinoid necrosis	% glomeruli showing fibrinoid necrosis
Extracapillary 1†	% glomeruli with cellular crescents
Extracapillary 2	% glomeruli with cellular/fibrocellular crescents
Extracapillary 3†	Mean cellular + fibrocellular crescent score
Extracapillary 4	% glomeruli showing fibrous crescents
Extracapillary 5†	Mean fibrous crescent score
Extracapillary 6	% glomeruli with fibrocellular + fibrous crescents
Karyorrhexis	% glomeruli with karyorrhexis

Figure 1 Continued.

Glomerular variables

Microthrombi*	% glomeruli with microthrombi
Pseudothrombi*	% glomeruli with pseudothrombi
Double contours	% glomeruli with double contours
Spikes/vacuoles	% glomeruli with spikes/vacuoles

Tubulointerstitial variables

Tubular atrophy‡	Tubular atrophy score
Acute tubular injury*	Acute tubular injury (+/-)
Tubular casts	Tubular casts (+/-)
Tubular luminal macrophages	Tubular luminal macrophages (+/-)
Tubular reabsorption droplets	Tubular reabsorption droplets (+/-)
Tubular regeneration*	Tubular regeneration (mitoses) (+/-)
Tubulitis*	Tubulitis (+/-)
Interstitial infiltration	Interstitial infiltration score
Interstitial lymphocytes†	Interstitial lymphocyte-dominant infiltrate (+/-)
Interstitial granulocytes	Interstitial granulocyte-dominant infiltrate (+/-)
Interstitial fibrosis‡	Interstitial fibrosis score (+/-)
Focal cortical atrophy*	Focal cortical atrophy (+/-)

Vascular variables

Vasculitis*	Vasculitis (+/-)
Fibrinoid necrosis in large vessels*	Fibrinoid necrosis in large vessels (+/-)
Thrombosis*	Thrombosis (+/-)
Hyaline arteriosclerosis*	Hyaline arteriosclerosis (+/-)
Fibrous intimal hyperplasia*	Fibrous intimal hyperplasia (+/-)
Arterial intimal fibrosis	Arterial intimal fibrosis (+/-)

For definitions of histopathologic lesions, see Appendix 5.1. Percentages represent the proportion of involved scorable glomeruli. Crescent score: a multiplication factor of 1 was used for segmental crescents, and 2 for circumferential crescents.

* Excluded from analyses because of low prevalence (≤ 5 patients). † Excluded from analyses because the variable was strongly correlated with another variable ($r/p > 0.8$). ‡ Interstitial fibrosis and tubular atrophy were combined to form the composite variable "IF/TA" (interstitial fibrosis/tubular atrophy; whichever was the higher value).

Clinical dataset

The following clinical parameters were recorded for each patient at the time of biopsy: age, sex, race, mean arterial pressure ([MAP] diastolic blood pressure + $\frac{1}{3}$ pulse pressure), antihypertensive medication, previous immunosuppression, induction immunosuppression, time since SLE diagnosis, estimated glomerular filtration rate (eGFR), 24h proteinuria, erythrocyturia, and the presence of antinuclear, anti-double stranded DNA, and antiphospholipid antibodies. The Cockcroft-Gault²⁶ (normalised to a body surface area of 1.73 m² and Schwartz²⁷⁻²⁹ formulas were used to calculate eGFR for adults and children, respectively. The eGFR and 24h proteinuria were registered at 1, 5, and 10 years (± 0.5 year) and at

the last follow-up. Clinical data were studied throughout follow-up for the occurrence of renal flare and/or ESRD.

Brief study outcomes and statistical methods

A complete outline of this section is given in **Appendix 5.2**. Outcomes were studied in two settings: (i) the complete cohort of patients with all observed LN classes who received various therapies and (ii) a subset of patients with class III or IV ($\pm$ V) LN who received induction immunosuppression including cyclophosphamide (CYC), mycophenolate mofetil (MMF), or azathioprine (AZA). Prespecified variables for multivariable analyses were: variables from the reduced histopathology dataset; interaction terms of these with race, age, and induction immunosuppression; and the clinical variables sex, race, time since SLE diagnosis, age₀, proteinuria₀, erythrocyturia₀, MAP₀, induction immunosuppression, and decade during which the patient was biopsied.

Renal flare and ESRD

Time to first LN flare was calculated for patients who achieved (partial) remission from the date of biopsy until the date of flare for patients who reached this endpoint; the remaining patients were censored at the last follow-up or at the time of ESRD. Time to ESRD was calculated analogously. Patients who reached ESRD before 10 years follow-up were regarded as having eGFR=0 mL/min/1.73 m² at the remaining time points. Multivariable Cox proportional-hazards models included the prespecified variables and were simplified by stepwise removal of the least significant variables.

eGFR during follow-up

The extent by which variables were associated with irreversible nephron loss was investigated by modelling eGFR during follow-up. The prespecified variables were tested for their potential to predict a change in the intercept of the adjusted average level of decline in multivariable random intercept/slope linear mixed-effects models, which were simplified by removing the least significant variables (Wald test) and comparing the goodness of fit of nested models (maximum likelihood ratio test).

Progressive eGFR decline

To investigate progressive eGFR decline that did not necessarily result in ESRD and/or renal flare, variables were analysed in association with progressive eGFR decline over 1, 5, and 10 years relative to its linear prediction based upon eGFR₀.

Normally distributed data were expressed as mean $\pm$ standard deviation (SD). Non-normally distributed data were expressed as median (25th–75th percentile). Correlations between clinical and histopathologic variables were assessed using Pearson and Spearman tests, as appropriate. Given the chance of false-positives by multiple correlations of histopathologic variables, Bonferroni correction was performed (**Appendix 5.3**). All other *P*-values were two-tailed and considered significant at *P*<0.05. All analyses were performed using SPSS 23.0 (IBM, Armonk, New York).

RESULTS

We retrieved 293 reports of LN patients from the pathology archives at the LUMC. Of these, 134 patients were not followed at any of the specified centres, and 54 did not have a retrievable renal biopsy or their biopsy was of insufficient quality. Thus, 105 patients were included. Baseline clinical and laboratory findings are summarised in **Table 1** and histopathologic findings in **Table 2**. A complete overview of histopathologic findings and assessment of scorable glomeruli are found in **Appendix 5.4**. The histopathology dataset (**Figure 1**) was reduced from 32 to 27 glomerular and 18 to 9 tubulointerstitial variables by excluding lesions occurring in ≤ 5 patients, excluding one of two strongly correlated variables, and combining interstitial fibrosis and tubular atrophy (IF/TA, see **Appendix 5.3**). Correlations between histopathologic variables and MAP_{G} , eGFR_{G} , and proteinuria₀ are shown in **Appendix 5.5**.

Treatment and outcome

The median follow-up was 9.9 years (25th–75th percentile, 5.9–13.8). Induction immunosuppression was given to 102 patients (**Table 1**). Patients biopsied before 2000 received significantly more frequently AZA and less often CYC or MMF than patients biopsied after 2000 (all $P < 0.001$). Five patients required dialysis at the time of renal biopsy and did not regain renal function during follow-up. Of 100 patients without ESRD at the time of biopsy, 99 achieved (partial) remission; of these, 47 experienced a renal flare during follow-up. Fifteen of the patients who experienced a renal flare eventually developed ESRD. In addition to the five patients with ESRD at the time of biopsy, 16 patients progressed to ESRD and did not regain renal function (**Figure 2**). Five patients died during follow-up due to renal failure ($n=1$), infection ($n=2$), cardiovascular disease ($n=1$), and trauma ($n=1$). A comparison between patients biopsied before and after 2000 revealed no difference in renal survival ($P=0.34$) and renal flare rate ($P=0.66$).

Table 1 Clinical characteristics of 105 patients with LN at the time of renal biopsy.

Characteristic	All patients (n=105)
Decade of renal biopsy, n (%)	
1980–1989	7 (7)
1990–1999	36 (34)
2000–2009	56 (53)
2010–2011	6 (6)
Age, years	
Mean $\pm$ SD	29.8 $\pm$ 13.2
<18 years, n (%)	22 (21)
Females, n (%)	83 (79)
Race, n (%)	
Caucasian	68 (65)
Asian	24 (23)
Afro-Caribbean	13 (12)
Previous diagnosis of SLE, n (%)	77 (73)
Years since SLE diagnosis, median (25 th –75 th percentile)	4.2 (1.1–7.9)
Diagnosis of SLE at the time of biopsy, n (%)	28 (27)
BMI, kg/m ²	
Mean $\pm$ SD	22.4 $\pm$ 4.4
Diastolic BP $\geq$ 90 mm Hg, n (%) [total=101]	31 (31)
MAP (mm Hg)	
Mean $\pm$ SD	97 $\pm$ 17
Taking antihypertensive medication, n (%) [total=103]	34 (33)
Treated with RAS blockade, n (%) [total=103]	19 (18)
Induction immunosuppression, n (%)	
No immunosuppression	3 (3)*
CS alone	4 (4)†
CS + CYC NIH	26 (25)
CS + CYC EuroLupus	27 (26)
CS + MMF	14 (13)
CS + AZA	31 (30)
eGFR _{cr} , mL/min/1.73 m ²	
Mean $\pm$ SD	76.5 $\pm$ 36.2
CKD stage, n (%)	
1	36 (34)
2	34 (32)
3	24 (23)
4	9 (9)
5	2 (2)
Proteinuria _{cr} , g/24h	
Median (25 th –75 th percentile)	2.48 (1.25–5.00)
Erythrocyturia, n	
–/+ /++ /+++	5/22/22/56
Previous immunosuppression, n (%)	19 (18)
ANA, n (%)	104 (99)
Anti-dsDNA, n (%) [total=101]	77 (76)
Antiphospholipid antibodies, n (%) [total=57]	29 (51)

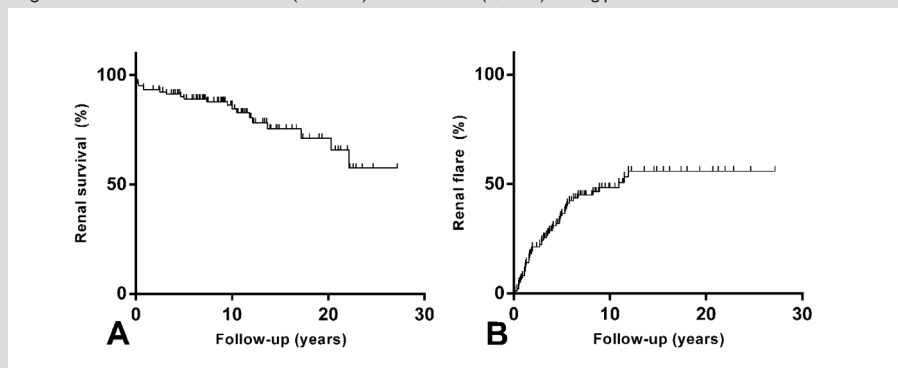
The number of patients with valid registrations is indicated if data were missing for some patients. –/+ /++ /+++ erythrocyturia score ([–] 0–18 erythrocytes/ μ L; [–+] 19–25 erythrocytes/ μ L; [+++] 26–40 erythrocytes/ μ L; [+++] >40 erythrocytes/ μ L). ANA, anti-nuclear antibody; anti-dsDNA, anti-double stranded DNA antibody; AZA, azathioprine; BP, blood pressure; CS, corticosteroids; CYC, cyclophosphamide; eGFR_{cr}, eGFR at the time of biopsy; MAP, mean arterial pressure; MMF, mycophenolate mofetil; RAS, renin-angiotensin system. *Patients (n=3) with class III, IV, and III+V LN. †Patients with class III/IV (n=2) and class V (n=2) LN.

Table 2 Distribution of selected histopathologic lesions in 105 patients with LN.

Scorable glomeruli, n	
Median (25 th –75 th percentile)	13 (10–19)
ISN/RPS class, n (%)	
I	1 (1)
II	3 (3)
III	24 (23)
IV	60 (57)
III/IV + V	12 (11)
V	5 (5)
Normal glomeruli, n (%)	
absent	47 (45)
I–24% of glomeruli	37 (35)
25–49% of glomeruli	16 (15)
≥50% of glomeruli	5 (5)
Global sclerosis, n (%)	
absent	71 (68)
I–24% of glomeruli	21 (20)
25–49% of glomeruli	10 (10)
≥50% of glomeruli	3 (3)
Mesangial hypercellularity, n (%)	
absent	22 (21)
I–24% of glomeruli	38 (36)
25–49% of glomeruli	34 (32)
≥50% of glomeruli	11 (10)
Endocapillary hypercellularity, n (%)	
absent	12 (11)
I–24% of glomeruli	26 (25)
25–49% of glomeruli	17 (16)
≥50% of glomeruli	50 (48)
Crescents, n (%)	
absent	38 (36)
I–24% of glomeruli	30 (29)
25–49% of glomeruli	24 (23)
≥50% of glomeruli	13 (12)
Interstitial infiltration, n (%)	
absent	56 (53)
I–24% of glomeruli	37 (35)
25–49% of glomeruli	4 (4)
≥50% of glomeruli	8 (8)
IF/TA, n (%)	
absent	67 (64)
I–24% of glomeruli	26 (25)
25–49% of glomeruli	8 (8)
≥50% of glomeruli	4 (4)

IF/TA, interstitial fibrosis or tubular atrophy.

Figure 2 Probabilities of renal survival (A, n=105) and renal flare (B, n=99) among patients in the cohort.



Time to end-stage renal disease (ESRD) and time to first renal flare are depicted according to the Kaplan-Meier method. A: during follow-up, 21 patients progressed to ESRD. The probability of renal survival was 93% at 1 year, 90% at 5 years, 85% at 10 years, and 71% at 20 years of follow-up. B: only patients were considered who achieved (partial) remission after renal biopsy and were not censored because they already reached ESRD. During follow-up, 47 patients experienced a renal flare. The probability of renal flare was 8% at 1 year, 35% at 5 years, and 48% at 10 years.

Predictors of renal outcome

Predictors of renal outcome were similar for the complete cohort and the subset (Tables 3, 4, and Appendix 5.6). Below, parameter estimates refer to the complete cohort.

Renal flare and ESRD

Non-Caucasian race (HR 2.23 [95% confidence interval (95% CI), 1.23 to 4.04]) and fibrinoid necrosis (HR 1.04 for each percent of glomeruli [95% CI, 1.00 to 1.07]) independently predicted renal flare (Table 3). The following variables independently predicted ESRD (Table 3): non-Caucasian race (HR 7.16 [95% CI, 2.34 to 21.91]), $eGFR_0$ (HR 0.98 for each mL/min/1.73 m² [95% CI, 0.97 to 1.00]), fibrous crescents (HR 1.09 for each percent of glomeruli [95% CI, 1.02 to 1.17]), fibrinoid necrosis (HR 1.08 for each percent of glomeruli [95% CI, 1.02 to 1.13]), and the presence of IF/TA $\geq 25\%$ (HR 3.89 [95% CI, 1.25 to 12.14]).

eGFR during follow-up

The adjusted mean $eGFR$ at the time of renal biopsy was 116.5 mL/min/1.73 m², with an average change of -0.7 mL/min/1.73 m² per year (Table 4). Cellular/fibrocellular crescents independently predicted an overall lower $eGFR$ (i.e. lower adjusted mean $eGFR$ at baseline and during follow-up) of -0.4 mL/min/1.73 m²/%glomeruli (95% CI, -0.6 to -0.2), as did fibrous crescents (-1.4 mL/min/1.73 m²/%glomeruli [95% CI -2.4 to -0.5]) and the presence of IF/TA $\geq 25\%$ (-40.5 mL/min/1.73 m² [95% CI, -56.2 to -24.8]). Conversely, each percent of normal glomeruli, including glomeruli with 1–3 leukocytes, independently predicted an overall higher $eGFR$ ($+0.27$ mL/min/1.73 m²/%glomeruli [95% CI, 0.1 to 0.4]). Clinically, non-Caucasian race and age₀ independently predicted an overall lower $eGFR$ (-11.4 mL/min/1.73 m² [95% CI, -21.9 to -0.8] and -0.7 mL/min/1.73 m²/year [95% CI, -1.2 to -0.4], respectively).

Table 3 Multivariable prediction models for renal flare and end-stage renal disease (ESRD).

Model	ISN/RPS Class I–V (n=105)		ISN/RPS Class III/IV (±V)* (n=91)	
	HR (95% CI)	P	HR (95% CI)	P
Renal flare (n=99)				
Non-Caucasian	2.23 (1.23; 4.04)	0.008	2.08 (1.09; 3.98)	0.03
% Fibrinoid necrosis (glomerular)	1.04 (1.00; 1.07)	0.04	1.04 (1.00; 1.08)	0.04
	HR (95% CI)	P	HR (95% CI)	P
ESRD (n=105)				
Non-Caucasian	7.16 (2.34; 21.91)	0.001	9.12 (2.85; 29.22)	<0.001
Age ₀ , y	1.02 (0.99; 1.06)	0.18	1.02 (0.99; 1.06)	0.23
eGFR ₀ , mL/min/1.73 m ²	0.98 (0.97; 1.00)	<0.05	0.99 (0.97; 1.00)	0.12
% Fibrinoid necrosis (glomerular)	1.08 (1.02; 1.13)	0.004	1.07 (1.02; 1.13)	0.01
% Fibrous crescents	1.09 (1.02; 1.17)	0.02	1.10 (1.02; 1.19)	0.01
IF/TA ≥25%	3.89 (1.25; 12.14)	0.02	4.53 (1.40; 14.73)	0.01

* Patients with class III/IV (±V) LN who received induction immunosuppressive treatment with cytotoxic drugs were analysed separately from the complete cohort. CI, confidence interval; eGFR₀, eGFR at the time of renal biopsy; HR, hazard ratio; IF/TA, interstitial fibrosis/tubular atrophy.

Table 4 Multivariable prediction model for the course of eGFR during follow-up[†].

Model	ISN/RPS Class I–V (n=105)		ISN/RPS Class III/IV (±V)‡ (n=91)	
	β (95% CI)	P	β (95% CI)	P
(Intercept)	116.5 (100.1; 132.8)	<0.001	116.4 (98.9; 133.9)	<0.001
(Time, y)	−0.7 (−1.5; 0.0)	0.06	−0.6 (−1.4; 0.2)	0.13
Baseline predictors				
Non-Caucasian	−11.4 (−21.9; −0.8)	0.04	−13.5 (−25.2; −1.7)	0.03
Age ₀ , y	−0.8 (−1.2; −0.4)	<0.001	−0.8 (−1.2; −0.4)	<0.001
% Normal glomeruli/minimal leukocyte influx	0.2 (0.1; 0.4)	0.01	0.2 (0.0; 0.5)	0.03
% Cellular/fibrocellular crescents	−0.4 (−0.6; −0.2)	0.001	−0.4 (−0.6; −0.1)	0.003
% Fibrous crescents	−1.4 (−2.4; −0.5)	0.004	−1.6 (−2.6; −0.5)	0.004
IF/TA ≥25%	−40.5 (−56.2; −24.8)	<0.001	−41.4 (−58.3; −24.4)	<0.001

β indicates eGFR in mL/min/1.73 m². eGFR at time t is given by: eGFR(t) = intercept + β_{Time}*t + Z, where Z is the value given by the baseline predictors of the patient: Z = β_{Age0}*Age₀ + β_{%Normal glomeruli/minimal leukocyte influx}*%Normal glomeruli/minimal leukocyte influx + β_{%cellular/fibrocellular crescents}*%cellular/fibrocellular crescents + β_{%fibrous crescents}*%fibrous crescents + β_{non-Caucasian} (if Non-Caucasian) + β_{IF/TA ≥25%} (if IF/TA ≥25%). † Mixed model analysis. ‡ Patients with class III/IV (±V) LN who received induction immunosuppression with cytotoxic drugs were analysed separately from the complete cohort. CI, confidence interval; IF/TA, interstitial fibrosis/tubular atrophy.

Progressive eGFR decline

Briefly, a decline of eGFR over 1 and 5 years was independently predicted by non-Caucasian race, age₀, fibrinoid necrosis, fibrous crescents, and IF/TA $\geq 25\%$. Contrastingly, wire loops and endocapillary lymphocytes were associated with eGFR recovery over 10 years follow-up (**Appendix 5.6**).

Influence of therapy, race, and age on the predictive values of histopathologic variables

In the complete cohort, cytotoxic immunosuppression and/or ACE inhibition were not associated with any of the renal outcomes (**Appendix 5.2**). Of the histopathologic variables, only segmental/global endocapillary hypercellularity ($P=0.03$) and cellular crescents ($P=0.05$) were associated with cytotoxic immunosuppression (CYC, MMF, or AZA). Therapy showed no interactions with histopathologic variables for the different outcomes, with the exception of global glomerulosclerosis with ESRD (HR 1.03 for each percent glomeruli [95% CI, 1.00 to 1.06]) only in patients treated with AZA within the class III/IV subset (**Appendix 5.7**). Spikes/vacuoles and tubular reabsorption droplets were associated with a lower eGFR during follow-up in patients with Afro-Caribbean race compared with other races (**Appendix 5.7**). None of the prespecified clinical variables were correlated both with spikes/vacuoles or tubular reabsorption droplets and race. Age₀ showed no interactions with histopathologic variables for the different outcomes (**Appendix 5.7**).

Immunofluorescence in relation to outcome

The intensities of the individual immunoglobulins (IgA, IgG, and IgM) and complement factors (C3 and C1q) were not associated with ESRD, renal flare, or eGFR during follow-up (data not shown); neither were different immunofluorescence patterns, including $< I + IgG$ and C1q or the full house pattern.

ISN/RPS classes in relation to outcome

ISN/RPS classes were not significantly associated with overall renal survival ($P=0.72$) and renal flare ($P=0.29$). LN classes were significantly associated with eGFR during follow-up ($P<0.05$), with the lowest eGFR in class IV-S LN. For detailed results, see **Appendix 5.8**.

DISCUSSION

Controversies surrounding the prognostic significance of histopathologic (sub)classes in the classification of LN indicate that the prognosticators should be restructured from scratch.^{19,20} Without preconceptions, we analysed clinical and histopathologic predictors of renal outcomes in 105 patients with class I–V LN, including a subset of 91 patients with class III or IV ($\pm V$) LN treated relatively uniformly with cytotoxic immunosuppression. Essentially, our analysis of the different outcomes in the complete cohort and its subset revealed two categories of clinical and histopathologic predictors: (i) variables predictive

of the level of eGFR during follow-up and (ii) variables predictive of renal flare, progressive eGFR decline, and ultimately ESRD. The variables that emerged as independent predictors in the complete cohort were consistent with the predictors in the class III or IV ($\pm$ V) LN subset. Here, we discuss the clinicopathologic predictors of renal outcome as identified among patients with class I–V LN.

In our study, the percentage of normal glomeruli with <4 leukocytes, in the absence of other abnormalities, was the only independent histopathologic predictor associated with a higher eGFR during follow-up. The prognostic significance of glomeruli that are normal by light microscopy most likely indicates that the relatively unaffected part of the kidney is vital in determining renal function. This notion is well known in the setting of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis, where biopsies in which $\geq 50\%$ of glomeruli are normal using identical definitions have been incorporated in a well-validated “focal class” in the classification.^{30–35} Based on our results, glomeruli that are normal by light microscopy may similarly transcend the LN classes and warrant incorporation into a separate index.

Only a limited number of active glomerular lesions had adverse prognostic value. Of the endocapillary lesions, wire loops and endocapillary lymphocytes were associated with eGFR recovery rather than decline over 10 years follow-up (**Appendix 5.6**), suggesting that the treatment of active endocapillary lesions was successful and damage was largely reversible for most patients. This result reflects the suggestion of the Oxford study on IgA nephropathy that endocapillary hypercellularity is a lesion more responsive to immunosuppression given the lack of its association with renal function decline among patients who received immunosuppression.²² In contrast with active endocapillary lesions, the association of cellular/fibrocellular crescents with a lower eGFR during follow-up suggests active extracapillary lesions may result in irreversible damage. Yet, like active endocapillary lesions, active extracapillary lesions were not associated with progressive eGFR decline. The prognostic significance of extracapillary lesions also implies a treatment effect, but more in terms of halting the progression. In the current classification of LN, active endocapillary and extracapillary lesions contribute equally to the assignment of class III or IV LN. Our findings indicate that more weight should be given to extracapillary lesions.

In contrast with other active glomerular lesions, fibrinoid necrosis was not associated with eGFR₀, but rather with renal flare, progressive eGFR decline, and ESRD. Interestingly, fibrinoid necrosis was correlated with segmental, but not global endocapillary hypercellularity. This finding is consistent with the notion that global and segmental lesions represent distinct entities in class IV LN,^{13, 14, 36} and that segmental lesions, with emphasis on fibrinoid necrosis, may have a different immunopathogenesis than global lesions.¹³

Apart from fibrinoid necrosis, chronic glomerular lesions were generally better predictors of eGFR and progressive eGFR decline than active lesions. Fibrous crescents were inde-

pendently associated with lower eGFR during follow-up, progressive eGFR decline, and ESRD. Correspondingly, it has been demonstrated that the composite chronicity index devised by Austin *et al.*³⁷ as well as its components individually, are excellent predictors of ESRD; whereas, the activity index and its components are weaker predictors.³⁷⁻⁴²

Currently, primarily the histopathologic class based on glomerular pathology determines the recommended clinical management of LN.⁴⁻⁶ Our results confirm that, in addition to glomerular variables, tubulointerstitial variables including IF/TA, interstitial infiltrates, tubular casts, and arterial intimal fibrosis,^{38,39,41-44} as well as clinical variables including non-Caucasian race, age, MAP₀, and eGFR₀, are also valuable predictors of renal outcome in LN.^{39,40,45,46}

Our study has some limitations. First, the predictors we identified apply to patients with the spectrum of clinical and histopathologic features observed in our cohort, in which the biopsies were scored by an experienced nephropathologist using our definitions. Indeed, a number of lesions, including microthrombi and vasculitis, were excluded from our analyses due to a low prevalence. These lesions may well have prognostic significance and should be evaluated in other cohorts. Second, we did not analyse electron microscopy (EM); which, if at hand, is the usual complement to light and immunofluorescence microscopy to comprehensively study LN pathology. Whereas in our study immunofluorescence microscopy did not confer prognostic significance, EM studies may reveal additional prognosticators. Third, our study is retrospective, whereas an ideal prognostic study would be prospective and standardise diagnostic and therapeutic procedures for all patients. However, our design allowed us to identify a cohort that could be followed to determine long-term outcomes.

Inherent to any histopathologic study investigating active LN, our results must be interpreted in the light of immunosuppression. Most patients (68%) in the class III or IV ($\pm$ V) subset were treated relatively uniformly with regimens including CYC or MMF according to guidelines. Importantly, 29 (31%) patients were treated with AZA. Within the subset, no interactions between pathology variables and treatment were found, with one exception: global glomerulosclerosis was significantly associated with ESRD for patients treated with AZA, whereas in patients who received CYC or MMF, it was not. AZA has been associated with increased risk of renal flare and inferior efficacy compared with CYC in terms of delaying the progression of chronic lesions.^{47,48} The adverse prognostic significance of global glomerulosclerosis among patients treated with AZA and of chronic lesions in general provides circumstantial evidence that AZA should not be recommended as induction therapy in case of chronic lesions.

In conclusion, our results show that while ISN/RPS classes were poorly associated with the clinically relevant outcomes, prognostication in LN may benefit from the specific assessment of clinical variables and lesions currently obscured in the classification. Normal glomeruli, cellular/fibrocellular crescents, fibrous crescents, and IF/TA were potent predictors of eGFR during follow-up. Importantly, particularly active endocapillary lesions were

likely responsive to immunosuppression since they were not associated with renal function deterioration. Contrastingly, fibrinoid necrosis, fibrous crescents, and IF/TA predicted progressive renal dysfunction even in the uniformly treated subset, implying that these lesions are unlikely to benefit from immunosuppression and thereby correspond to the clinically most relevant category of predictors. An evidence-based era of classifying LN is on the horizon, with an international effort to refine the histopathologic classification already on its way. Our results suggest that LN classes should be expanded with an evidence-based index, analogous to the MEST score in the Oxford classification, with special attention to defining clinically important categories of predictors. Awaiting further validation of a formal index, we suggest that at least fibrinoid necrosis, fibrous crescents, and IF/TA warrant explicit independent scoring to assess the risk of progressive renal dysfunction in conjunction with clinical findings.

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Chapter 6

Brain Histopathology in Patients with Systemic Lupus Erythematosus: Identification of Lesions associated with clinical Neuropsychiatric Lupus Syndromes and the Role of Complement

Danielle Cohen, Emilie C. Rijnink, Rob J.A. Nabuurs, Gerda M. Steup-Beekman, Maarten J. Versluis, Bart J. Emmer, Malu Zandbergen, Mark A. van Buchem, Cornelia F. Allaart, Ron Wolterbeek, Jan A. Bruijn, Sjoerd G. van Duinen, Tom W.J. Huizinga, Ingeborg M. Bajema

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ABSTRACT

Background

Neuropsychiatric (NP) involvement is a poorly understood manifestation of systemic lupus erythematosus (SLE). We studied post-mortem histopathology in relation to clinical NP-SLE syndromes and complement deposition in brains of NP-SLE and SLE patients, and control cases. Furthermore, we investigated the correlation between cerebral post-mortem histopathology and ex vivo 7-Tesla MRI findings in SLE and NP-SLE patients.

Methods

A nationwide search for autopsy material yielded brain tissue from 16 NP-SLE and 18 SLE patients. Brains obtained from 24 patients who died from acute cardiac events served as controls. Apart from a histopathologic evaluation, paraffin-embedded tissue from the cerebral cortex was stained for components of the classical and lectin complement pathways, as well as for the terminal complement complex.

Results

Diffuse vasculopathy, microinfarction, macroinfarction, vasculitis, and microthrombi occurred significantly more often in NP-SLE than SLE, and were absent in controls. Focal vasculopathy was found both in SLE patients and controls. Complement deposition was strongly associated with both SLE and NP-SLE, but not with controls ($P < 0.001$). Microthrombi were found uniquely in NP-SLE, and were associated with C4d and C5b-9 deposits ($P < 0.05$). 7-Tesla MRI was unable to detect most small vessel injury that was visible histopathologically.

Conclusions

Our study demonstrates that histopathologic lesions in NP-SLE represent a continuum, ranging from nonspecific lesions such as focal vasculopathy, to more specific lesions such as C4d- and C5b-9-associated microthrombi and diffuse vasculopathy related to clinical syndromes defining NP-SLE. Complement deposition may be a key factor in the interaction between circulating autoantibodies and thromboischemic lesions observed in NP-SLE. In conclusion, complement inhibition may have novel therapeutic potential in NP-SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a severe autoimmune disease characterised by circulating autoantibodies and immune complexes. SLE can manifest in virtually any organ system.¹ Nervous system involvement in SLE is commonly referred to as neuropsychiatric SLE (NP-SLE),² and occurs in 10–80% of SLE patients.^{3–7} NP-SLE can present with a variety of symptoms, including stroke and psychosis, which are frequently under-recognised despite being associated with increased morbidity and mortality.^{8,9} Because NP-SLE is clinically heterogeneous, and because we lack aetiologic insight and evidence-based therapeutic interventions, the clinical management is complex.

As part of the clinical workup of a potential NP-SLE patient, magnetic resonance imaging (MRI) of the brain plays a prominent role,¹⁰ although interpretation of scans is often hampered by the so-called “clinico-radiological paradox”, by which some patients with SLE and severe neurologic symptoms exhibit no or nonspecific abnormalities on MRI. In an analysis of MRI findings from 74 NP-SLE patients, 42% had no MRI abnormalities, despite having signs and symptoms of active cerebral disease. Otherwise, white matter hyperintensities were the most common MRI finding, which has been suggested to represent cerebral hypoperfusion and infarction.¹¹

The few studies performed to date regarding histopathology of NP-SLE revealed that most lesions are related to ischemic injury in the vicinity of small-diameter vessels.^{12–16} Specifically, microthrombosis, microinfarction, and microbleeds were generally found. Whereas SLE is characterised by autoantibody-mediated inflammation, the findings in the brain suggest a thromboischemic pathogenesis. Thus far, studies in patients have failed to provide clues for the interaction between the autoantibody-mediated inflammation and thromboischemic lesions in NP-SLE. Given the recent evidence that classical complement activation corresponds with the presence of glomerular microthrombi in lupus nephritis¹⁷ and other thrombotic microangiopathies,^{18–22} we hypothesised that complement activation may also underlie thromboischemic injury typically observed in brains of NP-SLE patients.

In a nationwide study using cerebral autopsy material from NP-SLE, SLE, and control cases, we examined post-mortem histopathology in relation to clinical NP-SLE syndromes and complement deposition. Furthermore, in order to evaluate whether histopathologic lesions could be detected clinically, we investigated the correlation between cerebral post-mortem histopathology and ex vivo 7-Tesla MRI in SLE and NP-SLE patients.

METHODS

Nationwide search for cerebral tissues from SLE patients

We conducted a nationwide search for cerebral autopsy tissue obtained from SLE patients with and without clinical signs of neuropsychiatric involvement. Samples were searched using the Dutch PALGA database, a histo- and cytopathology network and archive founded in 1971 to which all pathology laboratories within the Netherlands contribute.²³ Hence, our search included patients autopsied between 1971–2010. We excluded samples from patients who had cutaneous or discoid lupus only, and included all patients with SLE according to the 1982 American College of Rheumatology (ACR) revised criteria^{24,25} with cerebral autopsy tissue available.

Clinical data were collected by contacting the rheumatologists at the different locations where the patients had been hospitalised. Because banking of sera and cerebrospinal fluid was not routinely performed as part of the clinical workup for the patients in this study, analysis of these materials was not feasible. Patients were divided into the following groups by two rheumatologists with extensive experience in diagnosing NP-SLE (GMSB and TWJH): patients with neuropsychiatric syndromes attributable to SLE (NP-SLE group) and SLE patients without neuropsychiatric syndromes attributed to SLE (SLE group). The presence of neuropsychiatric syndromes in each patient was evaluated according to the ACR definitions,² and the existence of secondary factors causing these manifestations was assessed.

Controls were identified from the archives of the Leiden University Medical Center and the Reinier de Graaf Hospital (Delft, the Netherlands) and included previously healthy patients who died of an acute cardiac event (confirmed at autopsy) between 2006–2009.

Histopathology and immunohistochemistry

Sections were stained with haematoxylin and eosin using standard protocols. The following cerebral complement components were stained: C1q (classical pathway), Mannose Binding Lectin (MBL, lectin pathway), C4d, and C5b-9 (membrane attack complex). Deparaffinised sections were subjected to antigen retrieval using EDTA-TRIS (pH 9.0), 10 mM citrate buffer (pH 6.0), or proteinase (bacterial, type XXIV; Sigma-Aldrich, St. Louis, Missouri). The sections were then stained with antibodies against C1q (Dako Cytomation, Denmark, 1:800), C4d (Biomedica Gruppe, Austria, 1:50), and MBL (Sigma-Aldrich Biotechnology, 1:500), and C5b-9 (A239; Quidel, San Diego, California; 1:500). Staining was visualised using the appropriate secondary antibodies with diaminobenzidine as chromagen. Finally, sections were counterstained with haematoxylin. Optimal antibody dilutions and incubation times were predetermined empirically by titration experiments using positive controls.

Histopathologic and immunohistochemical evaluation

All sections were evaluated by a neuropathologist (SGvD) who was blinded with respect to clinical data. Each case was scored for the presence of microinfarction, macroinfarction, large haemorrhage, microbleeds, cerebral infection, vasculitis, and vasculopathy. Infarction was defined as sharply delimited regions of cellular death or tissue necrosis, sometimes with cavitation; the distinction between microinfarction and macroinfarction was made based upon the ability to detect these lesions by light microscopy or by gross inspection, respectively.²⁶ Infarction could be observed in the absence of (micro)thrombi. Microthrombi were defined as microscopic clumps of fibrin, platelets, and erythrocytes.²⁷ Microbleeds were defined as small (<5 mm) perivascular haemosiderin-deposits (usually within macrophages), generally associated with local vessel wall damage. Large haemorrhage was defined as rupture of the blood vessel wall and extravasation of erythrocytes without inflammation in acute stages and oedema, ischemia, infiltration of neutrophils, haemosiderin-laden macrophages, and necrosis in later stages.²⁷ Vasculitis was defined as an inflammatory infiltrate and destructive change in the blood vessel wall.²⁷ In contrast, vasculopathy was defined as endothelial cell proliferation, thickening of the vessel wall, and narrowing of the capillary lumen without an inflammatory infiltrate.¹⁵ Vasculopathy was scored semi-quantitatively as “absent” (<10% of vessels with vasculopathy per low-power field [LPF]), “focal” (10–50% of vessels with vasculopathy per LPF), or “diffuse” (>50% of vessels with vasculopathy in every LPF).

The immunohistochemically stained sections were scored by two independent observers who were blinded with respect to the clinical data. C1q and C4d primarily stained endothelial cells of small vessels in the white and grey matter, and were scored as “absent” (<10% of small vessels with C1q or C4d per LPF), “focal” (10–50% of small vessels with C1q or C4d per LPF), or “diffuse” (>50% of small vessels with C1q or C4d in every LPF). C5b-9 similarly stained vessels, as well as cells with the morphology of glial cells; thus, scoring for vessels was performed similar to C1q and C4d. Because MBL did not stain vessels, MBL-positive cells were scored as being either present or absent.

Whole formalin-fixed brains: Clinical case histories

From three patients, whole formalin-fixed brains were available, enabling a direct comparison between post-mortem ex vivo MRI and cerebral histopathology. Patient 1 was a 57-year-old female with NP-SLE, antiphospholipid syndrome, and severe cerebrovascular disease. Patient 2 was a 38-year-old male with NP-SLE, acute neurologic deterioration, and vasculitis. Patient 3 was a 63-year-old female with SLE without neuropsychiatric symptoms during the course of her disease who suffered from acute myocardial infarction. Detailed case histories are provided in **Appendix 6.1**.

Post-mortem neuroimaging and evaluation of the acquired images

The procedure of post-mortem 7-Tesla MRI scanning is detailed in **Appendix 6.2**. MRI scans were reviewed by two neuroradiologists with extensive experience in the field of

NP-SLE (MAvB and BJE), who then identified areas of interest.

Histopathologic analysis of post-mortem MRI-scanned brains

After radiological analysis, tissue blocks of areas of interest were sampled, embedded in paraffin, and stained with haematoxylin and eosin. The neuropathologist reported the histopathologic changes in each sample.

Ethical considerations

The ethics committee of the Leiden University Medical Center approved this study (P02.028). In accordance with the Dutch National Ethics guideline (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies), all tissue samples and patient data were coded and kept anonymous throughout the study.

Statistical analyses

Categorical variables were compared using chi-square test and its trend version for ordered categories (linear-by-linear analysis). Correlations between histopathologic variables were assessed by calculation of Pearson or Spearman correlation coefficients, as appropriate. All analyses were performed using SPSS Statistics 23.0 (IBM, Armonk, New York). *P*-values <0.05 were considered significant.

RESULTS

Our nationwide search for cerebral autopsy tissue of SLE patients yielded 296 hits. Of these, 48 fulfilled our inclusion criteria. From 14 of these patients, paraffin blocks were no longer available, or tissue quality was unsuitable for analysis. Thus, formalin-fixed tissue samples from 34 SLE patients (27 females, 7 males) autopsied between 1981–2009 were retrieved from 12 Dutch pathology laboratories and used for analysis. Twenty-four patients who died of an acute cardiac event were included as controls. **Table I** provides an overview of clinical characteristics of NP-SLE, SLE, and control cases.

Three patients in the SLE group had neuropsychiatric syndromes that could be attributed to factors other than SLE. Specifically, one patient suffered intracerebral haemorrhage associated with anticoagulant therapy, one patient had a severe cerebral mycotic infection associated with high-dose immunosuppressive therapy for lupus nephritis, and one patient died in a uraemic coma following acute renal failure secondary to lupus nephritis.

Histopathology

The majority of histopathologic lesions were located in the cerebral cortex, distributed equally across the white and grey matter. The patient groups (NP-SLE and SLE) differed significantly from the controls with respect to all histopathologic parameters. Microinfarction ($P=0.016$), macroinfarction ($P=0.002$), and vasculitis ($P<0.050$) were more frequent

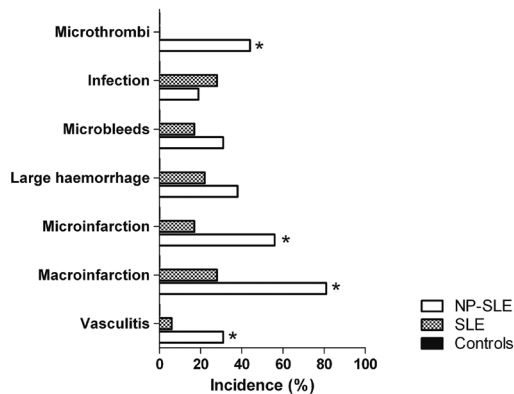
in NP-SLE patients than SLE patients. Microthrombi were found exclusively in NP-SLE patients ($P=0.002$, versus the other groups). Histopathologic findings in the three groups are shown in **Figure 1**.

Table 1 Patient characteristics.

Characteristic	NP-SLE (n=16)	SLE (n=18)	Controls (n=24)
Females, n (%)	15 (94)	12 (67)	10 (42)
Age at death in years, mean (SD)	44 (14)	46 (19)	47 (17)
Neuropsychiatric syndromes (ACR 99 definitions)*			
Cerebrovascular disease, n (%)	11 (69)	2 (11)	0 (0)
Movement disorder, n (%)	2 (13)	0 (0)	0 (0)
Seizures and seizure disorders, n (%)	1 (6)	0 (0)	0 (0)
Acute confusional state, n (%)	2 (13)	1 (6)	0 (0)
Cognitive dysfunction, n (%)	1 (6)	0 (0)	0 (0)
Psychosis, n (%)	1 (6)	0 (0)	0 (0)
No neuropsychiatric symptoms, n (%)	0 (0)	15 (83)	24 (100)
Primary versus secondary NP-SLE			
Primary NP-SLE, n (%)	16 (100)	0 (0)	0 (0)
Secondary NP-SLE, n (%)	0 (0)	3 (17)	0 (0)
Neurologic infection, n (%)	3 (19)	5 (28)	0 (0)
Brain mass in grams, mean (SD)	1308 (177)	1290 (137)	1437 (171)

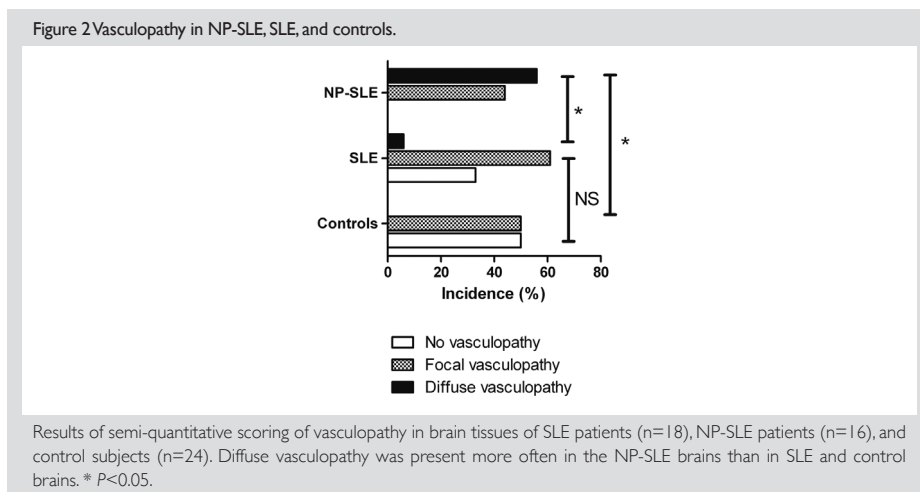
* Eleven NP-SLE patients had one syndrome, four patients had two syndromes, and one patient had three syndromes.

Figure 1 Histopathologic lesions in NP-SLE, SLE, and controls.



Frequency of the indicated lesions in brain tissue of SLE patients (n=18), NP-SLE patients (n=16), and control subjects (n=24). Microthrombi, microinfarctions, macroinfarctions, and vasculitis were present more often in the brains of patients in the NP-SLE group compared to those of patients in the SLE group (*, $P<0.05$ versus SLE). None of the seven parameters were present in the brains of control patients.

The presence of vasculopathy – and its distribution in either a focal or diffuse pattern – differed between the three groups based on linear-by-linear association ($P<0.001$; **Figure 2**).



Vasculitis was found in six patients (five NP-SLE, one SLE; $P<0.050$). Each NP-SLE patient with vasculitis also had vasculopathy (four diffuse, one focal). Vasculitis was associated with cerebrovascular disease in four NP-SLE patients and with acute confusional state in one NP-SLE patient. The patient from the SLE group with vasculitis had a severe mycotic infection associated with immunosuppressive therapy.

Typical examples of vasculopathy and cerebral microthrombi are shown in **Figure 3A** and **3B**, respectively.

Correlations between neuropathological findings in patients with SLE and NP-SLE

In patients with SLE (NP-SLE and SLE), vasculopathy was correlated with macroinfarction ($p=0.43$, $P=0.012$) and microthrombi ($p=0.57$, $P<0.001$). Furthermore, microthrombi were correlated with microinfarction ($r=0.54$, $P=0.001$) and microbleeds ($r=0.40$, $P=0.018$). Large haemorrhage was correlated with macroinfarction ($r=0.35$, $P=0.042$) and vasculitis ($r=0.38$, $P=0.027$), and microinfarction was correlated with microbleeds ($r=0.46$, $P=0.006$).

Immunohistochemistry

Figure 3C-J shows typical examples of immunohistochemical staining with C1q, C4d, MBL, and C5b-9 in patients and controls. C1q, C4d, and C5b-9 were observed on endothelial cells of small vessels (**Figure 3D, F, J**). **Table 2** shows C1q, C4d, and C5b-9 staining patterns in patients and controls. Additionally, C5b-9 was observed on cells with the morphology of glial cells (**Figure 3I**). Cellular staining of C5b-9 was not different between patients and

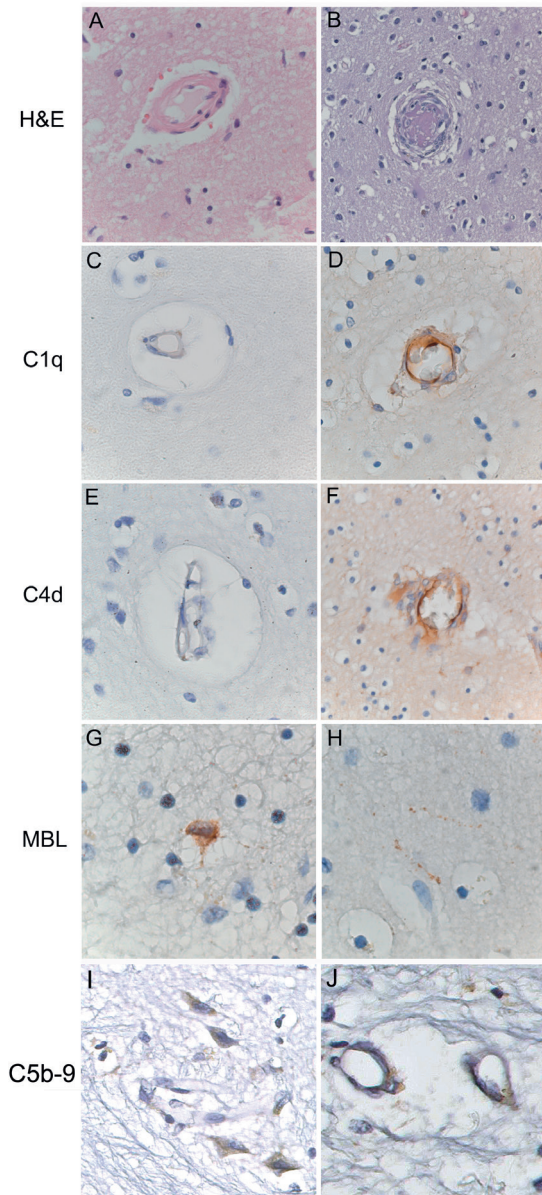
controls (data not shown). MBL deposits did not stain vessels; thus, co-localisation between MBL and C1q or C4d did not occur. Instead, MBL-positive staining was observed on cells resembling astrocytes. These MBL-positive cells (**Figure 3G–H**) were detected both in patients and controls (no difference between the groups; data not shown).

Table 2 Vascular C1q, C4d, and C5b-9 staining in patients and controls.

Staining pattern	NP-SLE (n=16)	SLE (n=18)	Controls (n=24)	P*
No C1q staining	0 (0)	1 (6)	15 (63)	
Focal C1q staining	11 (69)	10 (55)	9 (37)	
Diffuse C1q staining	5 (31)	7 (39)	0 (0)	<0.001
No C4d staining	2 (12)	2 (11)	18 (75)	
Focal C4d staining	11 (69)	14 (78)	6 (25)	
Diffuse C4d staining	3 (19)	2 (11)	0 (0)	<0.001
No C5b-9 staining	3 (19)	3 (17)	20 (83)	
Focal C5b-9 staining	9 (56)	12 (67)	4 (17)	
Diffuse C5b-9 staining	4 (25)	3 (17)	0 (0)	<0.001

All values are given as n (%). C1q: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.531$. C4d: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.801$. C5b-9: NP-SLE vs. controls $P<0.001$; SLE vs. controls $P<0.001$; NP-SLE vs. SLE $P=0.796$. *Chi-square test.

Figure 3 Examples of histopathologic lesions and immunohistochemical staining patterns.



A, B, D, F, H, I, J: staining patterns in an NP-SLE case. C, E, G: staining patterns in a control case. Stainings: haematoxylin and eosin (H&E, A–B), C1q (C–D), C4d (E–F), mannose-binding lectin (MBL, G–H), and C5b-9 (I–J). Magnification: 40X. A: vasculopathy (thickening of the vessel wall, no inflammatory infiltrate). B: cerebral microthrombus. C–D: negative and positive C1q staining, respectively. E–F: negative and positive C4d staining, respectively. C1q showed a linear pattern of intravascular deposits (D), similar to C4d (F). G–I: positive glial cell staining. J: C5b-9 staining showing a linear pattern of intravascular deposits.

Complement deposition in relation to neuropathological findings in patients with SLE and NP-SLE

We investigated the relationship between C1q, C4d, C5b-9, and the presence of microthrombi, microinfarctions, macroinfarctions, vasculitis, and vasculopathy. Only the presence of microthrombi was associated with C4d and C5b-9 staining ($P=0.047$ and $P=0.020$, respectively). Every patient with microthrombi also had either focal or diffuse C4d and C5b-9 staining. C4d and C5b-9 were strongly associated with microthrombi within the NP-SLE group (linear-by-linear association: $P=0.024$ and $P=0.008$, respectively).

Relationship between C4d, C1q, and C5b-9 in SLE and NP-SLE

To gain additional insight into the cascade of events in classical complement activation, the staining patterns of C1q, C4d, and C5b-9 were related to one another in the SLE and NP-SLE patient groups. In general, diffuse C1q staining was present more often than diffuse C5b-9 and C4d staining (35%, 21%, and 15%, respectively). Twenty-five of 34 (74%) patients with SLE and/or NP-SLE had concurrent positive (either focal or diffuse) vascular staining of C1q, C4d, and C5b-9. Twenty-nine of the 30 patients with positive C4d staining (either focal or diffuse) had concurrent C1q deposits and 25 had concurrent C5b-9 deposits (an overlap of 97% and 83%, respectively). Conversely, 29 of the 33 patients with positive C1q staining (either focal or diffuse) had concurrent C4d, and 28 had concurrent C5b-9 staining (an overlap of 89% and 85%, respectively). Of the 28 patients with positive C5b-9 staining, 25 (89%) had concurrent C1q and C4d staining. **Appendix 6.3** shows that the distributions of C1q, C4d, and C5b-9 were quite similar in both the SLE and NP-SLE group.

Post-mortem ex vivo MRI and correlations with histopathology and immunohistochemistry

The results of post-mortem ex vivo MRI scans and histopathology sections of two NP-SLE patients and one SLE patient are shown in **Figure 4**.

Patient 1: Post-mortem ex vivo MRI

MRI revealed extensive confluent periventricular and deep white matter lesions with notable sparing of U-fibres (**Figure 4A–B**). Furthermore, central lacunae in the deep white matter suggested tissue loss consistent with lacunar infarction. White matter lesions around confluent white matter lesions and adjacent to deep white matter lesions were perivascular in distribution.

Patient 1: Histopathology

Sections taken from both the deep and periventricular white matter lesions revealed areas of recent and less recent microinfarctions and macroinfarctions. In one deep white matter lesion, multiple microthrombi (**Figure 4C–D**) were identified. In all sections, prominent vasculopathy in both grey and white matter was found (**Figure 4E**). Vasculopathy was present within white matter hyperintensities and infarcted areas, as well as in normal-appearing white and grey matter. Furthermore, vast areas of atrophied cortex – particularly in the proximity of infarctions – had laminar necrosis (**Figure 4F**).

Patient 2: Post-mortem ex vivo MRI

MRI revealed normal grey and white matter differentiation (**Figure 4G–H**). Several linear hyperintensities were characteristic of normal Virchow-Robin spaces (**Figure 4G**). Unlike Patient 1, the white matter in Patient 2 was homogeneous, and cortical thickness was normal.

Patient 2: Histopathology

Because no abnormalities were identified on MRI, sections were obtained from several cortical areas. Each of these revealed diffuse vasculopathy (**Figure 4I–J**). Furthermore, lymphocytes had invaded vascular walls of several veins and venules, reflected by the presence of fragmented nuclei and fibrinoid material (**Figure 4K**). No intravascular microthrombi, infarctions, or gliosis were identified.

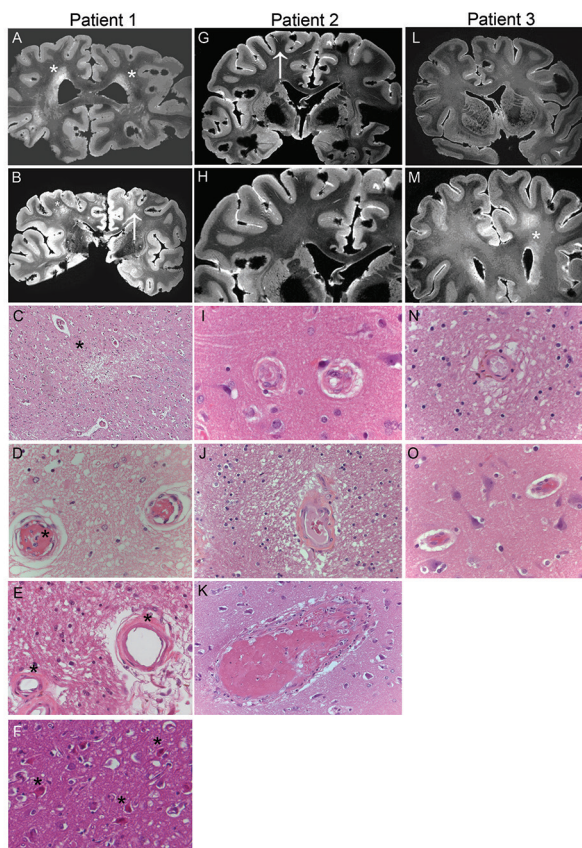
Patient 3: Post-mortem ex vivo MRI

MRI revealed a prominent Virchow-Robin space (**Figure 4L**). In addition, a linear perivascular white matter hyperintensity was present in the internal capsule. Another white matter lesion was identified in the frontal white matter (**Figure 4M**).

Patient 3: Histopathology

Sections taken from the frontal white matter lesion revealed focal vasculopathy. No other abnormalities were identified. Vasculopathy was present in a similar focal distribution pattern throughout all sections (**Figure 4N–O**).

Figure 4 Post-mortem 7-Tesla MRI in relation to histopathologic findings in NP-SLE and SLE patients.



Patients 1 and 2 were NP-SLE patients, patient 3 had SLE without neuropsychiatric symptoms. A: confluent periventricular and deep white matter lesions*. B: deep white matter lesions with sparing of U-fibres*; perivascular white matter lesions (arrow). C–F: sections from deep and periventricular white matter lesions. C: microinfarction*. D: microthrombi* in vicinity of white matter lesions. E: vasculopathy*. F: laminar necrosis*. G–H: normal grey/white matter with linear hyperintensities (normal Virchow-Robin spaces; G, arrow). I–K: sections from normal-appearing cortex. I–J: vasculopathy. K: venous vasculitis. L: prominent Virchow-Robin space and normal grey/white matter. M: frontal white matter lesion*. N: vasculopathy in section from M*. O: vasculopathy in section from normal-appearing area on MRI.

DISCUSSION

In this study, we described the injury in brains of patients with NP-SLE as compared to SLE and control cases. Furthermore, we studied brain injury in NP-SLE in relation to classical complement deposition. We were able to identify a number of histopathologic lesions that were specific to NP-SLE, and could thereby be linked to clinical NP-SLE manifestations as defined by the ACR.² Compared to SLE patients, NP-SLE patients had significantly more microinfarction, macroinfarction, vasculitis, and microthrombi, though in controls these and other neuropathological abnormalities were absent. Also, diffuse vasculopathy occurred significantly more often in NP-SLE than in SLE patients, whereas focal vasculopathy was found in all groups (NP-SLE, SLE, and controls). The common finding of vasculopathy and the absence of a relationship between vasculopathy and acute clinical NP-SLE are in accordance with previous findings.¹⁵ Our data show that the injury and clinical manifestations of NP-SLE represent a continuum, ranging from relatively nonspecific lesions found in most SLE patients such as focal vasculopathy – to specific and even pathognomonic lesions in this setting, including diffuse vasculopathy and microthrombi associated with clinical syndromes defining NP-SLE.²

Our study is the first to demonstrate that deposits of C1q and C4d, both components of the classical complement pathway, as well as the terminal complement complex (C5b-9), are concurrently present in the cerebral vessels of patients with NP-SLE and SLE significantly more often than in controls. This constellation of staining patterns indicates that activation of the classical complement pathway proceeded to completion, as demonstrated by the formation of the terminal complement complex. The role of complement activation in the development of thrombosis and ischemia has been studied extensively outside the field of SLE, where complement has been linked to microthrombotic injury in antiphospholipid syndrome^{19,22} and thrombotic microangiopathy.¹⁸ In these conditions, accumulation of antibodies in small vessels most likely leads to activation of the classical complement pathway, endothelial injury, and the subsequent formation of microthrombi.¹⁸ Given that vasculopathy, microinfarction, macroinfarction, vasculitis, and microthrombi may all evoke vascular occlusion and could thereby result in clinical abnormalities, the question arises as to why some of these lesions were found to be more specific in our study for NP-SLE than other lesions. Our findings suggest that thromboischemic injury may occur in all SLE patients but that overt NP-SLE ensues only after a certain threshold of complement-mediated injury is reached. The finding that, of all vascular lesions, only microthrombi were correlated with C4d and C5b-9, suggests that microthrombi represent a more progressed stage of complement-mediated injury. Conceivably, microthrombi were also present in patients with subclinical NP-SLE – though in smaller quantities and would therefore be more readily missed in these cases due to sampling error.

Although cerebral vasculitis is generally considered to be a rare finding in NP-SLE, we identified five NP-SLE patients (31%) with vasculitis in our study. This relatively high incidence as compared to previous studies¹²⁻¹⁵ may be explained by our clear distinction of SLE and NP-SLE cases. In four of the NP-SLE cases with vasculitis, vasculitis was associated with cerebrovascular disease. Interestingly, our neuropathological analyses revealed that vasculitis was correlated with large haemorrhage. Although according to the recommendations for the management of NP-SLE, cerebrovascular disease due to ischemic stroke and/or TIA comprises over 80% of cases and therefore does not require immunosuppressive therapy,¹⁰ we showed that vasculitis may still be an underlying factor in a substantial number of cases. Our findings emphasise that immunosuppressive therapy may occasionally be indicated in cases with a component of vasculitis underlying cerebrovascular disease as a manifestation of NP-SLE.

Diamond *et al.* focused on the role of autoantibodies in the development of neuropsychiatric symptoms in a murine model and found that anti-double stranded DNA antibodies derived from human SLE patients can cross-react with N-methyl-D-aspartate receptors on neurons causing neuronal death by excitotoxicity and apoptosis.²⁸⁻³⁰ However, in subsequent studies in which these antibodies were induced in mice, neuronal damage occurred solely when a breach in the blood-brain barrier was present.^{31,32} Our findings may indicate that continuously exposing the cerebral endothelium to autoantibodies can cause complement activation and endothelial injury in all SLE patients. Possibly, a second hit is required to develop overt clinical disease in the form of NP-SLE as defined by the ACR.² Infection, pregnancy, drug toxicity, and defects in complement regulation have all been described as triggering factors.^{20,21} Possibly, these evoke a breach in the blood-brain barrier together with the formation of C4d- and C5b-9-associated microthrombi as well as the other lesions we identified to be distinctive of NP-SLE. Since sera and cerebrospinal fluid (CSF) were not available from the patients in our study, it was not possible to further analyse the antibody profile in CSF in relation to the CSF/albumin ratio as a possible indicator of blood-brain barrier integrity in concert with the assessment of thromboischemic injury in NP-SLE and SLE cases. Whether these or other factors affecting the integrity of the blood-brain barrier play a role in NP-SLE will be the subject of future studies.

Apart from the clinicopathologic paradox of impressive histopathologic cerebral lesions that are sometimes not accompanied by overt clinical NP-SLE, there is also a clinicoradiological paradox. In contrast to the clinicopathologic paradox, the latter is defined by the absence of abnormalities on conventional MRI in patients with overt clinical NP-SLE, and this hampers assessment of the extent of cerebral injury in NP-SLE patients.¹¹ We investigated whether microvascular and thromboischemic injury could be detected clinically by analysing three whole brains of SLE patients using 7-Tesla MRI. High-field MRI can provide images at higher spatial resolution, resulting in more detailed information regarding microvascular injury compared to conventional MRI. Interestingly, for these brains (two NP-SLE, one SLE), even high-field MRI was unable to detect most small-vessel injury that was visible

histopathologically. The microvascular injury that could be detected was most prevalent in the vicinity of white matter hyperintensities. On the other hand, high-field MRI would detect nonspecific lesions more readily than conventional MRI.³³ White matter hyperintensities by conventional MRI may be found in 20 percent of the general population younger than age 50, and in 90 percent of people older than age 70.³³ Furthermore, white matter lesions may be observed in SLE patients who do not have neuropsychiatric symptoms. Indeed, white matter lesions were detected by 7-Tesla MRI in one SLE patient without NP-SLE in our study, which lesions corresponded to focal vasculopathy. Thus, although white matter hyperintensities may indicate an initial phase of vascular damage in some SLE patients, they should generally be considered nonspecific. Probably, more sensitive as well as more specific imaging tools are required for the diagnosis of NP-SLE. Future studies to determine the feasibility of, for instance, quantitative techniques such as volumetric magnetisation transfer imaging (MTI)^{34,35} to detect specific lesions in SLE and NP-SLE patients are called for, as brain biopsies remain unattainable in routine clinical practice.

Concluding, NP-SLE is a poorly understood manifestation of SLE, associated with significant morbidity and mortality. NP-SLE is usually treated with aggressive immunosuppression, which can be beneficial in some – but certainly not all – NP-SLE patients.^{10,36} Our finding that complement activation is present in both SLE and NP-SLE suggests that this mechanism may contribute to the development of injury. It remains to be elucidated whether the clinical manifestations of NP-SLE following injury are due to a thromboischemic pathomechanism, due to a breach in the blood-brain barrier facilitating antibody-mediated neuroinflammation, or due to a combination of both. Importantly, our novel finding of complement in association with injury in SLE and NP-SLE proposes the complement system as a promising new target in the treatment of NP-SLE. The finding that C5b-9 deposits were present in 82% of patients with SLE and NP-SLE in our study, suggests that at least this proportion of patients with NP-SLE – and perhaps also patients with SLE – may benefit from treatment with the terminal complement inhibitor eculizumab.³⁷⁻⁴² The efficacy of such treatment in NP-SLE and SLE remains to be investigated in future studies.

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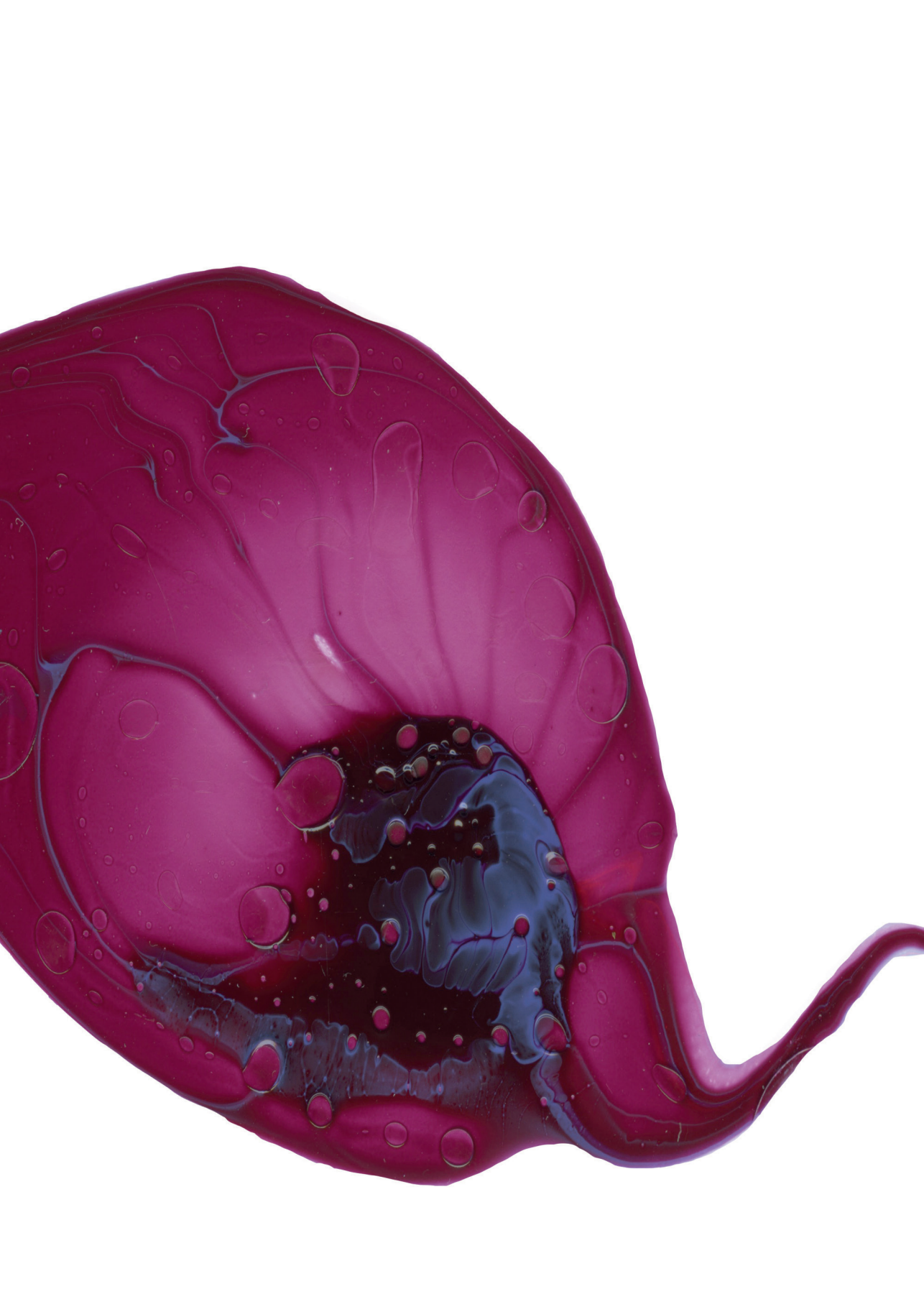
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Chapter 7

Tissue Microchimerism is Increased During Human Pregnancy: A Human Autopsy Study

Emilie C. Rijnink, Marlies E. Penning, Ron Wolterbeek, Suzanne Wilhelmus,
Malu Zandbergen, Sjoerd G. van Duinen, Joke Schutte, Jan A. Bruijn, Ingeborg M. Bajema

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ABSTRACT

Background

Microchimerism is the occurrence of small populations of cells with a different genetic background within an individual. Tissue microchimerism is considered to be primarily pregnancy-derived and is often studied relative to female-dominant autoimmune diseases, pregnancy complications, malignancies, response to injury, and transplantation outcomes. A particular distribution pattern of chimeric cells across various organs was recently described in a model of murine pregnancies. Our aim was to determine the frequency and distribution of tissue microchimerism across organs during and after pregnancy in humans.

Methods

We performed in situ hybridization of the Y chromosome on paraffin-embedded autopsy samples of kidneys, livers, spleens, lungs, hearts, and brains that were collected from 26 women who died while pregnant or within one month after delivery of a son. Frequencies of chimeric cells in various tissues were compared to those of a control group of non-pregnant women who had delivered sons.

Results

Tissue microchimerism occurred significantly more frequently in lungs, spleens, livers, kidneys, and hearts of pregnant women than non-pregnant women (all $P < 0.01$). We showed that some of the chimeric cells were CD3+ or CD34+. Corrected for cell density, the lung was most chimeric (470 Y chromosome-positive nuclei per million nuclei scored), followed by the spleen (208 Y+/10⁶ nuclei), liver (192 Y+/10⁶ nuclei), kidney (135 Y+/10⁶ nuclei), brain (85 Y+/10⁶ nuclei), and heart (40 Y+/10⁶ nuclei).

Conclusions

Data from this unique study group of women who died while pregnant or shortly after delivery provide information about the amount and physiologic distribution of chimeric cells in organs of pregnant women. We demonstrate that during pregnancy, a boost of chimeric cells is observed in women, with a distribution across organs that parallels findings in a mouse model.

INTRODUCTION

Microchimerism is defined as the occurrence of a small number of cells in an individual that originate from another individual.¹ One possible source of naturally occurring microchimerism is pregnancy. Knowledge about the source and dynamics of naturally occurring tissue microchimerism is relevant for interpreting its contribution to the pathogenesis of autoimmune disease, pregnancy complications, and malignancies; and for studying its role in response to injury and transplantation outcomes. Because of the association between certain autoimmune diseases and pregnancy, as well as their increased incidence in women of fertile age, the aim of many studies has been to investigate the role of microchimerism in the onset of autoimmune disease.² An adverse effect of pregnancy-acquired microchimerism has also been suggested in kidney transplantation. Here, female recipients of a spousal donor kidney who had not been pregnant had better graft survival than those who had been pregnant.³ On the other hand, a decreased incidence of malignancy in association with increasing parity has been found for various malignancies,⁴ suggesting a protective role for pregnancy-acquired microchimerism. To assess the significance of pregnancy-derived tissue microchimerism in autoimmune disease, malignancy, and other pregnancy-related conditions, however, its occurrence during pregnancy in humans must be established first.

During pregnancy fetal cells enter the maternal circulation (fetal microchimerism) and maternal cells enter the fetal circulation (maternal microchimerism). Fetal cellular microchimerism in the maternal circulation has been detected as early as seven weeks of gestation⁵ and remains detectable in maternal blood up to 27 years postpartum.⁶ Male cells of presumably fetal origin can differentiate and be incorporated into maternal tissues after pregnancy.⁷ We previously demonstrated that microchimerism, as measured by *in situ* hybridization of the Y chromosome, is present in the thyroid gland, lungs, skin, lymph nodes, kidneys, livers, and hearts of healthy non-pregnant women.^{8,9} Remarkably, these studies showed no significant association between microchimerism and parity, yet pregnancy is often assumed to be the main source of tissue microchimerism in women. Most studies have focused on microchimerism in relation to pregnancy in blood samples.¹⁰⁻¹⁴ Studies on microchimerism during pregnancy at the tissue level are limited to the demonstration of presumably fetal male microchimerism in affected tissues of pregnant women with polymorphic eruptions of pregnancy,¹⁵ pregnancy-associated breast cancer,¹⁶ melanoma,¹⁷ and appendicitis.¹⁸ However, no studies have systematically investigated human microchimerism in relation to pregnancy in healthy tissues.

Studying murine pregnancies, Fujiki *et al.* detected fetal cells in tissues of mouse dams after mating wild-type females with green fluorescent protein (GFP)-transgenic males.¹⁹ Interestingly, these authors found a particular distribution pattern of chimeric cells across various organs, identifying the greatest concentrations in the lungs, followed by the spleen, liver, and kidney. Remarkably, microchimerism in maternal lungs exceeded that found in other

organs 10- to 100 fold. Fujiki *et al.* suggested that the high rate of blood flow through lung tissue might explain this high rate of microchimerism in the lungs, in addition to the fact that the lung contains the first capillary bed a fetal cell crosses after entering the maternal uterine venous circulation. To further unravel the impact of microchimerism on maternal health by studying mouse models, the validity of these models to represent the human situation must be confirmed by studying the distribution of tissue microchimerism during human pregnancy.

The aim of the present study was to investigate the frequency and distribution of tissue microchimerism in lungs, spleens, livers, kidneys, brains, and hearts from women pregnant with sons and the influence of the postpartum interval on the amount of microchimerism. Our results provide information about the physiologic distribution of chimeric cells across the organs of pregnant women, allowing comparisons to animal models, and support a role for pregnancy as a source of tissue microchimerism.

METHODS

Patient selection and ethical approval

Tissue specimens came from autopsies performed on women in the Netherlands between 1990 and 2006. Samples were searched using the Dutch PALGA database, a histo- and cytopathology network and archive to which all pathology laboratories within the Netherlands contribute.²⁰ The search strategy included the parameters “autopsy”, “women”, and “age between 18 and 45 years”. All patients were included who died while pregnant with male fetuses or who died within one month after delivery of a son.

With this search strategy, we obtained formalin-fixed, paraffin-embedded tissue samples from 26 women, including samples from 19 lungs, 15 spleens, 19 livers, 19 kidneys, 17 hearts, and 5 brains. The number and availability of organ samples varied because of differences between laboratories. Also, some samples were excluded because of poor quality due to autolysis. A previously described group of non-pregnant women with sons served as historic controls.^{8,9} Patient characteristics of the pregnant women included in the current study and those of the historic control group are shown in **Table 1**.

Clinical data were obtained from autopsy reports and from the records of the National Maternal Mortality Committee of the Dutch Society of Obstetrics and Gynaecology. All samples were coded and handled anonymously in accordance with the Dutch National Ethics Guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies). In addition, this study was approved by the ethics committee of the Leiden University Medical Center (P12.107). **Table 2** shows pregnancy-related characteristics of the 26 pregnant women included in this study.

Table I Characteristics of pregnant women and controls.

Characteristic	This study	Koopmans et al. 2005	Koopmans et al. 2008
	<i>Pregnant women with sons</i>	<i>Non-pregnant women with sons</i>	<i>Non-pregnant women with sons</i>
Number of women	26	45	50
Age in years, mean (range)	32 (20–45)	64 (29–93)	64 (29–93)
Cause of death			
Infectious	0	9	11
Malignancy	0	14	13
Cerebral	1	8	10
Vascular/myocardial	14	12	13
Other	11, i.e. pre-eclampsia/ eclampsia (6), amniotic fluid embolism (2), liver cirrhosis, EUG, sarcoidosis	2, i.e. liver cirrhosis, cachexia	3, i.e. liver cirrhosis (2x), cachexia
Blood transfusion			
Yes	1	29	30
No	25	16	20
Organ samples studied by ISH			
Kidney	19	30	-
Liver	19	33	-
Heart	17	42	-
Spleen	15	29	-
Thyroid	-	-	43
Lung	19	-	37
Skin	-	-	20
Lymph node	-	-	6
Brain	5	-	-

EUG, extra-uterine gravidity; ISH, in situ hybridization.

In situ hybridization

The same protocol for in situ hybridization of the Y chromosome was used as previously described for our historic control group.^{8,9} Formalin-fixed, paraffin-embedded samples were cut into 4 µm slices, transferred to Superfrost plus glass slides (Menzel-Glaser, Germany), and dried overnight at 37 °C. A Y chromosome-specific DNA probe²¹ was labelled with digoxigenin (DIG) according to the standard Nick-translation protocol. After labelling, the probe was precipitated, dried, and dissolved in hybridization mixture (50% deionised formamide; 0.05 M sodium phosphate buffer, pH 7.0; 0.3 mol/L NaCl; 30 mmol/L Na citrate (2×SSC); and 10% dextran sulphate). Salmon sperm DNA, transfer RNA, and Cot-1 DNA were added to the hybridization mixture to prevent nonspecific binding of DNA.

Table 2 Pregnancy-related characteristics of cases.

Characteristic	Pregnant women (n=26)
Death postpartum, n	11
GA at birth, weeks	37.4 (29.9–39.1)
Death postpartum, hours	36.0 (0.2–432)
Death during pregnancy, n	15
GA at death, weeks	29.0 (10–40.3)
Death to autopsy time, hours	24.0 (1–48)
Sex of fetus	
Male, %	100
Female, %	0
Gravidity	2.2 (1–10)
Parity	1 (0–5)
Eclampsia/preeclampsia, n	6

Data are presented as mean (range), unless indicated otherwise. GA, gestational age.

The slides were deparaffinised in xylene and rehydrated in an ethanol series followed by a distilled water rinse. The slides were prepared with 0.05 M citrate buffer (pH 6.0) at 80 °C for 80 minutes and rinsed in prewarmed distilled water at 37 °C. Enzyme digestion was performed using 0.5% pepsin (Serva Electrophoresis GmbH, Heidelberg, Germany) in 0.01 M HCl at 37 °C for 20 minutes. The slides were dehydrated in upgraded ethanol and air-dried. Tissue sections on each slide were covered with 30 µL of hybridization mixture containing 5 ng/µL labelled probe. The slides were denatured for 10 minutes on a metal plate at 80 °C and incubated at 37 °C overnight. The following day, the slides were washed three times in 2×SSC/0.1% Tween at 37 °C to remove the cover slips and three times in 0.1×SSC at 60 °C. To visualise the DIG-labelled probe, the sections were incubated consecutively with a mouse anti-DIG monoclonal antibody (Sigma-Aldrich, St. Louis, Missouri, USA) and anti-mouse Envision-HRP (Dako, Glostrup, Denmark), followed by development with Nova Red Vector for 10 minutes. Haematoxylin staining was used as a background.

We confirmed by nested PCR and sequencing that the probe was specific for the Y chromosome.⁸ As a positive control, we used a male tissue sample in which the sensitivity of the Y chromosome signal was 58%. As a negative control, a male tissue sample was used on which the complete hybridization protocol was performed using the hybridization mixture without the Y chromosome probe.

Double staining

To study the phenotype of the chimeric cells in pregnant women, we performed CD3 and CD34 immunohistochemical staining in combination with in situ hybridization of the Y chromosome as described previously.²²

Scoring

Slides were scanned using a Panoramic Digital Slide Scanner (3DHISTECH Kft., Budapest, Hungary) and scored using Panoramic Viewer (3DHISTECH Kft., Budapest, Hungary). A predefined random area of 50 mm² was scored at 400× magnification by two observers. The Y chromosome was considered to be present when a red dot was visible within nuclei of similar size, colour, and intensity to those in positive controls. The degree of microchimerism in positive samples was scored quantitatively by counting the number of positive nuclei and expressing them as the number of positive nuclei per mm². To correct for differences in cell density among organs, image analysis using ImageJ (NIH) was performed to estimate the number of nuclei scored on each slide individually.

Because of the potential relationship between tissue injury and microchimerism, the degree of injury in each organ was scored semi-quantitatively by a pathologist. Haematoxylin and eosin-stained sections of the same tissue blocks on which in situ hybridization of the Y chromosome was performed were analysed by light microscopy. A score for active injury was composed of the following criteria: (0) no foci of inflammation, haemorrhage, and congestion; (1) minor focal inflammatory infiltrates, small foci of haemorrhage, and/or mild congestion; (2) more diffuse inflammatory infiltrates, diffuse signs of haemorrhage, and/or moderate congestion; (3) extensive inflammatory infiltrates, extensive haemorrhage, and/or extensive signs of congestion. Moreover, a score for chronic injury was based on the following criteria: (0) no calcifications, fibrosis, and steatosis; (1) minor foci of calcifications, fibrosis, and/or steatosis; (2) moderate signs of calcifications, fibrosis, and/or steatosis; (3) diffuse calcifications, fibrosis, and/or steatosis.

Statistical analyses

Fisher's exact tests were used to compare proportions of organs from pregnant women and non-pregnant women that were scored as positive. To correct for correlations among organ measurements within a patient, we used a linear mixed model to compare quantities of Y chromosomes in different organs. In our mixed model, we performed analyses with organ type and gravidity as fixed effects, as well as conducting separate analyses for organ type and maternal death either ante- or postpartum, organ type and gestational age, and organ type and either vaginal delivery or Caesarean section. Visual inspection of the distribution of residuals was performed to assess normality assumptions. We assessed correlations between active and chronic injury scores and number of Y chromosome-positive nuclei per million nuclei by calculating Spearman correlation coefficients (ρ). *P*-values <0.05 were considered significant. All analyses were done using SPSS Statistics 20.0 (IBM Corp., Armonk, New York).

RESULTS

Frequency of male cells in maternal organs

We performed in situ hybridization of the Y chromosome on 19 lungs, 15 spleens, 19 livers, 19 kidneys, 5 brains, and 17 hearts. Chimeric cells were found in all organs studied. **Table 3** shows that the proportion of women with male cells in their lungs, spleens, livers, kidneys, and hearts was significantly higher in this study of pregnant women with sons than in a historic control group of non-pregnant women with sons.^{8,9}

Table 3 Fractions of chimeric organs in pregnant versus non-pregnant women.

Organ	Pregnant women with sons (%)	Non-pregnant women with sons (%)	P
Kidney	16/19 (84)	5/30 (17)	<0.0001
Liver	18/19 (95)	6/33 (18)	<0.0001
Spleen	15/15 (100)	3/29 (10)	<0.0001
Heart	5/17 (29)	0/42 (0)	<0.01
Lung	19/19 (100)	10/37 (27)	<0.0001
Brain	5/5 (100)	-	-

Organ distribution of male cells

For the quantitative analyses of tissue microchimerism, the numbers of Y chromosome-positive cells per section were corrected for area and cell density. The spleen showed the most microchimerism per area with, on average, 0.45 positive Y chromosomes per mm², followed by the lung (0.30/mm²), kidney (0.27/mm²), liver (0.23/mm²), brain (0.06/mm²), and heart (0.02/mm²). However, after correction for cell density, the lung was most chimeric, with an average of 470 Y chromosome-positive nuclei per million nuclei scored. The lung was followed by the spleen (208 Y+/10⁶ nuclei), liver (192 Y+/10⁶ nuclei), kidney (135 Y+/10⁶ nuclei), brain (85 Y+/10⁶ nuclei), and heart (40 Y+/10⁶ nuclei). **Figure 1** shows the organ distribution of chimeric cells.

Lungs contained significantly more Y chromosome-positive cells corrected for cell density than any of the other organs studied (lung vs. any organ: $P<0.001$). Furthermore, the spleen and liver were significantly more chimeric than the heart ($P=0.032$ and $P=0.026$, respectively).

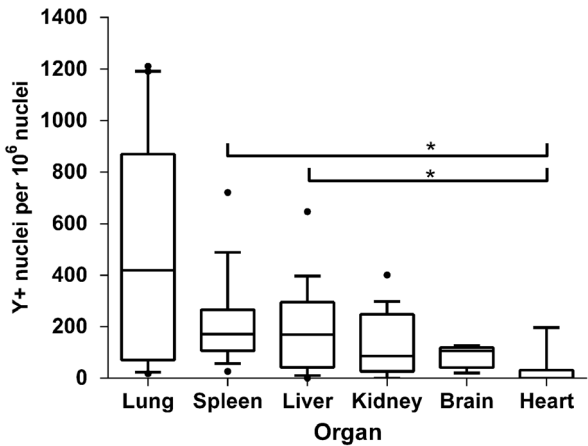
Location and phenotype of chimeric cells

Chimeric cells appeared to be both parenchymal cells and hematopoietic cells. **Figure 2** shows photographs of slides with chimeric cells as observed in the various organs. In lungs, chimeric cells were incorporated into the alveolar septa, bronchiolar walls, and blood vessels. In the spleen, chimeric cells seemed to be part of the hematopoietic cell population while in livers, chimeric cells resembled hepatocytes and were found in blood vessels. In

the kidney, chimeric cells occurred in tubules, glomeruli, and the endothelium of blood vessels. Male microchimerism was present in the cerebral cortex of all five women whose brain autopsy material was available. In the brain, chimeric cells appeared to be glial cells and possibly neurons and were also found in blood vessel endothelium. In hearts, chimeric cells resembled cardiomyocytes.

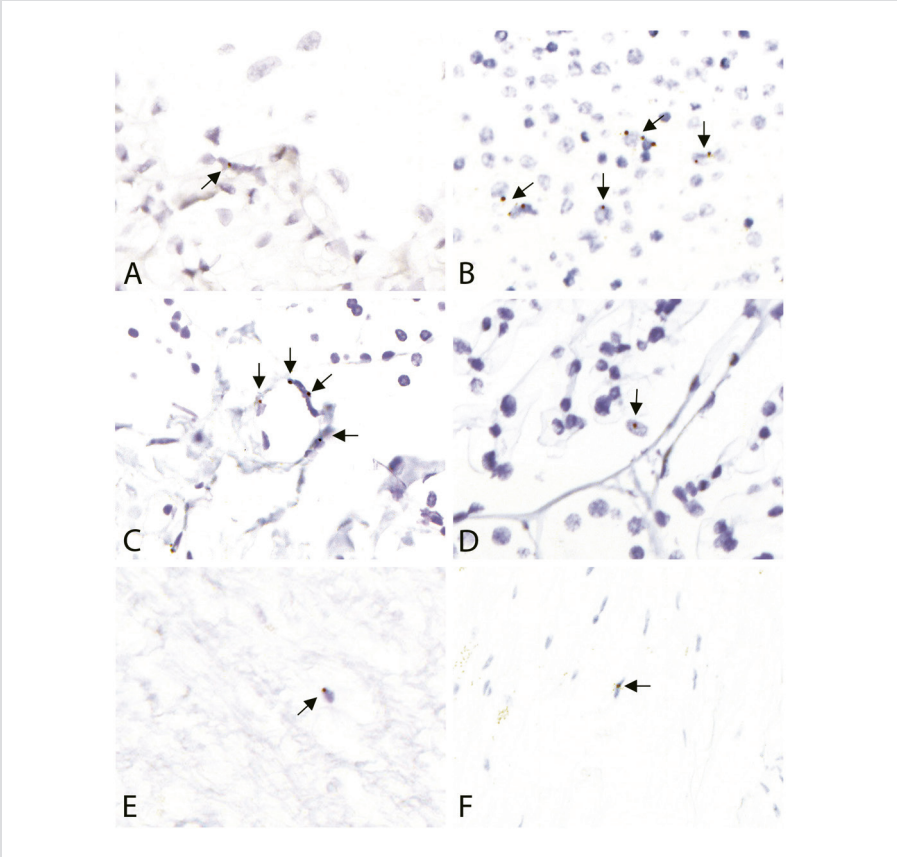
Double staining was performed on tissue sections showing high positivity after in situ hybridization of the Y chromosome from 2 lungs, 1 spleen, 1 liver, 2 kidneys, 1 heart, and 1 brain. In the lung, spleen, and kidney, some of the chimeric cells were CD3+, and in the lung, kidney, and liver, some were CD34+ (Figure 3). Also, following both double staining procedures, chimeric cells were found that did not show positivity for CD3 or CD34. In some instances, CD34+ chimeric cells lined a vascular wall, which could indicate that they are endothelial cells. Some of the CD34+ chimeric cells were found alone, which could imply that they have a stem cell phenotype.

Figure 1 Graph showing the numbers of Y chromosome-positive cells in maternal organs.



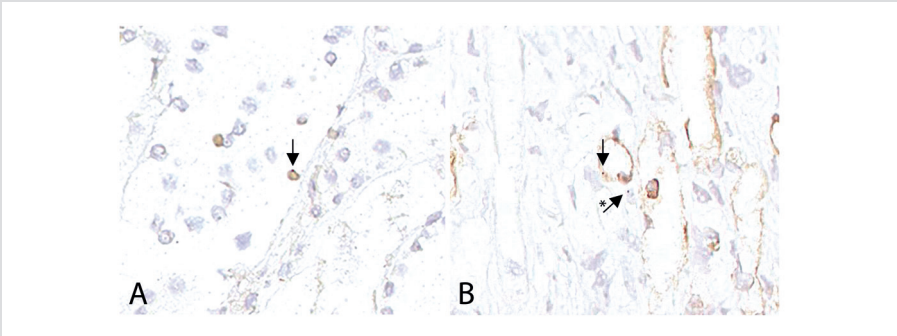
Lungs contained significantly more Y chromosome-positive cells corrected for cell density than any of the other organs studied (lung vs. any organ: $P < 0.001$). * $P < 0.05$. Horizontal lines represent median values, boxes represent 25th and 75th percentiles, whiskers represent 10th and 90th percentiles, circles represent outliers.

Figure 2 Y chromosome-positive nuclei in the lung (A), spleen (B), liver (C), kidney (D), brain (E), and heart (F).



Arrows indicate positively scored nuclei.

Figure 3 Double-staining by combination of in situ hybridization of the Y chromosome and immunohistochemical staining for CD3 and C34.



Cells that were both Y chromosome-positive and CD3+ (A) or CD34+ (B) were identified in kidney sections (A and B), as well as lungs, spleens, and livers (not shown). Y chromosome-positive, but CD3- (not shown) or CD34- (B, *) cells were also found after double staining.

Tissue microchimerism in relation to injury

The sections used for the in situ hybridization were given injury scores by a pathologist, who identified 15 out of 19 pulmonary sections with signs of active injury (congestion and inflammatory infiltrates), while none of the pulmonary sections had chronic injury. Of the splenic sections, six out of 15 had signs of acute congestion while none had chronic injury. A total of 15 out of 19 hepatic sections had active injury (congestion and inflammatory infiltrates), and two out of 19 had chronic injury (steatosis). Three out of 19 renal sections with active injury were identified (lymphocytic infiltrate and erythrocytes in tubuli), as well as three out of 19 sections with chronic injury (calcifications). From the hearts, five out of 17 sections contained active injury (acute ischemia), and one of 17 sections showed chronic injury (fibrosis). From the brains, one out of five sections was identified with active injury (Purkinje cell degeneration). Neither the active injury score nor the chronic injury score was significantly correlated with tissue microchimerism (as expressed by number of Y chromosome-positive nuclei per million) when all organs were analysed together (active injury: $p=0.077$, $P=0.5$; chronic injury: $p=0.017$, $P=0.9$). Active and chronic injury scores did not significantly correlate with quantities of microchimerism in organs studied individually, except for a significant correlation between active injury score and tissue microchimerism in the kidney ($p=0.475$, $P=0.04$).

Microchimerism in relation to pregnancy-related characteristics

The number of previous pregnancies did not associate with the amount of microchimerism ($P=0.9$). Women who died during pregnancy had on average more microchimerism than women who died after delivery; however, this difference failed to reach statistical significance ($P=0.4$). No relationship was found between gestational age and the amount of microchimerism ($P=0.7$), and the presence of preeclampsia/eclampsia did not differ relative to the amount of microchimerism ($P=0.6$). Only one woman had a known history of blood transfusion; therefore, the relationship between tissue microchimerism and blood transfusion history could not be evaluated. Women with a vaginal delivery had on average more microchimerism than women who delivered by Caesarean section; however, this difference was not statistically significant ($P=0.1$).

DISCUSSION

In this study, we investigated the frequency and distribution of chimeric cells in organs in a distinctive cohort of women who died during or shortly after pregnancy and of whom autopsy material was available. Male microchimerism occurred significantly more often in lungs, spleens, livers, kidneys, and hearts of women pregnant with sons than in non-pregnant women who had given birth to at least one son. We showed that some of the chimeric cells were CD3+ or CD34+. Moreover, we found a distribution pattern of microchimerism across organs of pregnant women that was similar to that identified in pregnant mice, with the lung being the organ in which microchimerism presented most

often and in greatest abundance.¹⁹

If microchimerism were mainly pregnancy-derived, one expected implication would be a negative correlation between the postpartum interval and the amount of tissue microchimerism. Moreover, because of the circulatory anatomical relationship between mother and fetus, the lung would be the candidate organ for having the highest amounts of microchimerism, as it contains the first capillary bed through which blood from the placenta passes. The current study has yielded two important observations that support the hypothesis that tissue microchimerism is mainly pregnancy-derived. First, tissue microchimerism occurs with higher frequency during pregnancy than in the years after pregnancy and is thus negatively correlated with the postpartum interval. Second, the lung harbours significantly more microchimerism than any other organ type after correction for cell density.

It is fascinating that we found the same distribution pattern of microchimerism across organs in our study in humans as in mice during pregnancy,¹⁹ with the lungs being the organs in which microchimerism presented most often and in greatest abundance, followed by the spleen, liver, kidney, and lowest amounts of microchimerism found in the heart and brain. Because we corrected our data for cell density, they are comparable to the flow cytometry results for mice.¹⁹ In mice, frequencies of microchimerism as measured by flow cytometry targeting GFP ranged from 0 to 60 GFP-positive cells per million cells. In our study, the range of observed frequencies of Y chromosome-positive cells was wider by a factor 20, from 0 to 1200 Y chromosome-positive cells per million cells. This difference could be explained by differences in pregnancies between mice and humans, such as duration, litter size, and uterine anatomy, or by variation in the sensitivity of the detection methods. In our study, the amount of microchimerism is likely underestimated because not all nucleated male cells will give a Y chromosome-positive signal: in histologic 4 μ m thick sections, the Y chromosome will be present in approximately 58% of all nuclei.⁹ Also, detection of tissue microchimerism from other sources in our study is limited to microchimerism derived from males. Microchimerism was also underestimated by Fujiki *et al.* using GFP in mice as a marker for microchimerism, as on average 48% of pups expressed GFP.

Several explanations are possible for the distribution of microchimerism across organs. One is that fetal cells are passively trapped in the lungs, which contain the first capillary bed that cells must cross after entering the uterine venous circulation. Another possible explanation is that the distribution of chimeric cells reflects blood flow, as expressed by percentage of cardiac output received. In fact, the fractional distribution of cardiac output corresponds to the pattern of distribution of chimeric cells across the organs of pregnant women, with lungs receiving 100% of cardiac output, followed by the liver (22.7%), kidneys (17.5%), brain (11.4%), and heart (4.0%).²³ Finally, chimeric cells could be actively accumulated in specific tissues because of favourable microenvironments, much like the “seed and soil” theory in cancer studies.²⁴ Accordingly, the high prevalence of microchimerism in the spleen and liver may arise from the sequestration of chimeric cells in these tissues.

In mice, allogenic fetal cells migrate to the spleen during pregnancy and may continue proliferation in the spleen long afterward.²⁵ Numerous studies in humans and mice have shown a preferential homing of chimeric cells to sites of injury.²⁶⁻²⁹ Pregnancy-associated cardiovascular events often have significant effects on target tissues, such as the lung.³⁰ It could therefore be argued that the high prevalence of microchimerism in lungs of deceased pregnant women is due to injury. A significant number of our cases died of vascular causes such as pulmonary embolism or preeclampsia, in which pulmonary injury could be anticipated. Indeed, 15 out of the 19 pulmonary sections contained signs of active injury. However, correlations between quantities of chimeric cells corrected for cell density with active injury scores were not significant in the lung sections analysed. The only organ with a significant correlation between tissue microchimerism and active injury scores was the kidney. Of interest, in gentamicin-induced kidney injury, fetus-derived chimeric cells engraft in the kidney as tubular cells, suggesting a role in the repair process.²⁷ In the setting of lupus nephritis, a repair function of microchimerism was recently supported by the demonstration of higher amounts of microchimerism in patients with better renal function.³¹ It is therefore conceivable that the kidney in particular is an organ prone to repair in the setting of microchimerism.

Chan *et al.* recently reported the existence of male microchimerism in the brains of 37 out of 59 (63%) autopsied women as measured by real-time quantitative PCR, but the women included in that study were not pregnant and had an unknown reproductive history.³² To our knowledge, we are the first to describe male microchimerism in the brains of pregnant women, having identified it in all 5 women studied. Because of small sample size, we cannot draw conclusions on increased prevalence of microchimerism in the brain during pregnancy. Changes in the permeability of the blood-brain barrier have been described during pregnancy and could therefore explain the establishment of a fetal cell population in the maternal brain.³³ In mice, Fujiki *et al.* did not observe fetal cells in the brain by flow cytometry.¹⁹ However, others using PCR have detected GFP+ fetal cells in the brains of mouse dams, resembling perivascular macrophages, neurons, astrocytes, and oligodendrocytes.^{34, 35}

Our results support a role for microchimerism in several pregnancy-related conditions, including autoimmune disease, pregnancy complications, and certain malignancies. In the setting of autoimmune disease, the difference between men and women in the incidence of autoimmune diseases makes pregnancy-derived microchimerism an intriguing candidate for causing these diseases. It has been suggested that chimeric CD3+ cells have a pathogenic role in systemic sclerosis by inducing a graft-versus-host-like reaction.³⁶ Because we found CD3+ chimeric cells in the tissues of pregnant women, it is possible that acquisition of these cells during pregnancy forms the basis for development of autoimmune diseases in which chimeric T cells are present. On the other hand, fetal CD34+ chimeric cells have been found in the circulation and tissues of healthy women many years after pregnancy, without the onset of autoimmunity,^{6, 37} and may therefore also be seen as innocent by-

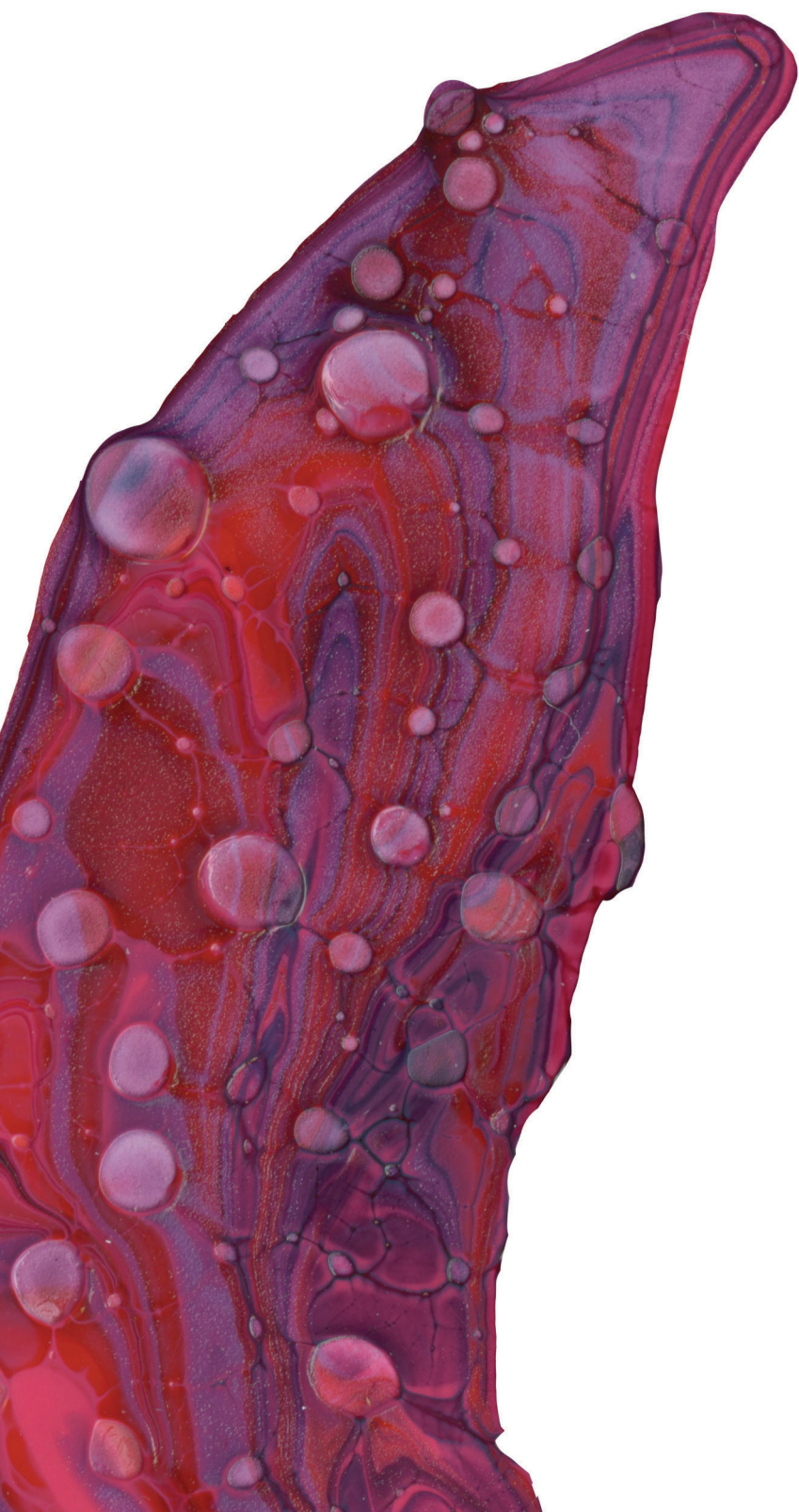
tanders. Further study is necessary to unravel the impact of fetal cell microchimerism on maternal health and should include analysis of microchimerism in different immunologic background strains of mice.

Concluding, we are the first to demonstrate the abundance of microchimerism in different tissues of pregnant women. Because we had a unique opportunity to study a group of women who died while pregnant or shortly after delivery, we could demonstrate that during pregnancy, a boost of pregnancy-derived chimeric cells is indeed observed in women, with a distribution across organs paralleling the distribution identified in a previous study in mice. Whether the distribution of chimeric cells across different organs reflects the occurrence of disease in these organs, as well as the immunologic mechanisms involved in the pathogenesis, will be the subject of ongoing research.

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Chapter 8

Increased Microchimerism in Peripheral Blood of Women with Systemic Lupus Erythematosus

Suzanne Wilhelmus, Emilie C. Rijnink, Malu Zandbergen, Juan D.N. Diaz de Pool,
Mathilde M. Almekinders, Jo H.M. Berden, Marc Bijl, Ernst C. Hagen,
Gerda M. Steup-Beekman, Hans J. Baelde, Jan A. Bruijn, Ingeborg M. Bajema

Submitted

ABSTRACT

Background

Microchimerism may play a role in the pathogenesis of systemic lupus erythematosus (SLE). Using an innovative technique permitting more sensitive and specific detection of chimeric cells than previous studies, we aimed to determine the origin and amount of microchimerism in peripheral blood of women with SLE and controls.

Methods

We investigated the relationship between microchimerism and disease onset, activity, and damage accrual. We included 11 SLE patients and 22 controls, their children, and if possible, their mothers. Quantitative PCR for insertion-deletion polymorphisms and null alleles was used to detect microchimerism in peripheral blood mononuclear cells and granulocytes.

Results

Microchimerism was detected more often in patients than controls (54.4% vs. 13.6%, $P=0.03$). In 50% of SLE patients with microchimerism, it originated from multiple relatives, whereas in controls microchimerism was always derived from one relative. Microchimerism was mostly of fetal origin, and the median number of fetal chimeric cells was $5/10^6$ in patients and $2.5/10^6$ in controls ($P=0.048$). We found no relationship between microchimerism and clinical or laboratory parameters.

Conclusions

Apart from demonstrating that microchimerism occurs more frequently in SLE patients than in controls, this study provides novel, thought-provoking evidence that microchimerism in SLE can be derived from multiple relatives.

INTRODUCTION

Microchimerism refers to the presence in an individual of a small number of genetically distinct cells of any type, originating from a different zygote. The most common (physiologic) source of microchimerism is pregnancy.¹ During pregnancy, fetal cells can enter the maternal circulation leading to fetal microchimerism in the mother. When maternal cells cross the placental barrier to the fetus, this can lead to maternal microchimerism. Pregnancies of all terms, including both miscarriages and pregnancies resulting in (live) birth, may lead to microchimerism.²⁻⁵

The role of microchimerism in health and disease is unclear. Microchimerism has been suggested to play a role in several autoimmune diseases, including systemic lupus erythematosus (SLE).⁶⁻⁹ SLE is an autoimmune disease that mainly affects women and has a peak incidence in the reproductive years.¹⁰ Studies in mice showed that, in selected parent-to-F1 combinations, injection of parental lymphocytes in their offspring led to a graft-versus-host response and a lupus-like disease.^{11, 12} These data suggest that pregnancy-acquired microchimerism may be of pathogenic significance in the development of SLE.

Women with SLE have a significantly higher prevalence of fetal Y chromosome-positive chimeric cells in tissue than healthy controls.¹³⁻¹⁵ A number of studies showed that also in peripheral blood, there is an increased frequency of fetal microchimerism in SLE patients compared to controls,^{16, 17} whereas other studies showed no differences between patients and controls.^{18, 19} Previous studies on fetal microchimerism in SLE were limited to the detection of male microchimerism, thereby underestimating the total amount of microchimerism. Furthermore, because microchimerism was mostly studied in whole blood, the phenotype of the chimeric cells could not be determined. Maternal microchimerism in SLE in peripheral blood was studied by Kanold *et al.*, and they did not find a difference between patients and controls.²⁰ However, their sensitivity of detecting chimeric cells was relatively low. In none of these studies were fetal and maternal microchimerism investigated together.

The aim of our study was to determine the presence and amount of microchimerism in peripheral blood of SLE patients and compare it to healthy controls. We studied peripheral blood mononuclear cells (PBMCs) and granulocytes separately to determine if microchimerism was present in either subset, or both. In our study we used insertion-deletion polymorphisms (indels) or null alleles for the detection of microchimerism, thus enabling us to study the origin of the chimeric cells: either fetal, maternal, or both. Also, we were able to establish whether microchimerism was derived from one relative or from multiple relatives. Furthermore, in order to find a clue as to the role of microchimerism in SLE, we investigated the relationship between disease activity and accumulated damage since the onset of SLE, and the presence of microchimerism. Finally, the temporal relationship between the chimerism-causing pregnancy and disease onset in SLE patients was studied.

METHODS

Ethics statement

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center (LUMC) (P09.047). All research was conducted in accordance with the ethical research standards of the LUMC and in compliance with the Declaration of Helsinki. Informed consent was obtained from all participants. The parents of minors gave written consent on their behalf.

Patients and controls

Eleven female SLE patients and 22 female controls were studied. From 2010 to 2015 SLE patients were recruited from four hospitals in the Netherlands: University Medical Center Groningen, Radboud University Medical Center Nijmegen, Bronovo Hospital The Hague, and Meander Medical Center Amersfoort. All included patients fulfilled at least four of the 1998 revised American College of Rheumatology Criteria for the classification of SLE.²¹ SLE disease activity was determined using the SLE Disease Activity Index 2000 (SLEDAI-2K).²² Accumulated damage since SLE onset was measured using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI).²³ The control group consisted of women without a history of autoimmune disease. For inclusion in the study, probands (SLE patients and controls) were required to have at least one child of at least 18 years old. The probands' children, as well as their mothers, were invited to participate. Peripheral blood samples were gathered from the probands, as well as either peripheral blood samples or buccal mouth swabs from their children and mothers. All probands were asked to fill out a questionnaire including their age, ethnicity, reproductive history, history of blood transfusion, use of immunosuppressive medication, and medical history.

Isolation of peripheral blood subsets

Peripheral venous blood samples were drawn in sodium-heparin solution vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) and processed to isolate peripheral blood mononuclear cells (PBMCs) by Ficoll amidotrizoate (pharmacy LUMC) with density gradient centrifugation 1.077 g/mL. Erythrolysis (Qiagen, Hilden, Germany) was performed to remove the erythrocytes from the remaining granulocytes. Samples were stored in 10% dimethyl sulfoxide in fetal bovine serum at -180°C until DNA extraction.

DNA extraction

DNA was extracted from PBMCs and granulocytes using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions with modifications. Briefly, we added 40 μL of proteinase K to 5×10^6 cells suspended in 200 μL phosphate-buffered saline. After adding 400 μL AL buffer, the suspension was incubated for 30 min (PBMCs) or overnight (granulocytes) at 56°C . After adding 200 μL of ethanol, the mixture was applied to the Mini spin column. Buffers AW1 and AW2 were used to wash the column,

after which 100 µl AE buffer was added and incubated at 70 °C for 10 minutes to elute the DNA. The eluate was reapplied for an optimal yield. All DNA samples were stored at 4 °C until quantitative PCR (qPCR). DNA extraction from buccal sterile OmniSwabs (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) was performed with the same kit and according to the manufacturer's instructions.

Allele informativity and genotyping

For the detection of fetal and maternal microchimerism a set of previously published indels and null alleles was used.²⁴⁻²⁶ In order to detect fetal and maternal microchimerism in the proband, and to discriminate between the proband's children and mother, informative alleles to distinguish the different family members were required. Maternal DNA was available for six of 11 patients and eight of 22 controls. There was no fetal DNA available for any of the miscarriages. Genotyping by qPCR was performed using the same protocol as described below, but with a DNA input of 20 ng. Of the published sets of null alleles and indels, 19 were informative in our study population: GSTM1, GSTT1, SRY, RhD (null alleles), and S01a, S01b, S03, S04a, S04b, S05b, S07b, S08b, S09b, S10a, S10b, S11a, S11b, Xq28 and R271 (indels). The primer sequences are listed in **Appendix 8.1**.

Detection of chimerism by qPCR

Fetal and maternal microchimerism were detected and quantified by qPCR. In all assays iQ SYBR Green Supermix (Bio-Rad, Hercules, California) was used, with 7.5 µM of each amplification primer. The amplification and melting conditions for all primers consisted of incubation at 96.5 °C for 10 min, followed by 44 cycles of 96.5 °C for 30 s and 60 °C for 1 min. The melting curve started at 65 °C for 5 s followed by 0.2 °C incremental increase, each lasting 5 s, to 95 °C. Amplification and melting data were collected by a Bio-Rad CFX96 detector and analysed by Bio-Rad CFX Manager version 3.1.

Primer specificity was ensured by Sanger sequencing of the amplification product and comparing the sequences to known genomic DNA sequences. Sensitivity was determined by testing serial dilutions of DNA positive for the indel or null allele in a background of DNA negative for the respective indel or null allele. A sensitivity of one genome equivalent (gEq, based on 6.6 pg DNA content per cell) in 100,000 gEq was reached for all primersets. Four aliquots, each containing 660 ng DNA (100,000 gEq), were tested in each subset (PBMC or granulocytes) for every proband. A standard curve for the specific assay was included to quantify the chimeric cells and validate the assay on each plate. The standard curve consisted of 100, 10, and 1 gEq spiked DNA per 100,000 gEq background DNA. Every sample was also tested for the housekeeping gene GAPDH. Results were expressed as the gEq of chimeric cells per one million gEq (gEq/10⁶). Negative controls consisting of either a water control or background DNA not carrying the indel or null allele tested, were included in each qPCR plate. Negative controls were consistently negative across all experiments. If there was any doubt as to the specificity of the amplification product, the length of the PCR product was compared to that of the positive control using QIAxcel Advanced System (Qiagen) according to the manufacturer's protocol.

Anti-contamination procedures

Strict anti-contamination procedures were employed during blood workup, DNA extraction, and qPCR preparation. Aerosol-resistant pipette tips and clean gloves were always used. The blood workup was performed in a laminar flow cabinet. Before DNA extraction or preparation of the qPCR, the cabinet used was thoroughly cleaned with DNA decontamination reagent (Sigma-Aldrich, St. Louis, Missouri), and irradiated with UV light for one hour. All lab consumables were certified DNA free, and also irradiated with UV light for one hour. For the qPCR, 8-well strips with individual lids were used.

Statistical analysis

For the comparison of categorical data, Fisher's exact tests were used (history of blood transfusion, presence of microchimerism). Student's *t*-tests were used to compare normally distributed data (age proband, age eldest child, age youngest child, SDI). For the comparison of non-normally distributed numerical data, Mann-Whitney U tests were used (number of pregnancies, number of children, number of chimeric cells, SLEDAI-2K). A *P*-value <0.05 was considered statistically significant. All analyses were performed using SPSS Statistics 20.0 (IBM, Armonk, New York).

RESULTS

Baseline characteristics of patients and controls are shown in **Table 1**. SLE patients had microchimerism more often than controls (54.4% vs. 13.6%, *P*=0.03). When microchimerism was present, the median total number of fetal chimeric cells per proband was higher in the patient group than in the control group (5 gEq/10⁶ vs. 2.5 gEq/10⁶ [*P*=0.048]) (**Table 2**). When comparing patients and controls with and without microchimerism, there was no difference in age, number of children, number of pregnancies, history of blood transfusion, disease activity (SLEDAI-2K) or accumulated damage since disease onset (SDI) (**Table 3**). Also, there was no difference in the use of immunosuppressive medication between patients with and patients without microchimerism (data not shown).

All patients and controls, except one, with detectable microchimerism had fetal microchimerism (**Table 4**). Of the eight controls with maternal DNA available, one had detectable maternal microchimerism. Of the six SLE patients with maternal DNA available, one had detectable maternal microchimerism, three did not, and of two the possible maternal microchimerism was indistinguishable from the fetal microchimerism that was present, due to an overlap in indels and null alleles.

Table 1 Baseline characteristics of SLE patients and controls.

Characteristic	SLE patients (n=11)	Controls (n=22)	P
Age proband, y	56.6 ± 5.5	57.2 ± 5.5	0.79 ^a
Age eldest child, y	31.4 ± 5.2	28.9 ± 5.7	0.24 ^a
Age youngest child, y	27.5 ± 5.3	24.4 ± 4.9	0.11 ^a
Number of children	2 (1)	2 (1)	0.60 ^b
Number of pregnancies	3 (2)	2.5 (1)	0.37 ^b
History of blood transfusion, %	72.7	13.6	0.001 ^c
SLEDAI-2K	0 (4)	-	n/a
SDI	2.2 ± 2.3	-	n/a

Results are shown as mean ±SD or as median (interquartile range), unless otherwise specified. P-values were assessed with ^aStudent's t-test, ^bMann-Whitney U test, or ^cFisher's exact test. SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Table 2 Microchimerism in SLE patients and controls.

Parameter	SLE patients (n=11)	Controls (n=22)	P
Microchimerism present in PBMCs or granulocytes, %	54.5	13.6	0.03 ^a
Microchimerism present in PBMCs, %	36.4	9.1	0.15 ^a
Microchimerism present in granulocytes, %	40.0 (n=10)	10.0 (n=20)	0.14 ^a
Total number of fetal chimeric cells/proband, when microchimerism is present, gEq/10 ⁶ gEq	5 (8.1) (n=6)	2.5 (n/a) (n=3)	0.048 ^b

Results are shown as median (interquartile range), unless otherwise specified. P-values were assessed with ^aFisher's exact test or ^bMann-Whitney U test. gEq, genome equivalents; n/a, not applicable because number of cases is too low to provide an interquartile range; PBMCs, peripheral blood mononuclear cells; SLE, systemic lupus erythematosus.

Table 3 Comparison of SLE patients and controls with and without microchimerism.

Characteristic	SLE patients			Controls		
	No micro-chimerism (n=5)	Micro-chimerism (n=6)	P	No micro-chimerism (n=19)	Micro-chimerism (n=3)	P
Age proband, y	57.4 ± 5.2	56.0 ± 6.3	0.70 ^a	57.6 ± 5.6	54.3 ± 4.3	0.33 ^a
Number of children	2 (1)	2.5 (1.25)	0.84 ^b	2 (1)	3 (n/a)	0.44 ^b
Number of pregnancies	2 (1.5)	3.5 (2)	0.33 ^b	2 (1)	3 (n/a)	0.71 ^b
Blood transfusion, %	60.0	83.3	0.55 ^c	10.5	33.3	0.37 ^c
SLEDAI-2K	0 (2.5)	2 (5.5)	0.37 ^b	-	-	-
SDI	1.8 ± 2.0	2.5 ± 2.6	0.64 ^a	-	-	-

Results are shown as mean ± SD or as median (interquartile range), unless otherwise specified. P-values were assessed with ^aStudent's t-test, ^bMann-Whitney U test, or ^cFisher's exact test. n/a, not applicable because number of cases is too low to provide an interquartile range; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Table 4 Origin of microchimerism in patients and controls.

Subject	Relatives ^a	Chimerism in PBMCs	
		Origin	Amount, gEq/10 ⁶
Patients			
1	Mother; daughter 1, miscarriage (n/a), daughter 2, son	Mother	16.5
		Daughter 1 or 2	2.5
		Son	2.5
2	Mother; daughter 1, 2 and 3	Daughter 1	5
3	Mother; daughter; son	Son	2.5
		-	-
4	Mother (n/a), miscarriages 1, 2 and 3 (n/a), daughter	-	-
5	Mother (n/a), daughter 1 (deceased, n/a), miscarriage (n/a), daughter 2 (n/a), daughter 3	-	-
6	Mother (n/a), son, daughter	Daughter	5
Controls			
1	Mother (n/a), daughter 1 and 2, son	Daughter 2	2.5
2	Mother (n/a), daughter; son	-	-
3	Mother; daughter 1, daughter 2, son	Mother	2.5

^aChronologically from old to young based on year of birth/miscarriage. ^bdue to technical problems with the material from the first blood draw, a second blood draw was done approximately 1 year later; gEq, genome equivalent; n/a, no DNA available; PBMCs, peripheral blood mononuclear cells; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

Also, we determined if the microchimerism originated from one relative or more. In all three controls with detectable microchimerism, it originated from one relative (either one child or mother). In contrast, in at least three of the six SLE patients the microchimerism originated from more than one relative, either from multiple children or from a child and mother (Table 4).

Finally, in patients with detectable microchimerism, we did not find a pattern in the temporal relationship between chimerism-causing pregnancies and the start of symptoms or diagnosis of SLE. One patient had her first symptoms prior to her first pregnancy, and experienced an exacerbation of symptoms during her first pregnancy, which resulted in a spontaneous miscarriage. Two patients experienced their first symptoms during their second pregnancy. In one of these patients, this pregnancy resulted in a spontaneous miscarriage. Therefore, it could not be determined if this pregnancy resulted in long-lasting microchimerism. In the other of the two patients, both her first and second pregnancy resulted in long-lasting microchimerism. Finally, three of six patients experienced their first symptoms one, seven, and ten years after the birth of their youngest child of whom microchimerism was detected.

Chimerism in granulocytes <i>Origin</i>	<i>Amount, gEq/10⁶</i>	Blood transfusion history	SLEDAI-2K	SDI
n/a	n/a	Yes	10	4
Daughter 3	7.5	Yes	4	0
Daughter 2 or mother	2.5			
Possibly daughter 1				
Daughter, and possibly mother	2.5	Yes	0 (1 st blood draw ^b)	2
-			0 (2 nd blood draw ^b)	2
Daughter	15	Yes	4	7
Daughter 3	5	Yes	0	1
-	-	No	0	1
Daughter 2	2.5	Yes	-	-
Daughter	2.5	No	-	-
-		No	-	-

DISCUSSION

Our study demonstrates that female SLE patients have detectable microchimerism in their peripheral blood more often than female controls. In almost all cases with detectable microchimerism, the origin of the chimeric cells was fetal. Additionally, in one patient and one control, maternal microchimerism was detected. The median total number of fetal chimeric cells in individuals with detectable microchimerism was higher in patients than in controls. Also, SLE patients often had chimeric cells originating from multiple relatives, in contrast to the controls, in whom the chimeric cells originated from only one relative.

In accordance with our results, two previous studies found fetal microchimerism in peripheral blood more often in SLE patients than in controls,^{16, 17} while two other studies did not.^{18, 19} Differences in the blood compartment tested (PBMCs or whole blood), the specificities and sensitivities of the different techniques used, and the numbers of patients and controls included, may account for these conflicting results. In contrast to our study, previous studies did not demonstrate a difference between SLE patients and controls

in the number of chimeric cells present. However, a limitation of these studies was that exclusively the presence of the Y chromosome was investigated, thereby only detecting male microchimerism. Our approach allowed us to detect both male and female fetal microchimerism, as well as maternal microchimerism. If, in our present study, we had only investigated microchimerism using the Y chromosome in women with at least one son, we would not have found a statistically significant difference in the occurrence of microchimerism between patients and controls (data not shown). Only one study investigated the presence of maternal microchimerism in SLE and found no difference between patients and controls with maternal microchimerism occurring in 6% and 3%, respectively.²⁰ This low prevalence of maternal microchimerism is in accordance with our results.

In the literature, there are indications that within one individual some sources of microchimerism lead to persistent microchimerism, while others do not. In one case report about a woman with hepatitis C, the detected chimerism in the liver seemed to originate from only one of her five pregnancies.²⁷ Also, after blood transfusions, it was shown that in the majority of cases with transfusion-associated microchimerism, there was evidence of only one or two non-recipient HLA-DR alleles, suggesting that the microchimerism commonly involves only one donor despite some patients receiving blood products from multiple donors.²⁸ However, in women with multiple children, it has not been systematically studied if there is a “favored-child” with regard to the persistence of microchimerism, i.e. if fetal microchimerism usually originates from one of the children, or from more children. Because we used indels and null alleles for the detection of microchimerism, we were able to show that at least half of the patients had persistent microchimerism from multiple relatives while all controls only had persistent microchimerism from one relative. The cause of this phenomenon is largely unknown. Studies in animals have demonstrated that syngenic or congenic matings resulted in more chimerism than allogenic matings, suggesting a role for HLA (mis)matches.^{29,30} In humans, in certain autoimmune diseases mothers and children were shown to have fewer HLA disparities,^{31,32} but these have not yet been significantly correlated to the presence of microchimerism.³¹ Nevertheless, having a certain HLA allele (HLA DQA1*0501) appears to be associated with the presence of fetal microchimerism.³³ Interestingly, HLA DQA1*0501 has also been associated with SLE.³⁵

The phenotype of a chimeric cell may affect the potential of a chimeric cell to result in to persistent microchimerism. We detected microchimerism in both PBMCs and granulocytes. Considering the relatively short half-life of granulocytes,³⁶ it is likely that the chimeric cells detected in this compartment are derived from stem cells. The existence of chimeric fetal progenitor cells was demonstrated in several studies (reviewed by Seppanen *et al.*³⁷). A higher prevalence of microchimerism in SLE patients than in controls can either mean that (i) more chimeric cells were acquired during pregnancy, (ii) more chimeric cells persisted after pregnancy, (iii) chimeric stem cells gave rise to more chimeric cells due to an unknown trigger, or (iv) a combination of aforementioned possibilities.

SLE patients had a blood transfusion in their history significantly more often than controls. However, within the groups of SLE patients and controls, we did not find a difference in blood transfusion history between subjects with and subjects without detectable microchimerism. In the literature, persistent chimerism was only described after blood transfusion following traumatic injury (reviewed by Bloch *et al.*³⁸), which was not the indication for a blood transfusion in any of our subjects. Furthermore, a recent study in patients having received a blood transfusion in the peripartum period, like some of our subjects, did not show microchimerism at six weeks and six months after pregnancy.³⁹ Therefore, it is unlikely that the difference in blood transfusion history between patients and controls explains our results.

In our study there was no difference in disease activity (SLEDAI-2K) or accumulated damage (SDI) between patients with and without microchimerism. The former result is in line with a previous study.¹⁹ Possible reasons for these findings are the absence of such associations or the small sample size. Also, many of the SLE patients were in clinical remission.

A limitation of our study is that we did not have maternal DNA available for all subjects. This means that we could not exclude a maternal source of the microchimerism in all cases. In a few patients of whom we did have maternal DNA available, it was not always possible to distinguish maternal microchimerism from the detected fetal microchimerism, due to an overlap in genetic markers. Furthermore, it was not possible to formally exclude all possible sources of microchimerism, such as unrecognised pregnancies or spontaneous abortions of which DNA was unavailable.

In summary, we detected microchimerism in peripheral blood more often and in higher numbers in female SLE patients than in female controls. The microchimerism detected was predominantly fetal in origin and was found in both PBMCs and granulocytes. Interestingly, this study provides the first evidence that SLE patients can have chimeric cells from multiple relatives, while all of the chimeric controls had chimeric cells from only one relative. Attempts to explain this phenomenon are speculative. It may depend on the immune response evoked by specific chimeric cells, possibly relating to HLA, or on the activation status of the immune system of the recipient in general. Future studies addressing the immunologic aspects of this phenomenon are called for. The exact role of chimeric cells in SLE is still unknown, but our data substantiate the hypothesis that chimeric cells do play a role in SLE.

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Chapter 9

SUMMARY AND GENERAL DISCUSSION

SUMMARY

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease causing damage in multiple organ systems. As a consequence, the clinical manifestations and outcomes of SLE are remarkably heterogeneous. In this thesis, issues relating to the diagnosis and prognosis of SLE were studied, focusing on the application of histopathologic evaluation in conjunction with clinical features in the setting of lupus nephritis (LN) and neuropsychiatric SLE (NP-SLE) in an effort to advance personalised medicine in SLE.

Given the protean manifestations of SLE, the list of differential diagnoses is extensive. Traditionally, classification criteria for SLE have been developed to distinguish patients with SLE encountered in rheumatology clinics from other patients with mainly rheumatic diseases. Given the heterogeneity of SLE, it is not unthinkable that the performance of these criteria in patients presenting at nephrology clinics is different. In the first part of this thesis, issues concerning the diagnosis of LN were investigated in the setting of nephritis patients with full house glomerular immune deposits – a finding considered to be very characteristic of LN.

In **chapter 2**, the diagnostic performance of the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria as compared to the American College of Rheumatology (ACR) criteria was investigated in a cohort of patients with full house glomerular deposits, aiming to resolve whether such patients can reliably be classified as having SLE using the SLICC criteria. Hundred forty-nine patients with full house glomerular immune deposits were identified, 117 of whom had clinical SLE, and 32 had membranous nephropathy (anti-PLA2R-positive, $n=1$; cancer-related, $n=3$), IgA nephropathy ($n=4$), infection-related glomerulonephritis ($n=2$), anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis ($n=2$), and idiopathic non-lupus full house nephropathy ($n=20$). The SLICC classification proved to be useful with regard to diagnostic sensitivity among these patients, since all 117 patients with clinical SLE fulfilled the SLICC classification criteria. The ACR criteria had a lower sensitivity in this setting (95%), the difference being that the SLICC criteria include hypocomplementaemia, whereas the ACR criteria do not. However, of the 117 SLE patients, none were classified as SLE according to the SLICC criteria because of its “stand-alone” criterion that allows classification of SLE in patients with biopsy-confirmed LN in combination with antinuclear or anti-double stranded DNA antibodies. Of the 32 patients with biopsies showing full house glomerular deposits and findings by light microscopy consistent with LN without clinical SLE, three met the SLICC criteria for SLE because of its “stand-alone” criterion, and none met ACR criteria. These findings support the usefulness of SLICC criteria with regard to diagnosing SLE among patients with nephritis, although the superior performance of the SLICC criteria (compared to the ACR criteria) was attributed to hypocomplementaemia as a criterion in the SLICC classification and not due to the “stand-alone” criterion.

Chapter 3 involves the same cohort of patients with full house glomerular immune deposits as was investigated in chapter 2. Chapter 3 is focused on the distinction between patients with nephritis and a renal biopsy showing full house glomerular immune deposits in the setting of clinically confirmed LN (lupus full house nephropathy [FHN]) and patients with this finding who do not have SLE and for whom the cause of full house immune deposits is idiopathic (idiopathic non-lupus FHN). In this chapter, a special focus is on the clinical, histopathologic, and prognostic differentiation between lupus and idiopathic non-lupus FHN, aiming to answer the question whether lupus and non-lupus full house nephropathy are clinically distinct entities. Of the 32 patients with non-lupus FHN, 20 patients had idiopathic non-lupus FHN, and in 12 patients secondary non-lupus FHN due to membranous nephropathy, IgA nephropathy, infection-related glomerulonephritis, and ANCA-associated glomerulonephritis was considered. Remarkable differences between lupus and idiopathic non-lupus FHN patients were noted: idiopathic non-lupus FHN patients were more frequently male, their renal biopsies more often showed a mesangial or membranous pattern of injury, and clinically they had more proteinuria, less erythrocyturia, and less complement consumption than lupus FHN patients. Most notably, multivariable analysis of patients with a LN class III or IV pattern of injury revealed that idiopathic non-lupus FHN compared to lupus FHN was an independent risk factor for end-stage renal disease. These results indicate that FHN is a pattern of renal injury most often encountered in SLE, but may also rarely be seen idiopathically as well as in a number of other diseases. The clinical recognition and distinction of idiopathic non-lupus FHN from lupus FHN is critical given the poor renal outcome of idiopathic non-lupus FHN.

Like SLE itself, renal manifestations of the disease are highly variable in their clinical presentation and outcome – as outlined in the second part of this thesis. This clinical variability parallels the broad spectrum of histopathologic abnormalities present in the renal biopsies of patients with LN. The glomerular histopathologic findings in renal biopsies of SLE patients are categorised according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification of LN. The most severe classes of LN – class III and class IV – are defined as renal biopsies showing fewer or more than 50% of glomeruli with endo- and/or extracapillary hypercellularity by light microscopy.¹ According to the current treatment guidelines for LN, patients with class III or IV LN – who are presumed to be at high risk of progression to end-stage renal disease – require aggressive immunosuppressive therapy consisting of a combination of corticosteroids and intravenous cyclophosphamide or mycophenolate mofetil. The categorisation of classes III and IV in the ISN/RPS classification reflects the notion that the severity and prognosis of LN are a function of the proportion of glomerular involvement and that there is a pathologic continuum from focal segmental to diffuse global glomerulonephritis. However, studies have shown that the lesions defining class III and IV LN themselves represent pathogenically heterogeneous lesions, with qualitatively and quantitatively different lesions of varied chronicity corresponding to different clinical outcomes.² The hypothesis that the definitions and assumptions inherent to the ISN/RPS classification may mask prognostic and pathogenic information

was investigated in chapters 4 and 5 of this thesis.

In **chapter 4**, the natural history of class III and IV LN was investigated, aiming to identify a subgroup of patients with a favourable prognosis eligible for treatment without cytotoxic immunosuppression. Hundred one patients with class III or IV LN were identified, three of whom did not receive cytotoxic immunosuppression according to guidelines. These three patients showed a favourable disease course during a follow-up period of 9–24 years. Importantly, the course of renal function during 10 years of follow-up in these three under-treated patients was not different from that of 98 patients with class III or IV LN from the same historic cohort who received guideline-recommended immunosuppression. These three patients provide evidence for the existence of a subgroup of patients with class III or IV LN with a favourable natural history. Thus, current guidelines risk overtreatment of LN patients by considering patients with class III or IV LN to be representative of a category uniformly requiring cytotoxic immunosuppression and by disregarding clinical parameters.

In **chapter 5**, the hypothesis that the lumped classes in the ISN/RPS classification may mask prognostic and pathogenic information was further explored by breaking down this classification and studying individual clinical and histopathologic variables in relation to renal outcome without preconceptions. Hundred five patients with class I–V LN were identified, and 50 histopathologic and 10 clinical variables were determined as candidate predictors for renal outcome. The results from this study clearly show that prognostication in LN may benefit from the specific assessment of lesions currently obscured in the classification. Normal glomeruli, cellular/fibrocellular crescents, fibrous crescents, and IF/TA were potent determinants of eGFR during follow-up. In addition, fibrous crescents, fibrinoid necrosis, and interstitial fibrosis or tubular atrophy predicted progressive renal function decline in the uniformly treated subset of patients with class III or IV LN. Importantly, these findings hint that these lesions were unresponsive, or incompletely responsive to the therapies given. Furthermore, clinical variables including ethnicity, age, renal function at baseline, and blood pressure may improve prognostication, warranting integration in clinical guidelines alongside the histopathologic classification of LN.

Whereas in LN knowledge about pathogenic mechanisms is central in establishing a histopathologic diagnosis and predicting the prognosis, little is known about the pathogenesis of NP-SLE. A major difficulty in the diagnosis of NP-SLE is the lack of clear diagnostic definitions, caused by a lack of pathognomonic features, inadequacy of diagnostic tools, and a vast heterogeneity of clinical symptoms. Due to the impracticability of performing a brain biopsy, histopathologic studies elucidating pathogenic mechanisms are limited, and a tissue diagnosis is generally not possible.

In the setting of NP-SLE, the complement system as a pathogenic mechanism in NP-SLE was investigated to provide a possible link between thromboischemic injury observed 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 NP-SLE was examined, and the association between complement and thromboischemic cerebral injury was assessed. Cerebral autopsy tissue was collected from SLE patients with and without NP-SLE, as well as from controls. Complement deposition was strongly associated with both SLE and NP-SLE, but not with controls. The results from this study demonstrate that histopathologic lesions in NP-SLE represent a continuum, ranging from nonspecific lesions such as focal vasculopathy, to more specific lesions including diffuse vasculopathy, microinfarction, macroinfarction, vasculitis, and C4d- and C5b-9-associated microthrombi related to clinical syndromes defining NP-SLE. Interestingly, the results from this study indicate that complement may be a key factor in the interaction between circulating autoantibodies and thromboischemic injury in NP-SLE and may have novel therapeutic potential. Furthermore, in one SLE and two NP-SLE patients, the correlation between cerebral post-mortem histopathology and ex vivo 7-Tesla MRI was investigated to evaluate whether histopathologic lesions may be detected clinically. 7-Tesla MRI could not detect most microvascular injury that was visible histopathologically, which is consistent with the clinicoradiological paradox of clinical symptoms in the absence of radiological findings by conventional MRI. Thus, more accurate diagnostic tools for NP-SLE are warranted.

Since making a diagnosis based on a common aetiology provides the highest level of conceptual understanding, we investigated microchimerism as a potential aetiological factor involved in SLE. To substantiate ongoing research relating microchimerism to SLE, as well as to other autoimmune diseases, pregnancy complications, malignancies, response to injury, and transplantation outcomes, the occurrence and distribution of tissue microchimerism during human pregnancy was investigated in **chapter 7**. In situ hybridization of the Y chromosome was performed on paraffin-embedded autopsy samples of kidneys, livers, spleens, lungs, hearts, and brains that were collected from 26 women who died while pregnant or within one month after delivery of a son. Frequencies of chimeric cells were compared to those of a control group of non-pregnant women who had delivered sons. Tissue microchimerism occurred significantly more frequently in lungs, spleens, livers, kidneys, and hearts of pregnant women than non-pregnant women. Remarkably, this distribution pattern replicates findings in pregnant mice, with the lungs being the organs in which microchimerism was present most often and in greatest abundance, followed by the spleen, liver, and kidney, with lowest amounts of microchimerism found in the heart and brain. Intriguingly, some of the chimeric cells were CD3+ or CD34+. Corrected for cell density, the lung was most chimeric, followed by the spleen, liver, kidney, brain, and heart. Data from this unique study group of women who died while pregnant or shortly after delivery provide important information about the amount and physiologic distribution of chimeric cells in organs of pregnant women and validate the use of mouse models to study microchimerism during pregnancy.

In **chapter 8**, the relationship between microchimerism and SLE was further studied by investigating the origin and amount of microchimerism in peripheral blood of women with SLE and controls, as well as the relationship between microchimerism and SLE onset, disease activity, and damage accrual. Eleven SLE patients and 22 controls were included, as well as their children and, if possible, their mothers. Quantitative PCR for insertion-deletion polymorphisms and null alleles was used to detect microchimerism in peripheral blood mononuclear cells and granulocytes. Microchimerism was detected more often in patients than controls (54.4% vs. 13.6%) and was found in both peripheral blood mononuclear cells and granulocytes. In 50% of SLE patients with microchimerism, it originated from multiple relatives, whereas in controls microchimerism was always derived from one relative. Microchimerism was mostly of fetal origin. No relationship was found between microchimerism and clinical or laboratory parameters. These results substantiate the hypothesis that chimeric cells play a role in SLE and provide novel, thought-provoking evidence that microchimerism in SLE can be derived from multiple relatives.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In the early days of medicine, systemic lupus erythematosus (SLE) was diagnosed based upon a constellation of observable clinical findings in predominantly young females showing similar clinical courses. With advancing knowledge and techniques, it became possible to diagnose SLE more specifically – reflecting a higher level of conceptual understanding of underlying pathophysiology. Because of the frequently poor outcome of SLE patients, scientists have long searched to identify prognostic indicators at the time of diagnosis, which may inform the patient and the physician about the preferred therapeutic strategy and expected outcomes, and may also be modifiable targets for treatment. In the case of lupus nephritis (LN), a renal biopsy currently plays a central role in the diagnostic process. For LN, a renal biopsy currently provides the best reflection of the severity of the disease process and often allows the exclusion of other diagnoses. Furthermore, a renal biopsy may provide a wealth of prognostic information and identify patients who are most likely to benefit from treatment. However, the central role of the renal biopsy in the diagnosis of LN may not result in the overlooking of clinical findings: clinical factors may be valuable predictors of outcome, not all histopathologic lesions may have prognostic significance, and not all lesions that resemble LN may clinically represent SLE. This has important implications for clinical practice and scientific research: a renal biopsy must always be interpreted in the setting of the clinical picture; and, in studying LN renal pathology, biopsy findings must always be interpreted in relation to clinical outcomes. In strong contrast with LN, neuropsychiatric SLE (NP-SLE) is a manifestation for which the performance of a biopsy of the affected tissue (the brain) is hardly feasible, and the pathology must therefore be assessed by less accurate diagnostic tools including radiological imaging. Nevertheless, the post-mortem histopathologic analysis of cerebral tissue is valuable to unravel pathogenic mechanisms. In this thesis, challenges relating to diagnosis and prognosis based on histopathologic findings in conjunction with clinical findings were investigated in two of the most severe manifestations of SLE: LN and NP-SLE – the results and implications for personalised medicine of which will be discussed here.

The diagnosis of LN

Has the time come for diagnostic criteria for SLE?

The first challenge addressed in chapter 2 of this thesis relates to the diagnosis of SLE focusing on patients with renal disease. Since diagnostic criteria are absent for SLE, classification criteria for SLE are of central importance – allowing physicians to arrange the heterogeneous manifestations of SLE into comprehensive categories for research purposes and provide a means for communication. Ideally, a classification groups together patients with identical manifestations, prognosis, and response to treatment. Indeed, a classification that does not function as such becomes an impediment for research and communication.

Underlining the importance of an accurate classification, classification criteria for SLE have been universally used as inclusion criteria for clinical trials and thereby reflect the patient population for which therapeutic guidelines are composed.

As emphasised in the introduction of this thesis, classification criteria for SLE are meant for research purposes and not for diagnosis. Theoretically, a diagnosis is not so much different from a classification in an individual patient. If classification criteria would have perfect sensitivity and specificity (100%), there would be no difference between diagnostic and classification criteria, and every single patient would be correctly diagnosed using the classification criteria.³ However, because disease manifestations in SLE are clearly not identical among patients and current classification criteria have imperfect accuracy, a certain proportion of patients would be misdiagnosed using the classification criteria. In these cases, only experienced physicians can establish a diagnosis by considering individual patient features (beyond those represented in the classification criteria), as well as other factors including the local prevalence of conditions that appear in the differential diagnosis.

In general, one must bear in mind that classification criteria, unless validated in multiple settings, reflect the applicability in the cohort of patients from which they were developed. Applying classification criteria in other settings requires consideration of the disease prevalence in a specific setting and the possibility of a different disease spectrum associated with particular organ manifestations. These elements were further elaborated in chapter 2, in which the diagnostic performance of the recently published SLICC classification of SLE was tested in the setting of patients with renal disease selected from the nephrology clinic. Here, the disease prevalence of SLE was relatively high and patients clearly had a different SLE disease spectrum than the SLE patients from the SLICC derivation cohort who were recruited from rheumatology clinics. Compared with patients in the SLICC derivation cohort, SLE patients with nephritis and full house nephropathy had a significantly lower frequency of alopecia, oral/nasal ulcers, and leukopenia, and a higher frequency of neurologic disorders, haemolytic anaemia, thrombocytopenia, anti-dsDNA antibodies, and hypocomplementaemia. These results are in agreement with those from a recently published large inception cohort comparing SLE patients with and without nephritis⁴ and support the notion that SLE is a disease with heterogeneous phenotypes.⁵ The results described in chapter 2 provide evidence for the usefulness of SLICC criteria for the diagnosis of SLE in patients with nephritis, although the added value of the SLICC criteria (compared with the ACR criteria) was uniquely due to hypocomplementaemia as a criterion and not due to the low threshold “stand-alone” criterion of biopsy-confirmed LN in combination with antinuclear or anti-dsDNA antibodies.

To resolve the inconsistencies of SLE classification criteria in different clinical settings, subspecialty-tailored criteria may be useful,⁶ taking into account the disease prevalence, differential diagnoses, and disease spectrum in each subspecialty. Thus, although evidence for the performance of the stand-alone criterion in the absence of at least four criteria

in the SLICC classification was missing,⁷ it may be that this criterion will indeed be useful to identify SLE patients among patients encountered in rheumatology clinics. Importantly, as investigated in this thesis, this low-threshold criterion in the setting of patients from the nephrology clinic with full house glomerular deposits compromised the specificity and should therefore be abandoned from SLE classification criteria used by nephrologists.

Non-lupus Full House Nephropathy: a red flag in the differential diagnosis of LN

Why should we be concerned about the suboptimal specificity (79%) of full house glomerular deposits for SLE and refrain from diagnosing LN in all patients with a seemingly renal-limited form of SLE? Making a clinical diagnosis only makes sense if patients have similar clinical features, a uniform prognosis, and response to treatment. In chapter 3, it was clearly demonstrated that patients with idiopathic and secondary non-lupus full house nephropathy (FHN) present with distinct clinical features and have a remarkably poor outcome as compared to lupus FHN patients. Here, the importance of taking note of the clinical characteristics rather than basing a diagnosis on tissue characteristics alone is clearly demonstrated. Besides the poor outcome of idiopathic and secondary non-lupus FHN patients, non-lupus FHN patients were clinically distinguished from lupus FHN patients by the absence of four or more clinical SLE signs and symptoms, higher levels of proteinuria, lower levels of erythrocyturia, and predominantly male sex. Also on the tissue level, some salient features were identified, including a predominantly membranous pattern of injury and less intense immunofluorescence staining compared to lupus FHN.

A number of factors may have contributed to the remarkably poor renal outcome of idiopathic non-lupus FHN patients. First, the severe clinicopathologic features at presentation and poor renal outcome in more than half of the patients with secondary non-lupus FHN raises the possibility that in some instances, idiopathic non-lupus FHN may represent severe forms of as yet unidentified other renal diseases that are accompanied by strong activation of the immune system, as has also been suggested previously.⁸⁻¹⁸ Second, idiopathic non-lupus FHN patients had a relative lack of immunosuppressive treatment, higher levels of proteinuria, and a tendency towards a higher chronicity score – although these factors did not explain the difference in renal survival comparing idiopathic non-lupus FHN and lupus FHN in the statistical models in chapter 3. The remarkably poor outcome of idiopathic non-lupus FHN urges future studies to unravel therapeutic strategies and aetiopathogenic mechanisms of immune complex deposition in idiopathic non-lupus FHN.

The prognosis of LN

Personalised medicine versus guideline-based medicine in LN

Making a correct diagnosis of LN and distinguishing other diagnoses such as non-lupus FHN are crucial, since a diagnosis of LN has considerable clinical implications. Current guidelines recommend treatment with intensive immunosuppression in those cases with glomerulonephritis classified as class III or IV LN according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification. Standard care for patients

with class III or IV LN includes intravenous cyclophosphamide or mycophenolate mofetil in combination with corticosteroids.¹⁹⁻²³ The intensive cytotoxic immunosuppressive regimens have considerable short-term and long-term toxicities.²⁴ Evidence-based guidelines for the treatment of LN are generated based on the body of clinical data available from therapeutic intervention studies in LN. The highest level of evidence in guidelines for LN is based on a number of landmark randomised controlled clinical trials (RCTs).¹⁹⁻²³ Historically, the first large RCT including LN patients performed at the National Institutes of Health (NIH) was not based on a consensus histopathologic classification, but included SLE patients with “proliferative LN” and declining renal function.²⁵ Since then, subsequent RCTs have formed the basis of current evidence-based treatment for proliferative LN, which generally included class III or IV ($\pm$ V). It is of interest that the evidence for the cut-offs defining patients with class III or IV ($\pm$ V) LN is lacking, and that these cut-offs have never been validated.

In general, RCTs have specific inclusion and exclusion criteria designed to represent a population large enough and sufficiently enriched regarding clinical abnormalities associated with an anticipated treatment effect to attain a number of end points and demonstrate a statistically and clinically significant difference in outcome. The usefulness of the guidelines may be questioned when individual patients within the heterogeneous population of LN patients in clinical practice differ in key characteristics from those of the trial population on which the guideline recommendation is based. In LN, inspection of the inclusion criteria of the landmark RCTs reveals that patients with only mildly disturbed renal function and/or mild proteinuria were frequently excluded from the RCTs,²⁵⁻³² and in some RCTs inclusion criteria were stricter for patients with class III than for class IV LN.²⁸⁻³⁰ Consequently, results from these large RCTs cannot unequivocally be applied to all patients with LN in a clinical setting, creating a gap in the treatment guidelines for LN patients with predominantly mild clinical features.

Since guidelines do not appear to be evidence-based across the clinical spectrum of LN, a guideline-based approach to the treatment of LN is problematic. As mentioned before, guidelines base their recommendations to initiate cytotoxic immunosuppressive treatment entirely on the lumped category of class III or IV ($\pm$ V) LN. In chapters 4 and 5 of this thesis, it was demonstrated that this lumped category obscures prognostic information that is hidden in individual clinical and histopathologic characteristics. Furthermore, the RCT-based guidelines in LN do not address pharmacogenetic differences between individual patients, which may also be of clinical importance.³³ The solution incorporating such characteristics of individual patients to predict outcome may be personalised medicine. Personalised medicine reflects the ability to classify individual patients into subpopulations who are similar in their clinical presentation, outcome, and response to treatment. Thus, the decision to initiate treatment is ideally focused on patients who will benefit from it, and be withheld from patients who will not. The toxicities of immunosuppressive regimens recommended in LN emphasise the importance of personalised medicine, to fulfil the physician's obligation

“to do good or to do no harm”. On the one hand, overtreatment of benign renal lesions subjects patients to severe side effects including infections, risk of malignancy and premature ovarian failure. On the other hand, delaying therapy because of presumed mild disease may be associated with increased glomerular injury, progressive tubulointerstitial fibrosis, glomerulosclerosis, and therefore a lesser response to immunosuppressive drugs.³⁴⁻³⁶

Scarce evidence from an early landmark NIH trial in LN²⁵ suggests that a subgroup of patients with class III or IV LN may be unlikely to benefit from cytotoxic immunosuppression. This hypothesis has never been tested prospectively in a separate clinical trial. Although a case for equipoise could be made for these cases, it is difficult to overcome the resistance of the medical community to actually investigate not treating these patients or choosing alternative treatment, since the guidelines recommend otherwise. Apparently, the development of evidence-based guidelines limits the subsequent application of personalised medicine. Clearly, the conflict between guideline-based and personalised medicine predominantly occurs in case of withholding recommended therapy. As a first step, it was attempted to identify the subpopulation of patients with class III or IV LN who retrospectively did not receive cytotoxic immunosuppressive therapy in chapter 4. The patients who were identified with class III or IV LN who did not receive immunosuppression according to guidelines provide evidence for the existence of a subgroup of patients for whom therapy regimens omitting cytotoxic drugs may suffice. Subsequent challenges will be to further characterise these patients eligible for milder therapy regimens and to test the utility of regimens omitting cytotoxic drugs in appropriate RCTs. As an alternative to not treating patients, an intervention study may be conceived including a wait-and-see approach. In this study design, the renal biopsy would be followed by a period during which LN patients are closely monitored, and possible introduction of anti-inflammatory treatment with corticosteroids followed by suppression of autoimmunity using cytotoxic immunosuppression only if deemed necessary. Indeed, older studies have demonstrated that anti-inflammatory doses of corticosteroids alone were as effective as corticosteroids plus cyclophosphamide in the early phase of LN treatment.^{25, 37}

A new era for the histopathologic classification of LN

In spite of the success and worldwide acceptance of the ISN/RPS histopathologic classification of LN,³⁸⁻⁴⁰ it is apparent that patients fulfilling classification criteria may have very different outcomes. Furthermore, diagnostic disagreement is common among pathologists due to unclear definitions.^{41, 42} Thus, the characterisation of prognostic subgroups in LN warrants refinement. The ISN/RPS classification was designed by a group of pathologists and clinicians combining expert opinion with the best available evidence provided by studies that identified prognostic factors from candidate factors selected by reasoning and considering the hypothesised pathway from the onset of disease to subsequent outcome (“candidate approach”). An evidence-based methodology in the design of a histopathologic classification as inspired by the Oxford classification of IgA nephropathy has paved the way for a new era of clinical pathology, basing classification systems on prognosticators

with evidence-based significance identified in a “hypothesis-free” approach to discover possible unsuspected factors. The prognosticators identified in chapter 5 that are currently obscured in the histopathologic classification of LN form a first step towards a revised histopathologic classification. An international initiative to revise the classification of LN must validate our results from chapter 5. It may well be that the time has come to refine historic classes, and incorporate a prognostic scoring system founded on lesions with evidence-based prognostic significance. This scoring system will likely include the lesions identified in chapter 5.

In the scoring system, it would be beneficial to specify the categories of prognostic significance associated with different lesions. Essentially, the analysis of the different outcomes in chapter 5 uniquely revealed two categories of clinical and histopathologic prognosticators: (i) variables predictive of eGFR during follow-up; and (ii) variables predictive of progressive renal function decline, renal flare, and ultimately ESRD. The first category of lesions represents injury that results in an irreversible loss of renal function that is not progressive or treated and halted successfully, whereas the second category of lesions represents injury resulting in progressive loss of renal function that is irreversible or impossible to halt using current treatment strategies. The results in chapter 5 suggest that particularly fibrinoid necrosis is a lesion in the latter category, raising the possibility that this lesion may have a different pathogenesis and may therefore benefit from treatment with a different therapeutic target.⁴³⁻⁴⁵ Similarly associated with progressive renal function decline, chronic lesions including fibrous crescents and interstitial fibrosis/tubular atrophy are unlikely to benefit from immunosuppressive therapy,⁴⁶ and may more likely benefit from a renoprotective approach including antihypertensive therapy.

In addition to the benefit of a histopathologic scoring system, prognostication for patients with LN might improve by considering other prognosticators. In chapter 5, it was shown that clinical variables including age, ethnicity, blood pressure and renal function at the time of biopsy have prognostic significance. Ideally, a prognostic model would incorporate these clinical variables in addition to histopathologic variables, as well as other possible predictors that have yet to be identified. Presently, it is unknown how new biomarkers of persistent inflammation may change the classification and personalised medicine in LN in the future, for instance investigations using urinary proteomics to detect surrogate markers of unrecognised nephron loss,⁴⁷ urinary flow cytometry to characterise the activation pattern of lymphocytes in persistent renal inflammation,⁴⁸ or measuring urinary cytokine/chemokine excretion.⁴⁹ Similar to, for example, the implementation of prediction tools such as “Adjuvant! Online”⁵⁰ and “PREDICT”⁵¹ in the clinical management of breast cancer, increasingly sophisticated prediction tools for LN may be on the horizon, incorporating a growing number of prognosticators and enabling a move from the categorisation of patients into broad prognostic groups to the realisation of providing survival estimates at the patient level.

The diagnosis of NP-SLE

NP-SLE as a spectrum

In marked contrast to LN, the lack of accurate diagnostic tools and a lack of knowledge about the pathogenesis challenge the diagnosis of NP-SLE. To gain insight into the pathogenesis and the injury at the tissue level, an autopsy study was performed (chapter 6). The results clearly illustrate that the injury and clinical manifestations of NP-SLE represent a continuum, ranging from relatively nonspecific lesions found in most SLE patients including focal vasculopathy, microinfarction, macroinfarction, and vasculitis – to specific and even pathognomonic lesions, including diffuse vasculopathy and microthrombi associated with clinical syndromes defining NP-SLE.⁵² Conceivably, thromboischemic injury occurs in all SLE patients but overt NP-SLE only ensues after a certain threshold of injury is reached.

Since a brain biopsy to detect possible specific lesions such as microthrombi remains unfeasible in clinical practice, NP-SLE syndromes are diagnosed in SLE patients *per exclusionem* supported by clinical and MRI findings. However, MRI often shows no abnormalities or nonspecific abnormalities such as small white matter hyperintensities⁵³ in the presence of clinical symptoms, known as the clinicroadiological paradox. Thus, in the diagnosis of NP-SLE, there is a need for sensitive and specific diagnostic tools that can distinguish NP-SLE from other diseases in patients with a clinical neuropsychiatric syndrome. At the same time, these tools should improve the assessment of the extent of injury.

In this thesis, the performance of 7-Tesla MRI was investigated as a diagnostic tool for NP-SLE as compared to histopathologic evaluation in two patients with NP-SLE and one patient with SLE without neuropsychiatric involvement. 7-Tesla MRI appeared to be less sensitive than histopathologic evaluation: post-mortem histopathologic evaluation of cerebral tissue of one NP-SLE patient revealed histopathologic injury in the absence of MRI findings. The specificity of both 7-Tesla MRI and histopathologic evaluation were limited: both revealed abnormalities in one patient without neuropsychiatric symptoms. The relevance of such subclinical injury in the setting of NP-SLE remains to be elucidated but the injury may well reflect the nonspecific histopathologic injury in SLE patients without neuropsychiatric syndromes. Future studies to determine the feasibility of, for instance, quantitative techniques such as volumetric magnetisation transfer imaging (MTI) detecting specific lesions in SLE and NP-SLE patients are called for,⁵⁴ as brain biopsies remain unattainable in routine clinical practice. Until then, the clinical evaluation will remain the mainstay in the diagnosis of NP-SLE.

The prognosis of NP-SLE

Because the heterogeneous manifestations of NP-SLE reflect a wide range of qualitative and quantitative lesions of which the diagnosis itself is often hampered, little is known about prognostic subgroups and factors that may be used to classify them. The identification of improved diagnostic tools and markers may pave the way for the identification and characterisation of prognostic subgroups in NP-SLE.

An inflammatory and a thromboischemic pathogenic mechanism have been implicated as separate autoimmune pathogenic mechanisms for NP-SLE, the distinction of which is often not feasible.⁵⁵ Cytotoxic immunosuppressive therapy is indicated in selected NP-SLE patients with a suspected inflammatory underlying disease mechanism. This therapy is relatively non-specific, anti-proliferative, and/or anti-metabolic in nature, and may therefore – like in LN – have severe systemic side effects, including sepsis, avascular bone necrosis, gonadal dysfunction, and malignancy.⁵⁶ Antiplatelet and/or anticoagulation therapy is indicated for patients with a suspected thromboischemic pathogenic mechanism. This type of therapy also carries the risk of significant haemorrhagic complications.⁵⁷ Clearly, there is a need for better tools to distinguish these pathogenic mechanisms to enable better targeting of treatment. In addition, the development of targeted therapeutics that abrogate the pathogenesis of NP-SLE more selectively is warranted to maximise treatment efficacy and minimise side effects.

Aetiopathogenesis of SLE: the common ground

Because the diagnosis of SLE is challenged by the clinical heterogeneity and overlap with other diseases, the identification of underlying aetiopathogenic mechanisms may provide specific disease indicators assisting in making the diagnosis as well as providing potential modifiable therapeutic targets that could possibly prevent subsequent disease or halt its progression.

Complement as a novel therapeutic target in NP-SLE

As described in the introduction of this thesis, the role of complement as a downstream effector of immune complex-mediated tissue injury in LN is well studied. In chapter 6, it was demonstrated that classical complement deposition is also strongly associated with NP-SLE and microthrombi in particular, providing a possible link between thromboischemic injury and inflammation in NP-SLE. Similar to what has been suggested in other entities such as thrombotic microangiopathy, accumulation of antibodies in small vessels most likely leads to activation of the classical complement pathway, endothelial injury, and the subsequent formation of microthrombi.⁵⁸ In other fields, the complement system has recently been introduced as a promising therapeutic target. The finding in chapter 6 that C5b-9 deposits were present in 82% of patients with SLE and NP-SLE, suggests that at least this proportion of patients with NP-SLE – and perhaps also patients with SLE in the absence of neuropsychiatric symptoms – may benefit from treatment with the terminal complement inhibitor eculizumab.

Eculizumab is a recombinant fully humanised IgG2/IgG4 monoclonal antibody that binds to complement component C5 and prevents the cleavage of C5 and thereby the formation of the anaphylatoxin C5a and the formation of the membrane attack complex (C5b-9). Eculizumab was shown to be efficacious in the prevention of erythrocyte lysis in paroxysmal nocturnal haemoglobinuria⁵⁹ and in the treatment of atypical haemolytic uraemic syndrome.⁶⁰ No RCTs have investigated the efficacy of eculizumab in the treatment of SLE

manifestations. However, animal studies have indicated that eculizumab may be beneficial: in lupus-prone mice, blockade of complement C5 has been shown to decrease proteinuria and renal dysfunction and prolonged animal survival.⁶¹ A number of case reports suggest that eculizumab may also be beneficial in the treatment of SLE in humans.^{62,63} Clearly, the complement system is a promising target in NP-SLE urging RCTs to investigate the efficacy of eculizumab in SLE patients with NP-SLE.

Chimerism in SLE

Little is known about the role of microchimerism in SLE, but the difference between men and women in the incidence of SLE makes pregnancy-derived microchimerism an intriguing aetiopathogenic candidate. In this thesis, microchimerism was investigated in association with SLE. In chapter 7, the rationale for studying microchimerism in SLE as well as a number of other pregnancy-related conditions, including other autoimmune diseases, pregnancy complications, and certain malignancies was investigated. Here, an increased occurrence of tissue microchimerism was demonstrated in women during pregnancy compared to after pregnancy, substantiating the rationale for investigating microchimerism in relation to SLE. Subsequently, the increased frequency (54.4% vs. 13.6%) of microchimerism in SLE patients was demonstrated in chapter 8. The results in these chapters show that microchimerism is associated with SLE, but may also occur as a physiologic phenomenon without the advent of autoimmunity.

The higher prevalence of microchimerism in SLE patients than in controls can be explained by (i) acquisition of more chimeric cells during pregnancy, (ii) persistence of more chimeric cells after pregnancy, (iii) chimeric stem cells giving rise to more chimeric cells due to an unknown trigger, or (iv) a combination of aforementioned possibilities. In chapter 8, an important difference between SLE patients and controls was that in 50% of SLE patients with microchimerism, it originated from multiple relatives, whereas in controls microchimerism was always derived from one relative. The increased frequency of microchimerism in SLE patients may be explained by HLA relationships. Studies in animals have demonstrated that syngenic or congenic matings resulted in more chimerism than allogenic matings, suggesting a role for HLA (mis)matches.^{64,65} In humans, in certain autoimmune diseases mothers and children were shown to have fewer HLA disparities,^{66,67} but these have not yet been correlated with the presence of microchimerism.⁶⁶

Concerning the hypothesis that pregnancy-acquired microchimerism may be an explanation for the high incidence of SLE in women, it is remarkable that the results in chapter 7 demonstrate that the lung was the organ harbouring most microchimerism in pregnant women, followed by the spleen, liver, kidney, brain, and heart. Remarkably, this distribution pattern replicates findings in pregnant mice, with the lungs being the organs in which microchimerism presented most often and in greatest abundance, followed by the spleen, liver and kidney, with lowest amounts of microchimerism found in the heart and brain. These observations demonstrate the validity of mouse models to represent human fetomater-

nal cell trafficking during pregnancy and substantiate the usefulness of mouse models to further unravel the impact of microchimerism on maternal health. In the pregnant women without autoimmune diseases who were studied, several factors including anatomy, cytokine production, cell turnover, injury occurrence, and tissue metabolic rates could explain this distribution. The notable organ distribution raises the question whether the preferential occurrence of microchimerism in specific organs reflects the increased incidence of disease in these organs.

A number of hypotheses have been proposed for the role of microchimerism in SLE, including a pathogenic role, a repair role, and an innocent bystander role of chimeric cells. Relating to the first hypothesis, the finding that male T cells (CD3+) were present in pregnant women (chapter 7) is compelling. Possibly, the acquisition of CD3+ cells during pregnancy forms the basis for development of SLE in which chimeric T cells are present. In the mouse model by Via and Shearer,⁶⁸ the injection of chimeric T cells resulted in a graft-versus-host disease that resembles human SLE. Alternatively, the chimeric cells may not themselves be effector cells, but they may rather be the target of an immune reaction, and/or undergo cell death providing a source of DNA resulting in sensitisation and triggering the development of antinuclear antibodies. The engraftment of the chimeric cells in tissues of pregnant women is intriguing in this setting, raising the possibility that these cells under specific circumstances become the target of an alloimmune response. Concerning the repair hypothesis, it has been demonstrated that in gentamicin-induced kidney injury, fetus-derived chimeric cells engraft in the kidney as tubular cells, suggesting a role in the repair process. In the setting of LN, a repair function of microchimerism was recently supported by the positive association between amounts of microchimerism and renal function.⁶⁹ In chapter 8, no relationship between microchimerism and clinical or laboratory parameters was found. In SLE patients, it has been demonstrated that microchimerism occurs more often in organs from patients with SLE who had experienced injury than in normal control organs, irrespective of whether the injury was SLE-related, non-SLE-related, or both – supporting the hypothesis that tissue chimerism is the result of a repair process.⁷⁰ In chapter 7, the only organ with a significant correlation between tissue microchimerism and active injury scores was the kidney. It is therefore conceivable that the kidney is particularly prone to repair by chimeric cells. However, the latter observation could also be explained by the innocent bystander hypothesis, with the increased microchimerism being the result of nonspecific recruitment of inflammatory cells including chimeric cells. The innocent bystander hypothesis is supported by the occurrence of chimeric cells in healthy women (chapters 7 and 8), demonstrating that chimerism is also a physiologic phenomenon, and may only be pathogenic under specific circumstances. Further study is necessary to unravel the impact of fetal cell microchimerism on maternal health and conditions under which chimeric cells may induce SLE.

Conclusion: towards personalised medicine in SLE

In the current thesis different challenges concerning the diagnosis and prognosis of SLE were investigated. A central conclusion from this thesis is that personalised medicine should be the framework of clinical care for SLE patients. A special focus should be on improving outcomes of individual SLE patients by considering prognostic factors and initiating treatment only in patients who will benefit from it and withholding treatment from those who will not. By this approach, the risk of adverse effects due to ineffective treatment is minimised. Thus, classifications should be developed starting with prognostic groups as identified by a constellation of clinical and histopathologic features. The finding of a prognostic difference between FHN patients with and without sufficient additional classification criteria for SLE (chapters 2 and 3) is the ultimate impetus to distinguish these patient groups by means of classification. Hence, patients with FHN without sufficient SLE criteria should not be regarded as representing a “renal-limited” form of SLE and should not be included in the classification. Currently, the ISN/RPS histopathologic classification of LN lacks accuracy in defining prognostic subgroups (chapters 4 and 5). For patients with LN receiving conventional immunosuppressive treatment, renal outcomes including progression to ESRD, renal flare, and eGFR during follow-up could be predicted by the histopathologic and clinical parameters described in chapter 5 – justifying the inclusion of these parameters in a revised version of the histopathologic classification. These parameters in fact define patients who were unresponsive or only partially responsive to conventional treatment. Future research may indicate that such patients may benefit from new therapeutic regimens focusing on different therapeutic targets.⁷¹ Insight in different pathogenic pathways may provide these new therapeutic targets. The complement system was identified as a promising therapeutic target in NP-SLE (chapter 6), and its role in LN may also warrant further investigation. Future research in the field of SLE should focus on the identification of aetiopathogenic factors and identification of new prognostic factors to define prognostic subgroups, to predict treatment response, to monitor disease progression, to improve stratification of patients in RCTs, as well as to develop new targeted therapies minimising the side effects.

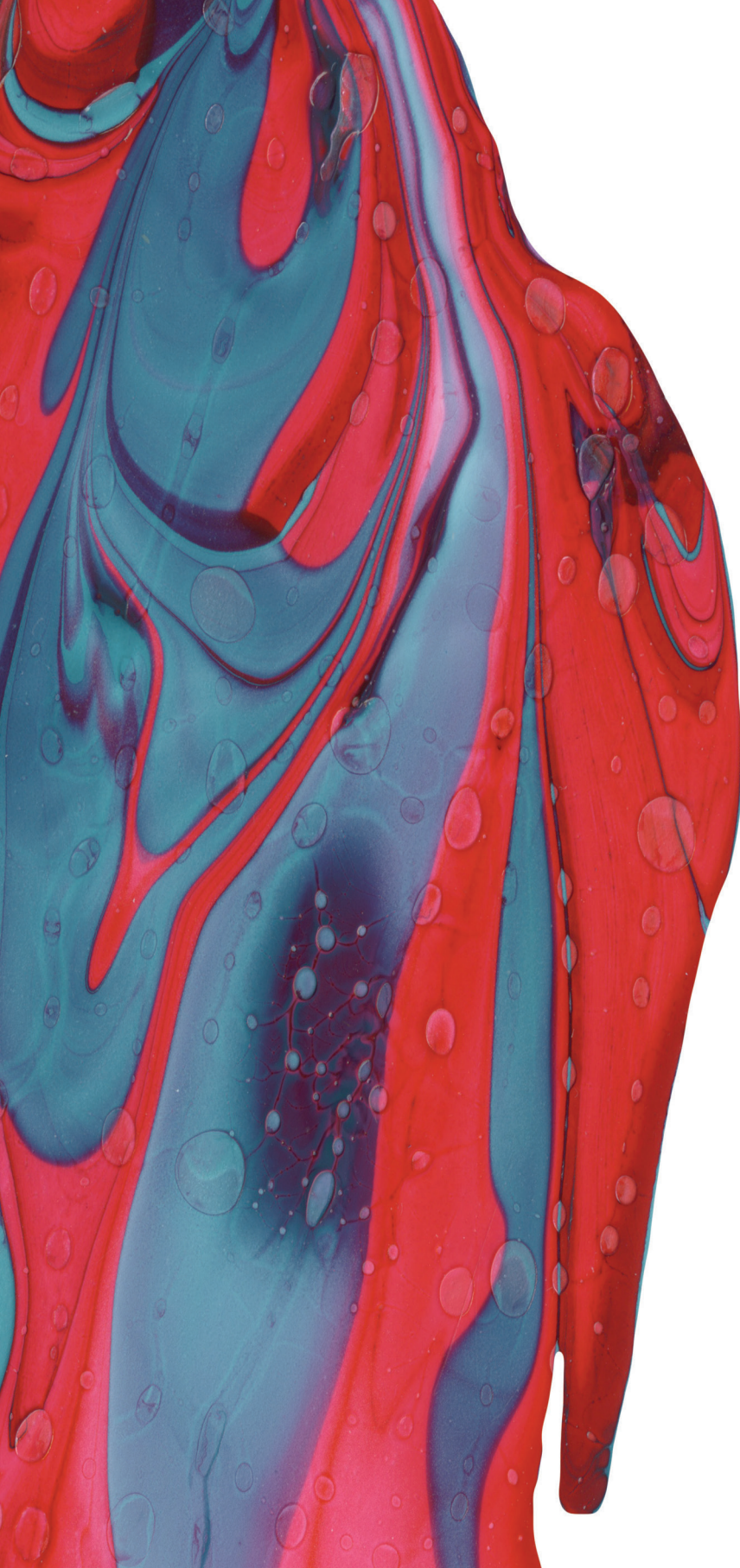
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Chapter 10

NEDERLANDSE SAMENVATTING

Systemische lupus erythematosus (SLE) of kortweg “lupus” is een auto-immuunziekte, dat wil zeggen een ziekte waarbij het immuunsysteem lichaamseigen stoffen aanvalt. SLE is een zeldzame ziekte (6 à 7 per 100,000 mensen) die met name jonge vrouwen treft. Het immuunsysteem zorgt ervoor dat we beschermd zijn tegen ziekmakende indringers, zoals virussen en bacteriën. Normaal gesproken is ons lichaam in staat om bestanddelen van lichaamsvreemde wezens te onderscheiden van lichaamseigen bestanddelen, maar in het geval van een auto-immuunziekte als SLE is dit onderscheidend vermogen gestoord. Als gevolg hiervan wordt het immuunsysteem geactiveerd, hetgeen onder andere leidt tot de vorming van afweerstoffen of “antistoffen” tegen lichaamseigen bestanddelen. In patiënten met SLE zijn deze antistoffen vaak gericht tegen bestanddelen van celkernen. Het aantonen van deze zogenaamde “antinucleaire antistoffen” (ANA, “nucleus” betekent celkern) vormt dan ook een belangrijke pijler om tot de diagnose SLE te komen. Deze afweerreacties en antistoffen kunnen verspreid door het hele lichaam ontstekingsreacties teweegbrengen, waardoor SLE uitingsvormen in veel verschillende organen kan hebben. Om deze reden is SLE ook wel “systemisch”, dat wil zeggen dat het hele lichaam of “systeem” betrokken kan zijn. De naam “lupus” stamt uit de Middeleeuwen, zo genoemd omdat de karakteristieke huidaandoening die bij een aantal lupuspatiënten kan worden gezien in het gelaat deed denken aan de beet van een wolf – ook wel *lupus* in het Latijn. Deze huidaandoening evenals een aantal andere huidaandoeningen bij lupus kenmerkt zich door een rode verkleuring, vanwaar de term “erythematosus” (rood) in de naam van deze ziekte is opgenomen.

Door het systemische karakter kent SLE veel verschillende uitingsvormen. De diagnose SLE wordt gesteld op basis van een combinatie van symptomen en bloeuitslagen, waarbij andere ziektes moeten worden uitgesloten. Artsen kunnen bij het stellen van de diagnose zich richten tot classificatiecriteria voor SLE zoals opgesteld door de *American College of Rheumatology* (ACR) en meer recent door de *Systemic Lupus International Collaborating Clinics* (SLICC). De meest recente SLICC criteria staan vermeld in **Tabel 1**. Om de diagnose SLE te stellen moet een patiënt ten minste aan vier criteria voldoen, waarvan ten minste een “klinisch criterium” en ten minste een “immunologisch criterium”. Klinische bevindingen in deze classificatie zijn bevindingen met name op basis van de anamnese (het uitvragen van de klacht) en het lichamelijk onderzoek. Immunologische afwijkingen kan de arts door middel van laboratoriumbepalingen vaststellen. Los van de conventionele eis om aan minstens vier criteria te voldoen, kan de diagnose SLE laagdrempeliger gesteld worden in het geval van nierontsteking passend bij lupus (op basis van een nierbiopsie) en de aanwezigheid van ANA of anti-DNA afweerstoffen.

Tabel 1 SLICC classificatiecriteria voor SLE.

criterium	Uitleg
Klinische criteria	
1. (Sub)acute cutane lupus	Vlinderexantheem: rode uitslag over de wangen en de neusrug (vlindervormig) Bulleuze lupus: huiduitslag met blaren passend bij lupus Maculopapuleus exantheem: rode bobbelige huiduitslag passend bij lupus Fotosensitiviteit: uitslag door zonlichtovergevoeligheid passend bij lupus Toxische epidermale necrolyse: afsterven van de bovenste huidlaag met blaarvorming Subacuut cutane lupus: huidafwijkingen lijkend op psoriasis
2. Chronische cutane lupus	Discoïde lupus: discusvormige, schilferende, verdikte huiduitslag op het gezicht, de hoofdhuid, oren, borst of armen passend bij lupus Lupus panniculitis: dieper gelegen huidontsteking Slijmvliesafwijkingen Chilblain lupus: door koude uitgelokte huidafwijkingen
3. Zweetjes/aften	Zweetjes (ulcera of aften) in de neus en/of mond
4. Niet verlittekenende alopecia	Hoofdhair dat verspreid dunner en/of breekbaarder wordt, waarbij gebroken haren te zien zijn
5. Artritis (≥2 gewrichten)	Gewrichtszwelling Gewrichtspijnen en ten minste 30 minuten ochtendstijfheid
6. Serositis	Pleuritis: ontsteking van het borstvlies (vlies om de longen) Pericarditis: ontsteking van het hartzakje (vlies om het hart)
7. Nefritis	Ontsteking van de nieren, zich onder andere uitend in eiwit en/of bloed in de urine
8. Neuropsychiatrische afwijkingen	Aantasting van het centrale zenuwstelsel (hersenen en ruggenmerg); onder andere epilepsie, concentratie- en geheugenproblemen en hallucinaties
9. Hemolytische anemie	Te weinig rode bloedcellen (bloedarmoede) door afbraak van rode bloedcellen
10. Leukocytopenie	Te weinig witte bloedcellen met daarbij een verhoogd infectierisico
11. Trombocytopenie	Te weinig bloedplaatjes met daarbij een verhoogd bloedingsrisico
Immunologische criteria	
1. Antinucleaire antistoffen (ANA)	Antistoffen tegen onderdelen van celkernen
2. Anti-dubbelstrengs DNA antistoffen (anti-dsDNA)	Antistoffen tegen DNA
3. Anti-Smith antistoffen (anti-Sm)	Anti-Sm antistoffen zijn genoemd naar de eerste patiënte in wiens bloed ze zijn aangetoond (haar naam was Smith). Deze antistoffen worden bijna uitsluitend in SLE patiënten gevonden
4. Antifosfolipide antistoffen	Antistoffen tegen het omhulsel van de cel. Aanwezigheid hiervan leidt tot een verhoogde stollingsneiging
5. Laag complement	Complementeiwitten worden gebruikt bij het opruimen van complexen met antistoffen in het bloed. Bij SLE kan het complement verlaagd zijn doordat het veel verbruikt wordt bij het opruimen van de antistofcomplexen.
6. Directe Coombs' test	Een test die gebruikt wordt voor het aantonen van antistoffen tegen rode bloedcellen

Buiten de verschijnselen die zijn opgenomen in de classificatiecriteria kent SLE nog een aantal andere uitingvormen die minder voorkomen, zoals ontsteking van de hartspeer, of die minder specifiek zijn voor SLE, zoals koorts en malaise. De grote variëteit aan uitingvormen van SLE en overlap van deze symptomen met een aantal andere ziektes kunnen de diagnose bemoeilijken. De diagnose wordt gesteld op basis van het voorkomen van een combinatie van verschillende symptomen en er bestaat geen test die op zichzelf zekerheid geeft. In dit proefschrift zijn verschillende problemen onderzocht die verband houden met het stellen van de diagnose SLE op basis van weefselonderzoek en klinische bevindingen. Weefselonderzoek in dit kader bestaat uit het onderzoeken met behulp van een microscoop van weefsel dat is verkregen door het nemen van een biopsie of postmortaal verkregen weefsel via een obductie. Klinische bevindingen zijn gegevens die de arts verzamelt op basis van de anamnese (het uitvragen van de klacht) en het lichamelijk onderzoek. Zoals blijkt uit de classificatiecriteria, is natuurlijk ook aanvullend laboratoriumonderzoek bij de klinische evaluatie van een patiënt met SLE van groot belang. Verder is in dit proefschrift onderzocht hoe men de uitkomst van betrokkenheid van de nieren bij SLE beter kan voorspellen, strevend naar de toepassing van het concept van “gepersonaliseerde geneeskunde” (personalised medicine). Personalised medicine is het concept waarbij wordt geprobeerd afzonderlijke patiënten in zo specifiek mogelijke subgroepen in te delen die gelijk zijn wat betreft hun ernst van de ziekte, verwachte beloop en verwachte respons op behandeling. Op deze manier kan een betere en nauwkeurigere beslissing worden genomen om een individuele patiënt te behandelen die baat zal hebben bij de behandeling, terwijl op deze manier ook wordt voorkomen dat een patiënt onterecht wordt behandeld en daarmee wordt blootgesteld aan mogelijk ernstige bijwerkingen van medicijnen.

Nierbetrokkenheid bij SLE, ook wel lupus nefritis genoemd, is een van de meest ernstige uitingen van SLE. Minstens 50% van de patiënten heeft op enig moment tekenen van lupus nefritis als uiting van SLE. De nieren zorgen ervoor dat afvalstoffen uit het bloed gefilterd worden en in de urine terecht komen, de vocht- en zoutbalans gereguleerd worden en dat bepaalde hormonen en vitamines gemaakt worden. Voorbeelden hiervan zijn erythropoïetine (“epo” zorgt voor aanmaak van rode bloedcellen) en vitamine D (zorgt voor calciumstofwisseling). Bij nierschade door lupus nefritis kan het zijn dat de ontstoken bloedfilterlichaampjes, “glomeruli” genoemd, niet meer goed werken. Hierdoor kunnen eiwitten en/of bloedcellen in de urine terecht komen als teken van schade aan de glomeruli. Verder kunnen de nieren dan niet meer goed het bloed van afvalstoffen ontdoen, de vocht- en zoutbalans op peil houden en in een laat stadium door hun verminderde functioneren leiden tot bloedarmoede en botontkalking. De nierfunctie kan in het laboratorium geschat worden door het meten van het “creatinine” in het bloed. Deze stof is een afbraakproduct van spieren en wordt volledig via de nieren uitgescheiden. Als de nieren minder functioneren, kan creatinine dus minder goed worden uitgescheiden via de urine en ophopen in het bloed. De uitscheiding of “klaring” van creatine wordt gebruikt als maat voor nierfunctie. Als de nierfunctie of “creatinineklaring” minder dan

$\pm 15\%$ bedraagt, is er sprake van eindstadium nierfalen. Op dat moment is dialyse of niertransplantatie noodzakelijk.

Omdat SLE valt onder de zogenaamde “reumatische” ziektes, waarbij er veelal ontsteking is van gewrichten, botten, spieren of bindweefsel, worden patiënten met SLE veelal behandeld door reumatologen. Classificatiecriteria voor SLE zijn dan ook gebruikelijk ontwikkeld om patiënten met SLE te kunnen onderscheiden van patiënten zonder SLE zoals men deze zou kunnen treffen in de spreekkamer van de reumatoloog. Echter, ook andere medisch specialisten komen in aanraking met patiënten met SLE. Het is duidelijk dat de patiëntengroep waarvan patiënten met SLE bij andere specialismes moeten worden onderscheiden andere soorten ziektes heeft dan de patiëntengroep in de reumatologieklinieken. Bijvoorbeeld, een reumatoloog moet een patiënt met “reuma” en gewrichtspijn kunnen onderscheiden van een patiënt met SLE met gewrichtspijn, terwijl een dermatoloog een patiënt met huiduitslag ten gevolge van SLE moet kunnen onderscheiden van een patiënt met een huiduitslag en een andere huidziekte. Waarschijnlijk kunnen criteria voor SLE niet zonder meer worden toegepast door verschillende specialisten.

In **hoofdstuk 2** van dit proefschrift is onderzocht hoe de SLICC en ACR criteria voor SLE presteren als deze worden toegepast bij patiënten met nierziekten die worden gezien door artsen die gespecialiseerd zijn in nierziekten, zogenoemde “nefrologen”. De patiënten in deze studie hadden een nierziekte waarvoor een nierbiopsie werd verricht om de diagnose te stellen. Heel suggestief voor lupus nefritis is het “full house” patroon bij immunofluorescentie. Immunofluorescentie is een techniek waarbij antistoffen en andere eiwitten van het immuunsysteem zichtbaar kunnen worden gemaakt op weefselniveau door middel van fluorescentie. Omdat SLE wordt gekenmerkt door de aanmaak van veel antistoffen en andere eiwitten van het immuunsysteem leidt dit in veel gevallen tot een sterk positieve aankleuring bij immunofluorescentie – vandaar het zogenoemde “full house patroon”. In hoofdstuk 2 zijn 149 patiënten met een dergelijk patroon onderzocht. Een deel van deze patiënten bleek daadwerkelijk SLE (79%) te hebben en een deel niet (21%). Deze verdeling betreft zogenaamde “klinische diagnoses”, oftewel diagnoses gesteld door de arts op basis van ervaring en expertise – los van de classificatiecriteria. Vervolgens werd gekeken hoe deze klinische diagnoses zich verhouden tot de diagnoses die gesteld zouden worden op basis van de classificatiecriteria. De SLICC criteria bleken van nut te zijn om patiënten met SLE te kunnen detecteren: alle patiënten met de klinische diagnose SLE voldeden aan ten minste vier SLICC criteria. De ACR criteria daarentegen detecteerden 95% van de patiënten met SLE. Het laagdrempelige criterium van de SLICC classificatie, waarbij alleen de aanwezigheid van lupus nefritis in combinatie met ANA en/of anti-DNA antistoffen genoeg is voor de diagnose, bleek niet van toegevoegde waarde te zijn. Er werden dus geen extra patiënten met SLE geïdentificeerd door het hanteren van dit laagdrempelige criterium die niet gedetecteerd zouden zijn geweest als er een hogere afkapwaarde (ten minste vier criteria zoals gebruikelijk) zou worden gesteld. Het bleek zelfs dat het toepassen van dit laagdrempelige criterium van de SLICC classificatie

resulteerde in het onterecht stellen van de diagnose SLE bij drie patiënten: deze patiënten hadden volgens de arts geen SLE, maar voldeden wel aan het laagdrempelige criterium. De bevindingen van deze studie laten zien dat de SLICC criteria nuttig zijn om SLE te diagnosticeren onder patiënten met nefritis in de nefrologiekliniek, alhoewel waakzaamheid is geboden bij het hanteren van het laagdrempelige niercriterium.

In **hoofdstuk 3** is dezelfde groep patiënten onderzocht als in hoofdstuk 2. De focus in hoofdstuk 3 lag op het onderscheid tussen patiënten met en zonder SLE. Het ging hier dus om een groep van 149 patiënten met een nierziekte waarbij de nierbiopsie met full house patroon zeer suggestief was voor lupus nefritis. Sommige artsen zouden wellicht stellen dat deze patiënten zelfs lupus nefritis hadden op basis van de bevinding bij nierbiopsie alleen. Van deze groep bleek 79% daadwerkelijk SLE te hebben volgens de arts; deze groep patiënten werd in de studie de “lupus full house nefropathie (FHN)” groep genoemd. De overgebleven 21% van de patiënten bleek volgens de arts geen SLE te hebben. De oorzaak van de nierziekte met full house immunofluorescentie was in de meerderheid (63%) van deze patiënten onbekend. Deze groep patiënten zonder bekende oorzaak werd de “idiopathische non-lupus FHN” groep genoemd. De overgebleven (38%) patiënten had een nierziekte passend bij een andere diagnose dan lupus nefritis; er werd dus een oorzaak gevonden. Het onderscheid tussen patiënten met lupus nefritis (lupus FHN groep) en patiënten zonder bekende oorzaak van de nierziekte (idiopathische non-lupus FHN groep) is onderzocht op het vlak van klinische kenmerken, afwijkingen op weefselniveau en uitkomsten op de lange termijn. De vraag is of (i) idiopathische non-lupus FHN een vorm van SLE is die zich beperkt tot de nier; (ii) patiënten met idiopathische non-lupus FHN later in het beloop van hun ziekte kenmerken van SLE ontwikkelen en uiteindelijk voldoen aan classificatiecriteria, of (iii) dat patiënten met FHN zonder SLE een compleet andere vooralsnog onbekende ziekte representeren. Er werden opvallende verschillen gevonden tussen lupus en non-lupus FHN patiënten. Patiënten met idiopathische non-lupus FHN waren vaker van het mannelijke geslacht, hun nierbiopsieën lieten een ander patroon van schade zien, klinisch hadden ze meer eiwit en minder rode bloedcellen in de urine en minder vaak verlaagde complementeiwitten dan patiënten met lupus FHN (zie **Tabel 1** voor definities). Opvallend was dat patiënten met een bepaald patroon van schade waarbij ontsteking werd gezien eerder eindstadium nierfalen bereikten als zij idiopathische non-lupus FHN hadden in vergelijking met lupus FHN. Deze resultaten laten zien dat hoewel het full house patroon een immunofluorescentiepatroon is dat het meest wordt gezien bij SLE, het van groot belang is te realiseren dat het bij een aantal andere nierziekten kan voorkomen evenals bij een idiopathische variant met een bijzonder slecht vooruitzicht wat betreft de nierfunctie.

Net als SLE zelf is lupus nefritis erg variabel wat betreft de klinische verschijnselen en beloop. Deze klinische variabiliteit reflecteert het brede spectrum van afwijkingen die men kan zien op weefselniveau in een nierbiopsie onder de microscoop – zogenaamde “histologische” afwijkingen. De histologische afwijkingen in lupus nefritis zijn geassocieerd

volgens de *International Society of Nephrology/Renal Pathology Society* (ISN/RPS) classificatie voor lupus nefritis. De meest ernstige klassen van lupus nefritis – de klassen III en IV – zijn gedefinieerd als nierbiopsieën waarbij respectievelijk minder of meer dan 50% van de glomeruli (bloedfilterlichaampjes) zijn ontstoken. Deze ernstige vorm van nefritis kan leiden tot eindstadium nierfalen en overlijden. Volgens de huidige richtlijnen voor de behandeling van lupus nefritis, moeten *alle* patiënten met klasse III/IV lupus nefritis worden behandeld met zware afweeronderdrukkende medicijnen, waaronder prednison en vormen van chemotherapie. Deze medicijnen kunnen gepaard gaan met ernstige bijwerkingen zoals onvruchtbaarheid, ontwikkeling van kanker en infecties. Echter, het op één hoop gooien van verschillende vormen en gradaties van ontsteking in klasse III/IV maskeert mogelijk kenmerken die wellicht zouden kunnen aangeven dat een patiënt een milder of juist een agressiever beloop van de ziekte zal hebben. Het gevolg van deze grote mengeling van afwijkingen in klasse III/IV is dat er wordt opgelegd dat alle patiënten behandeld moeten worden met deze zware medicijnen ongeacht de ernst van de ontsteking. Als kenmerken van een milder of juist agressiever beloop zouden bestaan, zouden sommige patiënten wellicht een minder zware behandeling kunnen krijgen, terwijl andere patiënten misschien zelfs nog zwaarder behandeld zouden moeten worden. Deze hypothese is onderzocht in het tweede deel van dit proefschrift.

Het natuurlijke beloop (zonder therapie) van klasse III/IV lupus nefritis is onderzocht in **hoofdstuk 4**. Het doel van deze studie was om een subgroep aan te tonen van patiënten met een gunstige uitkomst die wellicht zouden kunnen profiteren van behandeling zonder de zware afweeronderdrukkende medicijnen. Uit de archieven zijn 101 patiënten met klasse III/IV lupus nefritis geïdentificeerd, waarvan drie patiënten in het verleden om verschillende redenen in strijd met de behandelrichtlijnen niet behandeld bleken te zijn geweest met afweeronderdrukkende medicijnen. Deze drie patiënten lieten een gunstig beloop zien wel 9–24 jaar na nierbiopsie. Interessant genoeg was het beloop van nierfunctie van deze drie patiënten ook niet verschillend van het beloop van de andere 98 patiënten in de studie die wel waren behandeld met afweeronderdrukkende medicijnen. Deze drie patiënten zijn bewijs voor het bestaan van de subgroep van patiënten met klasse III/IV lupus nefritis met een gunstig natuurlijk beloop. De huidige richtlijnen leiden daarom in een aantal gevallen tot overbehandeling van patiënten met lupus nefritis.

In **hoofdstuk 5** is verder onderzocht welke kenmerken mogelijk van belang kunnen zijn om de uitkomst van patiënten met lupus nefritis te kunnen voorspellen. In deze studie zijn 105 patiënten met verschillende klassen lupus nefritis volgens de eerdergenoemde ISN/RPS classificatie onderzocht. Vijftig histologische kenmerken en tien klinische kenmerken werden getest of deze al dan niet verband hielden met verschillende uitkomsten. Deze uitkomsten waren de hoogte van de nierfunctie op de lange termijn, het doormaken van een opvlaming van lupus nefritis en het bereiken van eindstadium nierfalen. Dit onderzoek liet duidelijk zien dat het voorspellen van deze uitkomsten beter kan dan momenteel gebeurt aan de hand van ISN/RPS klassen. Specifieke histologische kenmerken

van de nierbiopsie, zoals de aanwezigheid van verlittekening en celdood, zijn met name van belang om een slechte uitkomst te voorspellen, evenals klinische kenmerken als niet-westerse afkomst en hogere leeftijd. Een arts kan aan de hand van deze kenmerken dan bepalen of een patiënt waarschijnlijk een agressief beloop van de ziekte zal hebben en zware behandeling moet starten, of dat er een wat meer terughoudende aanpak op zijn plaats is.

In tegenstelling tot lupus nefritis, waarover relatief veel bekend is, is neuropsychiatrische lupus waarschijnlijk de minst begrepen uitingvorm van SLE. Ongeveer 15–80% van de patiënten met SLE krijgt op enig moment een uiting van neuropsychiatrische lupus. Bij neuropsychiatrische lupus is er ontsteking van de hersenen of van zenuwen, hetgeen kan leiden tot uiteenlopende verschijnselen zoals hoofdpijn, epilepsie, psychose, een hersenbloeding of -infarct, depressie en aantasting van zenuwen in ledematen. De moeilijkheid om neuropsychiatrische lupus te diagnosticeren kan worden verklaard door de afwezigheid van duidelijke definities voor de diagnose, de afwezigheid van een test die met 100% zekerheid neuropsychiatrische lupus kan vaststellen en de grote verscheidenheid aan neuropsychiatrische symptomen. Omdat het nemen van een biopsie uit het brein vaak praktisch niet mogelijk en niet wenselijk is, is er ook weinig bekend over wat daadwerkelijk de patronen van schade en ontsteking zijn op weefselniveau.

In dit proefschrift is getracht om de schade op weefselniveau van neuropsychiatrische lupus beter in kaart te brengen (**hoofdstuk 6**). Hiervoor is een studie uitgevoerd gebruikmakend van hersenweefsel van overleden lupuspatiënten met en zonder uitingen in het centraal zenuwstelsel, alsmede van overleden patiënten zonder lupus. Opvallend is de schade bij neuropsychiatrische lupus gekenmerkt door de aanwezigheid van kleine stolsels in vaten en zuurstoftekort van het omliggende weefsel. Echte ontsteking met de aanwezigheid van immuuncellen, zoals in de nier vaak wordt gezien bij lupus nefritis, zijn in de hersenen vaak afwezig. Als mechanisme van ontsteking is het complementsysteem onderzocht, omdat dit systeem mogelijk een link kan zijn tussen ontsteking door antistoffen en het ontstaan van stolsel en zuurstoftekort. Zoals eerder genoemd worden eiwitten van het complementsysteem gebruikt bij het opruimen van verbindingen of “complexen” met antistoffen in het bloed (zie **Tabel 1**). In hoofdstuk 6 is beschreven dat de zogenaamde “klassieke” complementeiwitten in verhoogde mate werden gevonden in de hersenen van SLE-patiënten zowel met als zonder neuropsychiatrische manifestaties, maar niet in patiënten zonder SLE. Kleine stolsels werden alleen gevonden in breinen van patiënten met neuropsychiatrische lupus en niet in breinen van andere SLE-patiënten. Een belangrijke conclusie van dit onderzoek is dat het complementsysteem een belangrijke speler is betrokken bij het berokkenen van neurologisch schade in neuropsychiatrische lupus en mogelijk ook een belangrijk doelwit van behandeling kan zijn. Complementremmers worden momenteel al toegepast bij een aantal andere ziektes, maar kunnen mogelijk ook goed werken bij neuropsychiatrische lupus.

Aangezien de oorzaak van SLE nog steeds onbekend is en beter begrip van oorzakelijke factoren kan helpen om de diagnose te stellen of om gerichte behandelingen aan te wijzen, is hier verder onderzoek naar gedaan. De observaties dat SLE met name voorkomt bij vrouwen in de vruchtbare levensfase en zwangerschap van invloed is op het ontstaan en beloop van SLE, zijn er veel theorieën over het verband tussen zwangerschap en het ontstaan van SLE. In de jaren 90 is voor het eerst aangetoond dat tijdens de zwangerschap cellen van het ongeboren kind of “foetus” via de placenta in de bloedstroom van de moeder terecht kunnen komen en wel tot 27 jaar na de bevalling daar kunnen blijven. Dit fenomeen wordt ook wel “chimerisme” genoemd, een fenomeen omschreven als het voorkomen van cellen in een individu die afkomstig zijn van een ander individu. De voornaamste bron van chimerisme is zwangerschap, waarbij er uitwisseling van cellen van moeder naar kind en vice versa plaatsvindt via de placenta. Bijna alle zwangere vrouwen zijn chimeer, maar ook jaren na de zwangerschap kunnen nog chimere cellen in de bloedstroom van de moeder worden gevonden. Zo is het mogelijk dat chimere cellen verkregen tijdens de zwangerschap een immuunreactie teweeg brengen, resulterend in een auto-immuun ziekte als SLE. Echter is nooit aangetoond dat de hoeveelheid chimere cellen daadwerkelijk is verhoogd in organen van zwangere vrouwen. In **hoofdstuk 7** is gevonden dat de hoeveelheid mannelijke chimere cellen (met een Y chromosoom) in organen van vrouwen overleden tijdens de zwangerschap van een zoon is verhoogd ten opzichte van de hoeveelheid lang na de zwangerschap. Deze bevinding maakt chimerisme verkregen via de zwangerschap een intrigerende mogelijke oorzaak van een auto-immuunziekte als SLE. Opvallend was dat chimere cellen met name gevonden werden in de long, gevolgd door de milt, de nieren, het hart en het brein. Dit patroon van verdeling van chimere cellen over organen weerspiegelt het patroon dat al eerder is gevonden in een muismodel, hetgeen bevestigt dat proeven met muismodellen geschikt zijn om chimerisme tijdens de zwangerschap verder te onderzoeken in relatie tot ziekte.

De hoeveelheid chimerisme die achterblijft na de zwangerschap verschilt tussen vrouwen en is mogelijk gerelateerd aan de reactie van het immuunsysteem van de moeder op de cellen afkomstig van het kind. Weinig is bekend over hoe het immuunsysteem normaal reageert op cellen afkomstig van een kind en hoe de balans kan verschuiven in het geval van auto-immuunziekte. Het doel van de studie beschreven in **hoofdstuk 8** was om het voorkomen en oorsprong van chimere cellen in vrouwen met SLE in kaart te brengen vergeleken met gezonde vrouwen. We vonden dat chimerisme vaker voorkwam in het bloed van vrouwen met SLE vergeleken met gezonde vrouwen. Chimerisme was in de moeders vaker afkomstig van hun kind dan van hun eigen moeder. In vrouwen met SLE kon het chimerisme afkomstig zijn van verschillende familieleden, terwijl in gezonde vrouwen het chimerisme altijd afkomstig was van slechts een familielid. Deze resultaten ondersteunen de hypothese dat chimerisme een rol speelt in SLE.

Conclusie: op naar “personalised medicine” bij patiënten met SLE

In dit proefschrift zijn verschillende uitdagingen onderzocht die verband houden met het stellen van de diagnose en het voorspellen van de prognose van SLE. Een belangrijke conclusie van dit proefschrift is dat “personalised medicine” de maatstaf moet zijn voor patiënten met SLE. Er moet speciale aandacht zijn om uitkomsten van individuele patiënten met SLE te verbeteren door het bestuderen van verschillende voorspellende of “prognostische” factoren die zijn geassocieerd met verschillende uitkomsten. Op deze manier moeten alleen patiënten behandeld worden die baat zullen hebben bij behandeling. Classificaties moeten daarom ontwikkeld worden uitgaande van prognostische subgroepen gekenmerkt door een combinatie van verschillende klinische en histologische kenmerken. Kennis over oorzakelijke factoren kan helpen bij het stellen van de diagnose, maar kan ook inzichten brengen met betrekking tot mogelijke gerichte behandelingen. Verder onderzoek zal zich moeten richten op de identificatie van oorzakelijke factoren die mogelijk ook doelwitten kunnen zijn voor gerichte therapie, nieuwe prognostische factoren die prognostische subgroepen beter kunnen onderscheiden en factoren die de respons op behandeling kunnen voorspellen.

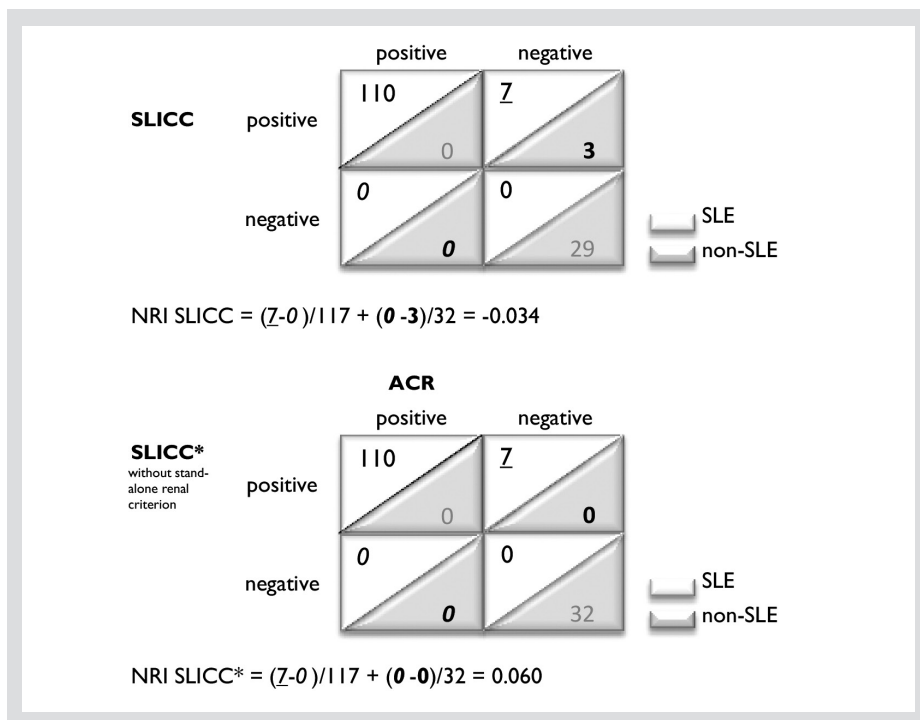
Appendix

Chapter 2	210
Appendix 2.1. Reclassification tables	210
Appendix 2.2. Prevalence of individual SLICC criteria	211
Chapter 3	212
Appendix 3.1. Clinical characteristics of patients with non-lupus full house nephropathy	212
Appendix 3.2. Electron microscopy findings in patients with non-lupus FHN	216
Chapter 5	217
Appendix 5.1. Histopathology definitions	217
Appendix 5.2. Detailed study outcomes and statistical methods	219
Appendix 5.3 Correlations between histopathologic variables	223
Appendix 5.4 Distribution of histopathologic lesions across patients with LN	226
<i>Correlations between histopathologic variables and the number of scorable glomeruli</i>	
Appendix 5.5. Correlations between histopathologic variables and mean arterial pressure, eGFR, and proteinuria at the time of renal biopsy	227
Appendix 5.6. Analysis of progressive eGFR decline	228
Appendix 5.7	230
<i>Interactions between histopathologic variables and race</i>	230
<i>Interactions between histopathologic variables and age</i>	230
<i>Interactions between histopathologic variables and therapy</i>	230
Appendix 5.8. ISN/RPS classes in relation to outcome	231
Chapter 6	232
Appendix 6.1. Case histories	232
Appendix 6.2. Post-mortem neuroimaging and evaluation of the acquired images	233
Appendix 6.3. Relationship between C1q, C4d, and C5b-9 in SLE and NP-SLE	234
Chapter 8	235
Appendix 8.1. Primers	235

Chapter 2

Appendix 2.1. Reclassification tables

Reclassification tables of cases with and without SLE used for calculation of the net reclassification improvement (NRI)



The NRI is based on reclassification tables constructed separately for patients with and without a reference standard clinical diagnosis of SLE ([SLE(+)] and [SLE(-)], respectively), and quantifies the correct movement in categories: [up] for fulfilling classification criteria and [down] for unfulfilling classification criteria as compared with reference classification criteria. The NRI can be expressed as follows:

$$NRI = \frac{\sum up|SLE(+) - down|SLE(+)}{\#SLE(+)} + \frac{\sum down|SLE(-) - up|SLE(-)}{\#SLE(-)}$$

Appendix 2.2. Prevalence of individual SLICC criteria

Prevalence of individual SLICC criteria in the full house cohort and the SLICC derivation cohort

Criterion	Patients with clinical SLE			Patients without clinical SLE		
	Full house cohort (n=117)	SLICC derivation cohort (n=310)	P	Full house cohort (n=32)	SLICC derivation cohort (n=392)	P
SLICC clinical criteria	n/total (%)	%		n/total (%)	%	
Acute cutaneous lupus	66/117 (56.4)	65.2	0.116	0/32 (0)	19.9	0.002
Chronic cutaneous lupus	14/117 (12.0)	19.7	0.065	0/32 (0)	6.4	0.242
Non-scarring alopecia	21/117 (17.9)	31.9	0.004	0/32 (0)	4.3	0.629
Oral/nasal ulcers	25/117 (21.4)	44.2	<0.001	3/32 (9.4)	7.9	0.734
Arthritis	86/117 (73.5)	79.0	0.243	1/32 (3.1)	56.4	<0.001
Serositis	44/117 (37.6)	35.2	0.652	1/32 (3.1)	2.8	1.000
Renal disorder	117/117 (100.0)	32.9	*	32/32 (100)	3.6	*
Neurologic disorder	18/117 (15.4)	5.5	0.002	0/32 (0)	1.0	1.000
Haemolytic anaemia	20/117 (17.1)	7.1	0.003	0/32 (0)	0.5	1.000
Leukopenia	36/117 (30.8)	46.4	0.004	0/32 (0)	5.2	0.385
Thrombocytopenia	28/117 (23.9)	13.5	0.013	0/32 (0)	2.0	1.000
SLICC immunologic criteria						
Antinuclear antibody	116/117 (99.1)	96.5	0.193	3/26 (11.5)	3.2	0.070
Anti-dsDNA	80/109 (73.4)	57.1	0.029	0/28 (0)	4.1	0.614
Anti-Sm	18/56 (32.1)	26.1	0.414	0/8 (0)	1.3	1.000
Antiphospholipid antibody	41/81 (50.6)	53.6	0.708	0/5 (0)	14.0	1.000
Hypocomplementaemia	94/109 (86.2)	59.0	<0.001	2/23 (8.7)	7.4	0.686
Direct Coombs' test	25/75 (33.3)	-	-	0/13 (0)	-	-

SLICC validation cohort derived from Petri et al.¹ Fulfilment of SLICC classification criteria in the full house cohort was registered up to and including the moment of renal biopsy. "Clinical SLE" refers to the reference standard clinicopathologic diagnosis of SLE. Anti-dsDNA, anti-double stranded DNA antibody. * Different by selection.

Chapter 3

Appendix 3.1. Clinical characteristics of patients with non-lupus full house nephropathy at the time of renal biopsy

Patient number	Clinicopathologic diagnosis	Serology at the time of biopsy	SLE criteria
Idiopathic non-lupus FHN			
1	Idiopathic minimal change-like nephropathy	ANA(-), ASO(-)	(-)
2	Idiopathic minimal change-like nephropathy	ANA(-), ASO(-)	(-)
3	Idiopathic mesangioproliferative nephropathy	ANA(-), ASO(-)	(-)
4	Idiopathic proliferative glomerulonephritis	ANA(-), HBsAg(-)	Hypocomplementaemia*
5	Idiopathic proliferative glomerulonephritis	ANA(dubious), anti-dsDNA(-), anti-GBM(-)	Nasal ulcers†
6	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), HBsAg(-)	Arthritis†, pleuritis*
7	Idiopathic proliferative glomerulonephritis	ANA(-), ASO(-)	(-)
8	Idiopathic proliferative glomerulonephritis	n/a	(-)
9	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), anti-GBM(-), ANCA(-), ASO(-)	(-)
10	Idiopathic proliferative glomerulonephritis	ANA(dubious), ANCA(-), anti-dsDNA B(-),	ANA (12)
11	Idiopathic proliferative glomerulonephritis	ANA(-)	(-)
12	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), ASO(-)	Arthritis (9)
13	Idiopathic MPGN + MN	n/a	(-)
14	Idiopathic proliferative glomerulonephritis	ANA(-), ANCA(-)	(-)
15	Idiopathic MN	ANA(-), HBsAg(-)	(-)
16	Idiopathic MN + TIN	ANA(-), HBsAg(-)	(-)
17	Idiopathic MN	ANA(-), ASO (-)	(-)
18	Idiopathic MN	n/a	(-)
19	Idiopathic proliferative glomerulonephritis	ANA(-), anti-dsDNA(-), HBsAg(-)	Serositis (2), arthritis (2)
20	Idiopathic focal segmental glomerulosclerosis	ANA(-), ASO(-)	(-)
Secondary non-lupus FHN			
21	IgA-like nephropathy	ANA(-), ASO(-)	(-)
22	Crescentic IgA-like nephropathy	HBV IgG(+)/IgM(-)/HBsAg(-)	(-)
23	Crescentic IgA-like nephropathy	ANA(-), HBsAg(-), anti-HCV(-), anti-HIV(-)	(-)
24	Crescentic IgA-like nephropathy	ANA(-), ASO(-)	(-)

Co-morbidity	Induction therapy	ESRD/death§	Follow-up, y
(-)	CS	(-)	34
DM II†	(-)	HD (2), deceased (13)	11
DM II (27), hypertension (27)	(-)	(-)	38
Alpha-thalassaemia*	CS	HD*, Tx (2)	24
Pulmonary silicosis†, nasal ulcers†	CS	CAPD*, Tx (1), deceased (11)	8
Seronegative RA*	CS	CAPD (1), deceased (11)	1
(-)	(-)	Tx (2), deceased (20)	20
Migraines (11 years of analgesic use)†, DM II (36)	(-)	CAPD (26), Tx (29)	41
Haemoptysis (9)	(-)	CAPD (15)	24
Pneumonia (Mycoplasma)*, gout*, pancytopenia (14)	Cyclosporin + CS	(-)	9
Malignant hypertension†, primary Raynaud's phenomenon (35)	(-)	Tx (39)	39
RA†, episcleritis*, cutaneous vasculitis*, pleuritis (4), pericarditis (7)	AZA + CS	Deceased (13)	13
Return of MPGN in kidney graft (5, 20), myocardial infarction (18)	AZA + CS	CAPD (4), Tx (5), deceased (28)	28
(-)	(-)	CAPD (3), Tx (7)	26
Migraine†, para-aortal lymphadenopathy (9)	(-)	Deceased (16)	15
Hypertensive retinopathy†, CAD (2)	CS	(-)	2
Coeliac disease (31), Hashimoto's thyroiditis (31)	(-)	(-)	34
Erysipelas*, hypogammaglobulinemia*, cocaine/cannabis abuse (6)	AZA + CS	CAPD (7), Tx (16)	21
Myocardial infarction†	(-)	Deceased (16)	7
Hashimoto's thyroiditis (13)	(-)	CAPD (16), Tx (20)	34
IgA nephropathy in transplanted kidney (14), prostate carcinoma and renal mass (35)	CS	Tx (14)	36
IgA nephropathy in transplanted kidney (16), CAD (17), DM II (23), Guillain-Barré syndrome (32)	Unknown	CAPD (14), Tx (16), deceased (36)	36
Preeclampsia†, malignant hypertension*, IgA nephropathy in transplanted kidney (3)	(-)	HD*, Tx (3)	12
IgA nephropathy in transplanted kidney (4, 13)	CS	Tx (4)	35

Appendix 3.1. Continued.

Patient number	Clinicopathologic diagnosis	Serology at the time of biopsy	SLE criteria
Secondary non-lupus FHN			
25	Infection-related glomerulonephritis	Anti-dsDNA(-), anti-amoeba(+), anti-malaria(-), HAV IgG(+)/IgM(-)	(-)
26	Infection-related glomerulonephritis	n/a	(-)
27	ANCA-associated glomerulonephritis	PR3-ANCA(+), ANA(-), anti-GBM(-), anti-dsDNA(-), HBsAg(-), anti-HCV(-)	Hypocomplementaemia*, nasal ulcers*
28	ANCA-associated glomerulonephritis	ANA(-), anti-ENA(-), anti-cardiolipin(-), PR3-ANCA(+)	ANA† ([–] during FU), nasal ulcers*
29	MN (anti-PLA2R)	ANA(-), anti-PLA2R(+), aCI q(-)	(-)
30	MN (cancer-associated) + FSGS	ANA(-), ASO(-)	(-)
31	MN (cancer-associated) + secondary glomerular sclerosis	ANA(+), anti-dsDNA(-), HBsAg(-), anti-HCV(-)	ANA*, ANA (4)
32	MN (cancer-associated) + minimal endocapillary proliferation	ANA(+), anti-dsDNA(-), aSS-A(+)	ANA* (not tested during follow-up)

Numbers in parentheses represent years since renal biopsy showing full house immunofluorescence. * indicates that the phenomenon occurred at the time of renal biopsy showing full house immunofluorescence; † indicates that the phenomenon occurred >6 months before the renal biopsy showing full house immunofluorescence; § Patients 2, 12, 22, and 31 died of cardiovascular disease, patients 30 and 32 of malignancy, patient 7 of terminal renal insufficiency, patient 13 of sepsis, and patients 4, 5, 15, 19, and 26 of unknown causes. (+), present; (-), absent; n/a, not available; ESRD, end-stage renal disease; ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; ASO, anti-streptolysin O; AZA, azathioprine; CAD, coronary artery disease; CAPD, chronic

Co-morbidity	Induction therapy	ESRD/death§	Follow-up, y
Malaria†, amoebic liver abscess *, DM II (20)	(-)	(-)	20
Pyelourethral obstruction†, enterococcal sepsis*, cardiomyopathy (2), pericarditis (5)	(-)	Tx (3), deceased (13)	13
Nasal ulcers*, cerebral vasculitis*, cavitating pulmonary lesions (15)	CYC + CS	HD*,Tx (5)	19
ENT-limited PR3-ANCA-associated vasculitis*	CYC + CS	(-)	8
Multiple pulmonary emboli*	CS	(-)	4
Squamous cell carcinoma lung*	(-)	Deceased (8)	0.4
Prostate carcinoma*	(-)	Deceased (9)	20
Metastasised cervical carcinoma†	CS	Deceased (3)	3

ambulant peritoneal dialysis; CS, corticosteroids; CYC, cyclophosphamide; DM, diabetes mellitus; dsDNA, double stranded DNA; ENA, extractable nuclear antigen; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HD, haemodialysis; HIV, human immunodeficiency virus; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; PLA2R, anti-phospholipase A2 receptor; RA, rheumatoid arthritis; TIN, tubulointerstitial nephritis; Tx, transplantation.

Appendix 3.2. Electron microscopy findings in patients with non-lupus (full house nephropathy) FHN

Patient number	Immune deposits	Electron microscopy Podocyte foot process effacement
<i>Idiopathic non-lupus FHN</i>		
1	(-)	(+)
2	n/a	n/a
3	n/a	n/a
4	mes-sed-sep	(+)
5	n/a	n/a
6*	mes-sed-tm-sep	(+)
7	n/a	n/a
8	n/a	n/a
9	sep	(+)
10	sep	(+)
11	n/a	n/a
12	sep	(+)
13	mes-sed-sep	n/a
14	tm-sep	(+)
15	n/a	n/a
16	n/a	n/a
17	n/a	n/a
18	sep	(+)
19	n/a	n/a
20	n/a	n/a
<i>Secondary non-lupus FHN</i>		
21	n/a	n/a
22	n/a	n/a
23	sed	(+)
24	n/a	n/a
25	mes-sed	(+)
26	mes-sed-tm-sep (humps)	(+)
27	n/a	n/a
28	n/a	n/a
29	sep	(+)
30	mes-sep	(+)
31	sep	(+)
32*	sep	(+)

N/a, not available; mes, mesangial; sed, subendothelial; tm, transmembranous; sep, subepithelial. *Tubuloreticular inclusions were identified by electron microscopy.

Chapter 5

Appendix 5.1. Histopathology definitions

Definitions for glomerular lesions

Scorable glomeruli: Glomeruli that remained after excluding those which had less than 3 mesangial fields because they were too small, on the edge of the biopsy and therefore incomplete, or otherwise artefactually damaged.

Focal: Involving <50% of glomeruli.

Diffuse: Involving ≥50% of glomeruli.

Segmental: Lesion involving less than half of the glomerular area inside Bowman's capsule.

Global: Lesion involving more than half of the glomerular area inside Bowman's capsule.

Normal glomerulus: Glomerulus without a lesion. A normal glomerulus may show subtle changes as a result of ischemia.

Minimal leukocyte influx: Occurrence of <4 neutrophils, lymphocytes, or monocytes in an otherwise normal glomerulus, in the absence of endothelial cell swelling.

Global sclerosis: Sclerosis of the entire glomerulus (obliteration of the capillary lumen by increased extracellular matrix, with or without hyalinosis or foam cells).

Segmental sclerosis: Less than 100% of the glomerulus is sclerosed (obliteration of the capillary lumen by increased extracellular matrix, with or without hyalinosis or foam cells).

Ischemic glomerulus: A glomerulus showing one or more of the following lesions: wrinkling of the glomerular basement membrane (GBM), collapse of the capillary tuft, thickening/splitting of Bowman's capsule.

Mesangial hypercellularity: Four or more nuclei in the contiguous matrix of a peripheral mesangial segment. Note: mesangial hypercellularity is scored for each glomerulus by assessing the most cellular mesangial area. Mesangial areas immediately adjacent to the vascular stalk should not be scored. Scoring categories: (0) <4 nuclei; (1) 4–5 nuclei; (2) 6–7 nuclei; (3) >7 nuclei.

Mesangial matrix expansion: Width of extracellular matrix exceeding 2 mesangial cell nuclei in ≥2 glomerular lobules.

Endocapillary hypercellularity: Hypercellularity due to an increased number of cells within glomerular capillary lumina (leukocytes or endothelial cells), causing narrowing of the lumina. Indicate whether lesion is segmental or global.

Endocapillary inflammatory infiltrate: ≥4 inflammatory cells in the glomerulus – either granulocytes, lymphocytes, or monocytes.

Endothelial cell swelling: Prominence of endothelial cells in capillary lumens with narrowing of the lumen.

Wire loop: Capillary wall thickening characterised by subendothelial immune complex deposits as demonstrated in the PAS staining.

Adhesion: Area of continuity between glomerular tuft and Bowman's capsule separate from extracapillary lesion or from area of segmental sclerosis.

Crescent: one of the following lesions involving > 10% of circumference of Bowman's capsule:

- **Cellular crescent:** Extracapillary cell proliferation of ≥ 3 cell layers with $\geq 50\%$ of the lesion occupied by cells.
- **Fibrocellular crescent:** Extracapillary lesion comprising cells and extracellular matrix, with $< 50\%$ cells and $< 90\%$ matrix.
- **Fibrous crescent:** Extracapillary lesion composed of $\geq 90\%$ matrix.
- **Segmental crescent:** Lesion occupying less than 50% of the circumference of Bowman's capsule.
- **Circumferential crescent:** Lesion occupying 50% or more of the circumference of Bowman's capsule.

Fibrinoid necrosis: Disruption of the GBM with fibrin exudation and karyorrhexis.

Karyorrhexis: Presence of fragmented nuclei including apoptosis.

Microthrombus: A microscopic clump of fibrin, platelets, and red blood cells.

Pseudothrombus: Eosinophilic, rounded aggregates in glomerular capillaries due to immune complex precipitates rather than fibrin, also known as hyaline thrombi.

Double contour/tram track: Double layer of GBM separated by clear zone on silver or PAS stains.

Spikes/vacuoles: Extensions of glomerular basement membrane between deposits (egg racks).

Definitions of tubulointerstitial lesions

Interstitial infiltration: Inflammatory cells within the cortical interstitium in more than 5% of the cortical area. Specify dominant cell type of infiltrate: either lymphocytes, granulocytes, or other. Scoring categories: (0) $< 5\%$; (1) 5–24%; (2) 25–49%; (3) $\geq 50\%$ of the cortical area.

Interstitial fibrosis: Extracellular matrix separating tubules in more than 5% of the cortical area. Scoring categories: (0) $< 5\%$; (1) 5–24%; (2) 25–49%; (3) $\geq 50\%$ of the cortical area.

Focal cortical atrophy: Subcapsular ischemic cortical atrophy, sharply demarcated from normal cortex.

Tubular atrophy: Loss of cytoplasmic organelles, accompanied by a decreased diameter of tubules and thick irregular tubular basement membrane (TBM). Scoring categories: (0) $< 5\%$; (1) 5–24%; (2) 25–49%; (3) $\geq 50\%$ of tubules.

Acute tubular injury: Necrosis of tubular epithelial cells (coagulation necrosis, karyorrhexis, pyknosis), swelling and clear vacuolation of tubular epithelium; can be accompanied by separation/detachment of tubular epithelium from TBM.

Tubular casts: Presence of proteinaceous structures within the lumen of the tubules; may contain cellular debris; only scored when present in nonatrophic tubuli.

Tubular luminal macrophages: Presence of macrophages in tubular lumina; to be distinguish from sloughed epithelial cells.

Tubular regeneration: Regeneration following acute tubular injury usually characterised by the presence of mitotic figures.

Tubular reabsorption droplets: PAS/silver-positive resorption droplets in the proximal tubular epithelium.

Tubulitis: Lymphocytes/other inflammatory cells within the epithelial layer of tubules.

Definitions of vascular lesions

Vasculitis: Inflammation in an arterial/arteriolar wall, characterised by the presence of inflammatory cells and/or fibrinoid necrosis.

Fibrinoid necrosis: Homogeneous, fibrin-like, deeply eosinophilic area with disruption of the architecture of the arterial/arteriolar vascular wall.

Thrombosis: Total occlusion of vessel with fibrin.

Hyaline arteriosclerosis: Accumulation of glassy, refractive, strongly PAS-positive material in the arteriolar intima and/or media.

Fibrous intimal hyperplasia: Cellular and fibroelastic intimal thickening with a fibrous intimal projection or cushion bulging into the lumen.

Arterial intimal fibrosis: Concentric thickening of the intima by deposition of collagen.

Appendix 5.2. Detailed study outcomes and statistical methods

Outcomes were studied in two settings: (i) the complete cohort of patients with all observed LN classes who received various therapies and (ii) a subset of patients with class III or IV ($\pm$ V) LN who received induction immunosuppression including cyclophosphamide (CYC), mycophenolate mofetil (MMF), or azathioprine (AZA). Prespecified variables for multivariable analyses were: variables from the reduced histopathology dataset; interaction terms of these variables with race, age, and induction immunosuppression; and the clinical variables sex, race, time since SLE diagnosis, age₀, proteinuria₀, erythrocyturia₀, MAP₀, induction immunosuppression, and decade during which the patient was biopsied. The models described below make no assumptions about the distribution of independent variables and the dependent variable, eGFR, was normally distributed; therefore, the variables were entered in the models without transformation.

Renal flare and ESRD

The outcomes “renal flare” and “ESRD” relate to time-to-event analyses. We studied the time to a first LN flare in patients who achieved (partial) remission after induction therapy. Time to first renal flare was calculated from the date of biopsy until the date of flare for patients who reached this endpoint. For patients who did not, the follow-up time was from the time of biopsy until the last follow-up or until the patient reached ESRD. The definition of ESRD was dialysis-dependence for >3 months or renal transplantation. Time to ESRD was calculated analogous to time to renal flare. Patients who reached ESRD before the last follow-up were regarded as having eGFR=0 mL/min/1.73 m² at the remaining time points. Outcomes were assessed using Kaplan-Meier analysis and Log-Rank tests. To ascertain independent predictors of ESRD and renal flare, multivariable Cox proportional-hazards models were designed including the prespecified variables. The multivariable models were simplified by stepwise removal of the least significant variables. Hazard ratios and 95% confidence intervals were estimated.

eGFR during follow-up

The extent by which variables were associated with irreversible nephron loss was investigated by modelling eGFR during follow-up. An adjusted average level (intercept) and rate (slope) of decline of renal function during follow-up were modelled. Baseline variables were tested for their potential to predict a change in the intercept of this adjusted average level of decline in random intercept/slope linear mixed-effects models. Variables were tested in univariable models in the complete cohort (**Table A5.2.1**). A full model including the prespecified variables was designed and simplified by removing the least significant variables (Wald test) and comparing the goodness of fit of nested models (maximum likelihood ratio test). The distribution of data and the homogeneity of variance were assessed using graphical evaluation of residuals.

Progressive eGFR decline

To investigate progressive eGFR decline that did not necessarily result in ESRD and/or renal flare, variables were analyzed in association with progressive eGFR decline over 1, 5, and 10 years relative to its linear prediction based upon $eGFR_0$. The linear relationship between $eGFR_0$ and the predicted eGFR at time t ($eGFR_{Predicted(t)}$) was defined as: $eGFR_{Predicted(t)} = eGFR_0 * \beta_{(t)} + constant$. Progressive eGFR decline relative to the $eGFR_{Predicted(t)}$ was assessed by calculating the corrected eGFR ($eGFR_{CORR(t)}$), which was defined as the difference between the observed $eGFR(t)$ and predicted $eGFR(t)$.² This procedure created a corrected value that was independent of the starting value. Variables were first tested in univariable linear regression models for the outcomes $eGFR_{CORR(1)}$, $eGFR_{CORR(5)}$, and $eGFR_{CORR(10)}$ in the complete cohort (**Table A5.2.2**). For the complete cohort and the selected subset, a prediction model for these outcomes was designed using automated backward linear regression starting with the prespecified variables.

Table A5.2.1 Univariable predictions of eGFR during follow-up in 105 LN patients.

eGFR in mL/min/1.73 m² during follow-up†

<i>Clinical variables</i>	β (95% CI)
Female sex	-5.5 (-21.5; 10.4)
Age ₀ , y	-0.8 (-1.3; -0.4)*
Race	
Caucasian	0.5 (-13.1; 14.1)
Asian	-0.8 (-16.3; 14.7)
Afro-Caribbean	0.3 (-19.3; 20.0)
Years since diagnosis SLE ₀	-1.2 (-2.3; 0.0)*
Proteinuria ₀ , g/24h	-0.4 (-2.1; 1.4)
Erythrocyturia ₀ > 1+	-11.9 (-42.3; 18.5)
MAP ₀ , mm Hg	-0.5 (-0.9; -0.1)*
ACE inhibitor (after Bx)	-2.2 (-19.1; 14.7)
Cytotoxic immunosuppressive (after Bx)	-13.1 (-39.0; 12.8)
Biopsied after 2000	15.4 (2.5; 28.3)*

Table A5.2.1 Continued.

<i>Glomerular variables</i>		β (95% CI)
% Normal glomeruli/minimal leukocyte influx§		0.4 (0.2; 0.7)*
% Global sclerosis		-0.8 (-1.2; -0.4)*
% Segmental sclerosis		3.3 (-0.3; 6.9)
% Ischemic glomeruli		-0.7 (-1.1; -0.2)*
% Mesangial hypercellularity		0.0 (-0.1; 0.1)
% Mesangial matrix expansion		0.1 (-0.3; 0.4)
% Endocapillary hypercellularity	Any	0.0 (-0.2; 0.2)
	Segmental	0.1 (-0.2; 0.4)
	Global	-0.1 (-0.3; 0.1)
% Endocapillary infiltration	Lymphocytes	0.0 (-0.2; 0.2)
	Monocytes	0.0 (-0.3; 0.2)
	Granulocytes	0.1 (-0.4; 0.6)
% Crescents	Cellular/fibrocellular§	-0.4 (-0.6; -0.1)*
	Fibrous	-1.6 (-2.8; -0.4)*
% Wire loops		0.0 (-0.3; 0.2)
% Adhesions		-0.4 (-1.1; 0.3)
% Fibrinoid necrosis		-0.2 (-1.1; 0.6)
% Karyorrhexis		-0.1 (-0.6; 0.3)
% Double contours		-0.1 (-0.5; 0.2)
% Spikes/vacuoles		0.1 (-0.2; 0.3)
<i>Tubulointerstitial variables</i>		β (95% CI)
IF/TA	5–24%	-3.9 (-14.0; 11.1)
	25–49%	-37.5 (-60.8; -14.2)*
	≥50%	-50.1 (-82.4; -17.7)*
Interstitial infiltration	5–24%	-11.8 (-25.2; 1.6)
	25–49%	13.1 (-20.7; 46.9)
	≥50%	-34.1 (-57.8; -10.5)*
Tubular casts		-19.5 (-32.0; -7.1)*
Tubular macrophages		-15.7 (-32.4; 0.9)
Tubular reabsorption droplets		-10.2 (-23.2; 2.8)
Arterial intimal fibrosis		-44.7 (-67.6; -21.7)*

β represents the mean change in the level of eGFR decline over time with one unit change of the variable.

* $P < 0.05$. † univariable random intercept/random slope mixed-effects models. § The composite variables "normal glomeruli/minimal leukocyte influx" and "extracapillary 2" were used rather than their individual components, as effect sizes of components were in strong accordance (data not shown).

Abbreviations: Bx, biopsy; IF/TA, interstitial fibrosis or tubular atrophy; MAP, mean arterial pressure.

Table A5.2.2 Univariable predictions of progressive eGFR decline (in mL/min/1.73 m²) over 1, 5, and 10 years follow-up in 105 LN patients.

		eGFR decline over 1 year (eGFR _{CORR(1)}) [‡]	eGFR decline over 5 years (eGFR _{CORR(5)}) [‡]	eGFR decline over 10 years (eGFR _{CORR(10)}) [‡]
		β	β	β
Clinical variables				
Female sex		-9.0 (-22.1; 4.1)	6.1 (-13.1; 25.2)	-6.2 (-32.5; 20.1)
Age ₀ , y		-0.5 (-0.9; -0.1)*	-0.9 (-1.5; -0.4)*	-1.2 (-1.9; -0.4)*
Race	<i>Caucasian</i>	10.2 (-0.8; 21.3)	11.2 (-7.1; 29.6)	13.7 (-11.2; 38.6)
	<i>Asian</i>	-2.9 (-15.7; 9.8)	5.3 (-10.8; 21.3)	3.5 (-18.6; 25.5)
	<i>Afro-Caribbean</i>	-16.6 (-32.6; -0.7)*	-28.5 (-50.9; -6.1)*	-28.5 (-59.1; 2.2)
Years since diagnosis SLE ₀		-1.0 (-2.0; -0.1)*	-2.4 (-3.6; -1.2)*	-2.2 (-3.9; -0.5)*
Proteinuria ₀ , g/24h		0.2 (-1.3; 1.6)	-0.5 (-2.5; 1.5)	1.2 (-1.5; 3.8)
Erythrocyturia ₀ >1+		1.6 (-23.5; 26.8)	-2.8 (-41.1; 35.5)	-10.2 (-98.6; 78.2)
MAP ₀ , mm Hg		-0.1 (-0.4; 0.2)	-0.3 (-0.8; 0.2)	-0.9 (-1.4; -0.3)*
ACE inhibitor (after Bx)		0.0 (-13.9; 14.0)	2.1 (-17.9; 22.1)	9.9 (-18.9; 38.7)
Cytotoxic immunosuppressive (after Bx)		1.2 (-20.3; 22.7)	4.4 (-27.2; 36.0)	13.4 (-27.3; 54.1)
Biopsied after 2000		7.6 (-3.3; 18.5)	10.2 (-5.2; 25.6)	7.0 (-14.3; 28.2)
Glomerular variables				
% Normal glomeruli/minimal leukocyte influx§		0.0 (-0.2; 0.2)	0.1 (-0.2; 0.4)	0.1 (-0.3; 0.5)
% Global sclerosis		-0.5 (-0.8; -0.2)*	-0.7 (-1.1; -0.2)*	-0.7 (-1.3; -0.1)*
% Segmental sclerosis		-0.8 (-3.9; 2.3)	2.3 (-2.3; 6.8)	3.0 (-2.5; 8.5)
% Ischemic glomeruli		-0.2 (-0.6; 0.2)	-0.3 (-0.9; 0.3)	-0.6 (-1.5; 0.3)
% Mesangial hypercellularity		-0.1 (-0.2; 0.1)	-0.1 (-0.2; 0.1)	0.0 (-0.2; 0.2)
% Mesangial matrix expansion		-0.1 (-0.3; 0.2)	-0.1 (-0.4; 0.3)	0.0 (-0.5; 0.5)
% Endocapillary hypercellularity	<i>Any</i>	0.2 (0.0; 0.3)	0.2 (-0.1; 0.4)	0.3 (0.0; 0.6)
	<i>Segmental</i>	0.0 (-0.3; 0.2)	0.1 (-0.3; 0.4)	0.4 (-0.1; 0.8)
	<i>Global</i>	0.2 (0.0; 0.4)	0.2 (-0.1; 0.5)	0.1 (-0.3; 0.5)
% Endocapillary infiltration	<i>Lymphocytes</i>	0.1 (-0.1; 0.3)	0.1 (-0.2; 0.3)	0.4 (0.0; 0.7)
	<i>Monocytes</i>	0.2 (0.0; 0.4)	0.2 (0.0; 0.5)	0.4 (0.0; 0.7)*
	<i>Granulocytes</i>	0.4 (0.0; 0.8)*	0.6 (0.0; 1.1)*	0.7 (-0.1; 1.5)
% Crescents	<i>Cellular/fibrocellular§</i>	-0.1 (-0.3; 0.2)	-0.1 (-0.5; 0.2)	-0.1 (-0.6; 0.3)
	<i>Fibrous</i>	-0.6 (-1.6; 0.5)	-0.9 (-2.4; 0.7)	-1.0 (-3.2; 1.2)
% Wire loops		0.2 (-0.1; 0.4)	0.3 (0.0; 0.6)	0.3 (-0.1; 0.8)
% Adhesions		-0.1 (-0.8; 0.5)	-0.4 (-1.2; 0.5)	-0.1 (-1.3; 1.0)
% Fibrinoid necrosis		-1.0 (-1.7; -0.3)*	-1.0 (-2.0; 0.1)	-0.2 (-1.8; 1.5)
% Karyorrhexis		0.2 (-0.2; 0.5)	0.0 (-0.7; 0.6)	-0.6 (-1.7; 0.5)
% Double contours		0.0 (-0.3; 0.3)	-0.2 (-0.7; 0.3)	-0.1 (-0.7; 0.4)
% Spikes/vacuoles		0.0 (-0.3; 0.2)	-0.1 (-0.5; 0.2)	0.0 (-0.4; 0.4)

Table A5.2.2 Continued.

		eGFR decline over 1 year (eGFR _{CORR(1)})‡	eGFR decline over 5 years (eGFR _{CORR(5)})‡	eGFR decline over 10 years (eGFR _{CORR(10)})‡
		β	β	β
<i>Tubulointerstitial variables</i>				
IF/TA	5–24%	3.2 (–9.3; 15.6)	–6.4 (–23.8; 10.9)	–13.5 (–37.0; 10.0)
	25–49%	–17.1 (–37.0; 2.8)	–26.5 (–57.6; 4.6)	–35.5 (–79.9; 9.0)
	≥50%	–27.9 (–55.4; –0.5)*	–33.7 (–71.4; 3.9)	–48.2 (–98.7; 2.3)
Interstitial infiltration	5–24%	–2.5 (–13.8; 8.8)	0.7 (–15.2; 16.5)	–5.5 (–27.0; 16.0)
	25–49%	9.3 (–18.7; 37.2)	4.7 (–33.6; 42.9)	35.5 (–26.9; 98.0)
	≥50%	–11.7 (–31.8; 8.3)	–22.5 (–53.8; 8.7)	–30.2 (–81.6; 21.1)
Tubular casts		–9.8 (–20.4; 0.9)	–14.1 (–29.1; 0.9)	–11.3 (–32.5; 9.8)
Tubular macrophages		8.4 (–5.8; 22.5)	–3.6 (–23.6; 16.4)	10.6 (–16.3; 37.6)
Tubular reabsorption droplets		–5.9 (–16.7; 5.0)	–17.0 (–32.2; –1.9)*	–15.9 (–37.1; 5.3)
Arterial intimal fibrosis		–34.5 (–53.7; –15.3)*	–38.0 (–64.7; –11.3)*	–31.1 (–63.5; 1.4)

Interpretation eGFR_{CORR}^{1/5/10} (in mL/min/1.73 m²): β represents the change in eGFR_{CORR} with one unit change of the variable. * $P < 0.05$. ‡ Univariable linear regression models. § The composite variables “normal glomeruli/minimal leukocyte influx” and “extracapillary 2” were used rather than their individual components, as effect sizes of components were in strong accordance (data not shown).

Abbreviations: Bx, biopsy; IF/TA, interstitial fibrosis or tubular atrophy; MAP, mean arterial pressure.

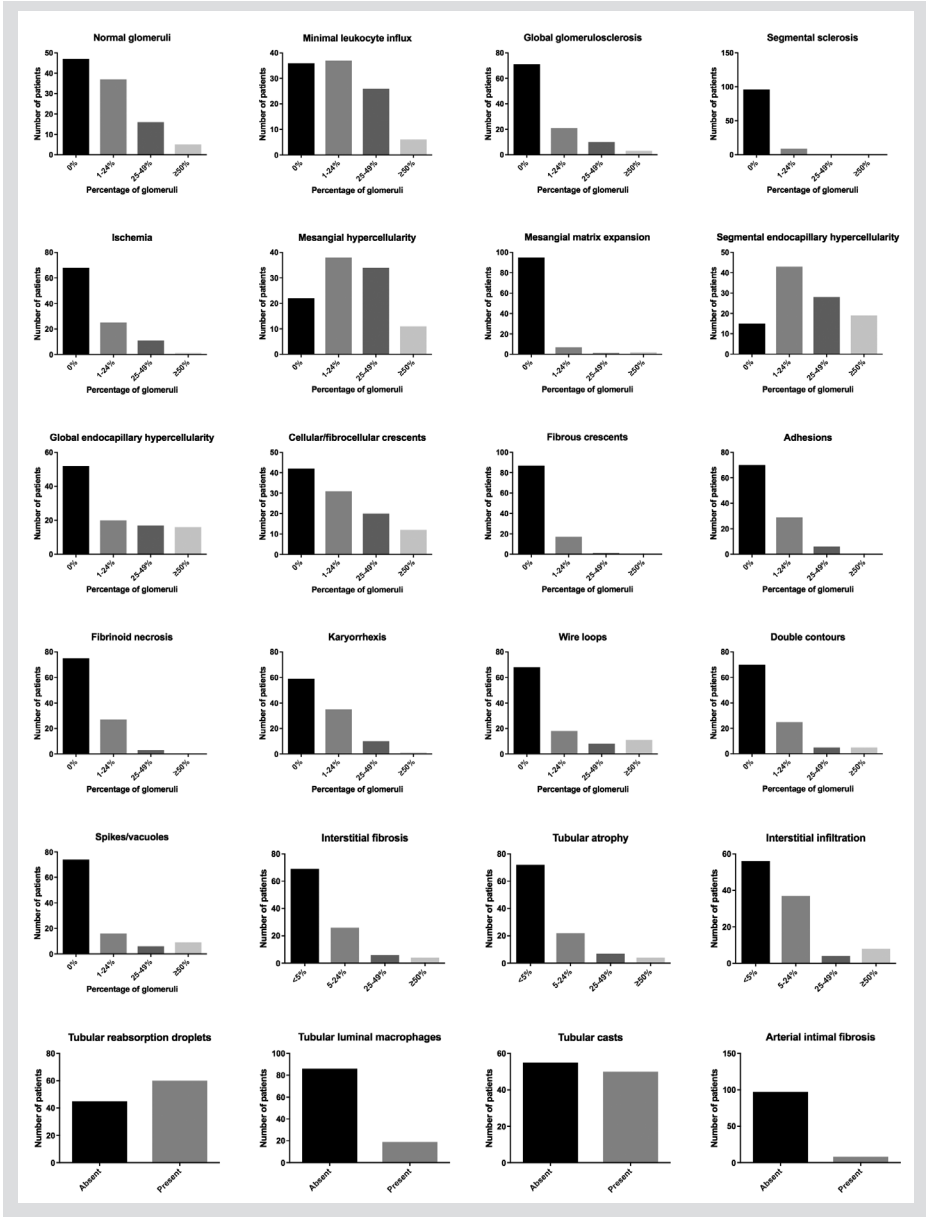
Appendix 5.3 Correlations between histopathologic variables

To prevent the inclusion of strongly correlated variables in our analyses of the various outcomes, we assessed correlations between 29 glomerular and 9 tubulointerstitial variables (occurring in >5 patients), excluding normal glomeruli. Significant correlation coefficients between histopathologic variables after Bonferroni correction are shown in the table below. The following variables that were strongly correlated with other variables ($r/p > 0.8$) were dropped: “mesangial 1” and “extracapillary 3/5” because of relatively laborious scoring; “endocapillary 3” because “minimal leukocyte influx” was encompassed by “normal glomeruli/minimal leukocyte influx”; “endothelial swelling”; “endocapillary inflammatory infiltrate”; and “endocapillary monocytes” because they were encompassed by “endocapillary 1/2/4”; “extracapillary 1” because cellular crescents were encompassed by “extracapillary 2”; and “interstitial lymphocytes” because they were encompassed by “interstitial infiltration”. “Interstitial fibrosis” and “tubular atrophy” were combined (whichever was the higher value) into a composite variable: “interstitial fibrosis/tubular atrophy” (IF/TA). Thus, 29 histopathologic variables remained to be tested in relation to outcomes.

	Mes2	End2	End3	EndSw	EndInf	EndGran	EndLym	EndMon	WL	Extr1
GlobGS			-0.5							
Isch										
Mes1	0.9									
Mes3										0.3
MinLeu		-0.4			-0.4					
End1		0.5	0.6	0.5	0.5					
End2		—	0.8	0.99	0.9	0.5	0.7	0.8	0.5	
End3			—	0.8	0.8		0.6	0.6	0.5	
EndSw				—	0.9	0.5	0.7	0.8	0.5	
EndInf					—	0.5	0.8	0.8	0.4	
EndGran						—		0.4		
EndLym							—	0.6		
EndMon								—	0.5	
Extr1										
Extr2										
Extr3										
Extr4										
TA										
IntInf										

Because 666 comparisons were assessed, the significance was set to $P=8 \cdot 10^{-5}$ by Bonferroni correction ($P=0.05/666$). Only correlation coefficients with $P < 8 \cdot 10^{-5}$ are shown. Numbers in the cells represent Pearson/Spearman correlation coefficients. Correlation coefficients >0.8 are bold-printed. Abbreviations: DC, double contours; End1/2/3: endocapillary 1/2/3; EndInf, endocapillary inflammatory infiltrate; EndGran, endocapillary granulocytes; EndLym, endocapillary lymphocytes; EndMon, endocapillary monocytes; EndSw: endothelial cell swelling; Extr1/2/3/4/5: extracapillary 1/2/3/4/5; GlobGS, global sclerosis; IF, interstitial fibrosis; ArtIF, arterial intimal fibrosis; IntInf, interstitial infiltration; IntLym, interstitial lymphocytes; IntGran, interstitial granulocytes; Mes1/2/3: mesangial 1/2/3; MinLeu, minimal leukocyte influx; TA, tubular atrophy; WL, wire loops.

Appendix 5.4 Distribution of histopathologic lesions across patients with LN



Correlations between histopathologic variables and the number of scorable glomeruli

None of the glomerular or tubulointerstitial variables were correlated with the number of scorable glomeruli. Patients who did not have more ($\geq$) than 10 scorable glomeruli in their renal biopsies – as is the minimum biopsy requirement according to the ISN/RPS – had 5 ($n=5$), 6 ($n=3$), 7 ($n=2$), 8 ($n=6$), or 9 ($n=3$) scorable glomeruli. A comparison between patients with fewer ($<$) or more ($\geq$) than 10 scorable glomeruli revealed that only the distribution of karyorrhexis, endocapillary granulocytes, and monocytes was different between the groups (all $P<0.05$; higher scores in patients with ≥ 10 scorable glomeruli). Following these results, we decided to uphold our initial threshold of ≥ 5 scorable glomeruli. Karyorrhexis, endocapillary granulocytes, and endocapillary monocytes were not univariably associated with any of the outcomes studied. These variables were not incorporated in our statistical models.

Appendix 5.5. Correlations between histopathologic variables and mean arterial pressure (MAP), eGFR, and proteinuria at the time of renal biopsy₀ in 105 patients with LN

	eGFR ₀ , mL/min/1.73 m ²		Proteinuria ₀ , g/24h		MAP ₀ , mm Hg		
<i>Glomerular variables</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	
% Normal glomeruli	0.3	<0.001*	-1.1	0.31	-0.2	0.02*	
% Minimal leukocyte influx	0.3	<0.001*	-0.2	0.06	-0.3	0.007*	
% Normal glomeruli/minimal leukocyte influx	0.4	<0.001*	-0.2	0.05*	-0.3	0.001*	
% Global sclerosis	-0.2	0.02*	0.0	0.78	0.1	0.27	
% Ischemic glomeruli	-0.2	0.01*	0.0	0.82	0.0	0.78	
% Endocapillary hypercellularity	Any	-0.2	0.11	0.2	0.02*	0.3	<0.001*
	Segmental	0.1	0.36	0.0	0.85	0.2	0.08
	Global	-0.3	0.008*	0.3	0.007*	0.3	0.009*
% Wire loops	-0.1	0.20	0.2	0.11	0.2	0.02*	
% Cellular/fibrocellular crescents	-0.2	0.03*	0.2	0.10	0.2	0.02*	
<i>Tubulointerstitial/vascular variables</i>	<i>ρ</i>	<i>P</i>	<i>ρ</i>	<i>P</i>	<i>ρ</i>	<i>P</i>	
IF/TA	-0.3	0.001*	0.1	0.67	0.2	0.03*	
Interstitial infiltration	-0.3	0.002*	0.4	<0.001*	0.1	0.23	
Tubular reabsorption droplets	-0.1	0.34	0.2	0.07	0.2	0.03*	
Tubular casts	-0.3	0.007*	0.2	0.10	0.2	0.14	
Tubular macrophages	-0.3	0.008*	0.3	0.005*	0.1	0.18	
Arterial intimal fibrosis	-0.2	0.03*	0.0	0.78	0.2	0.04*	

Only variables with any significant correlation with eGFR₀, proteinuria₀, and/or MAP₀ are shown.**P*<0.05. IF/TA, interstitial fibrosis or tubular atrophy; *r*, Pearson's correlation coefficient; *ρ*, Spearman's correlation coefficient.

Appendix 5.6. Analysis of progressive eGFR decline

Results

Results for the complete cohort and the subset were comparable. In the complete cohort, a decline of eGFR over 1 and 5 years was independently predicted by non-Caucasian race (1 year: -17.7 mL/min/ 1.73 m²; 5 years: -17.8 mL/min/ 1.73 m²), age₀ (1 year: -0.5 mL/min/ 1.73 m²/year; 5 years: -0.9 mL/min/ 1.73 m²/year), fibrinoid necrosis (1 year: -1.4 mL/min/ 1.73 m²%glomeruli; 5 years: -1.5 mL/min/ 1.73 m²%glomeruli), fibrous crescents (1 year: -1.2 mL/min/ 1.73 m²%glomeruli; 5 years: -1.6 mL/min/ 1.73 m²%glomeruli), and the presence of IF/TA $\geq 25\%$ (1 year: -21.7 mL/min/ 1.73 m²; 5 years: -30.3 mL/min/ 1.73 m²). Over 10 years follow-up, MAP₀ was associated with eGFR decline (-1.1 mL/min/ 1.73 m²/mm Hg), and endocapillary lymphocytes and wire loops were associated with eGFR recovery (both $+0.4$ mL/min/ 1.73 m²%glomeruli).

Multivariable prediction models for progressive eGFR decline in mL/min/1.73 m².

	ISN/RPS Class I–V (n=105)	ISN/RPS Class III/IV (±V)‡ (n=91)
Progressive eGFR decline over 1 year (eGFR_{CORR(1)})† (n=99)		
<i>Variables</i>	<i>β</i> (95% CI)	<i>β</i> (95% CI)
(Constant)	30.6 (17.0; 44.1)	33.1 (18.2; 47.9)
Non-Caucasian	−17.7 (−27.7; −7.7)	−21.1 (−32.2; −10.0)
Age ₀ , y	−0.5 (−0.9; −0.2)	−0.5 (−0.9; −0.1)
% Fibrinoid necrosis (glomerular)	−1.4 (−2.0; −0.8)	−1.4 (−2.0; −0.8)
% Fibrous crescents	−1.2 (−2.1; −0.2)	−1.3 (−2.3; −0.2)
IF/TA ≥25%	−21.7 (−36.2; −7.2)	−25.2 (−40.8; −9.5)
Tubular macrophages present	9.7 (−2.6; 21.9)	11.1 (−2.1; 24.4)

Progressive eGFR decline over 5 years (eGFR_{CORR(5)})† (n=98)		
<i>Variables</i>	<i>β</i> (95% CI)	<i>β</i> (95% CI)
(Constant)	45.7 (26.2; 65.2)	45.2 (24.4; 66.0)
Non-Caucasian	−17.8 (−33.0; −2.7)	−22.9 (−39.1; −6.6)
Age ₀ , y	−0.9 (−1.5; −0.4)	−0.8 (−1.4; −0.3)
% Fibrous crescents	−1.6 (−3.0; −0.2)	−1.7 (−3.3; −0.1)
% Fibrinoid necrosis (glomerular)	−1.5 (−2.5; −0.5)	−1.5 (−2.6; −0.5)
IF/TA ≥25%	−30.3 (−52.5; −8.1)	−31.7 (−54.4; −9.1)

Progressive eGFR decline over 10 years (eGFR_{CORR(10)})† (n=71)		
<i>Variables</i>	<i>β</i> (95% CI)	<i>β</i> (95% CI)
(Constant)	91.1 (40.4; 141.8)	93.6 (35.6; 151.6)
MAP ₀ , mm Hg	−1.1 (−1.6; −0.6)	−1.1 (−1.7; −0.6)
% Endocapillary lymphocytes	0.4 (0.0; 0.7)	0.4 (0.0; 0.7)
% Wire loops	0.4 (0.0; 0.9)	0.5 (0.0; 0.9)

eGFR_{CORR(t)} is the renal function deterioration (or improvement) in mL/min/1.73 m² at *t* years relative to the expected value based on the eGFR at baseline and the unadjusted mean decline of eGFR over *t* years. $eGFR_{CORR(t)} = eGFR_{Observed(t)} - eGFR_{Predicted(t)}$. For a given patient, the eGFR at time *t* is given by: $eGFR_{Predicted(t)} + eGFR_{CORR(t)}$. Estimations of eGFR_{Predicted(t)} were: $eGFR_{Predicted(1\text{ year})} = 29.9 + 0.67 * eGFR_0$; $eGFR_{Predicted(5\text{ years})} = 42.0 + 0.53 * eGFR_0$; and $eGFR_{Predicted(10\text{ years})} = 33.9 + 0.50 * eGFR_0$. Estimations of eGFR_{CORR(t)} are given in the table; e.g. $eGFR_{CORR(1)} = \text{Constant} + \beta_{Age0} * Age_0 + \beta_{\%Fibrous\ crescents} * \%Fibrous\ crescents + \beta_{\%Fibrinoid\ necrosis} * \%Fibrinoid\ necrosis + \beta_{Non-Caucasian} \text{ (if Non-Caucasian)} + \beta_{IF/TA \geq 25\%} \text{ (if IF/TA} \geq 25\%)$.

Appendix 5.7

Interactions between histopathologic variables and race

Compared with patients with Afro-Caribbean race, spikes/vacuoles were more positively associated with eGFR in patients with Caucasian or Asian race ($\beta=0.9$ mL/min/1.73 m² for each percent of glomeruli [95% CI, 0.2 to 1.5]). Moreover, the relationship between tubular reabsorption droplets on eGFR was different between ethnicities: Caucasian and Asian patients with tubular reabsorption droplets had a higher average eGFR than Afro-Caribbean patients ($\beta=55.6$ mL/min/1.73 m² [95% CI, 16.8 to 94.4]).

Interactions between histopathologic variables and age

Age at the time of renal biopsy was significantly correlated with endocapillary granulocytes ($r=-0.22$, $P=0.026$), IF/TA ($\rho=0.21$, $P=0.04$), tubular casts ($\rho=0.26$, $P=0.008$) and arterial intimal fibrosis ($\rho=0.31$, $P=0.001$). A comparison between children (age <18 years) and adults revealed that children showed significantly less global glomerulosclerosis ($P<0.001$) and ischemia ($P=0.03$), and more endocapillary granulocytes ($P<0.001$). No differences in tubulointerstitial/vascular parameters were noted. No interactions between age and histopathologic variables were found.

Interactions between histopathologic variables and therapy

To study whether cytotoxic immunosuppressive therapy influenced the predictive value of histopathologic lesions, induction therapy was divided in three categories: (i) no therapy/prednisolone only; (ii) guideline-recommended therapy³⁻⁵ including intravenous cyclophosphamide or mycophenolate mofetil (MMF); and (iii) azathioprine with prednisolone. No differences in the predictive value of histopathologic variables were noted between the treatment categories in mixed models for eGFR and in linear regression models for renal function recovery/deterioration in the complete cohort. In the subset of patients with class III or IV LN treated with cytotoxic drugs, analysis by treatment category for the outcomes ESRD and renal flare revealed no differences between predictive values of pathology variables, with one exception. Global glomerulosclerosis was significantly associated with ESRD for patients treated with azathioprine (HR 1.03 per %glomeruli, $P=0.01$), whereas in patients who received cyclophosphamide or MMF it was not. Time to first renal flare was not different between patients in each treatment category during 10 years follow-up ($P=0.5$). Twenty-five of 53 patients with ≥ 10 years follow-up did not experience a first renal flare during 10 years follow-up: of these, 3 patients who received induction immunosuppression with azathioprine ($n=10$) and none of the patients in the other treatment categories ($n=11$) experienced a first renal flare after this period ($P=0.2$).

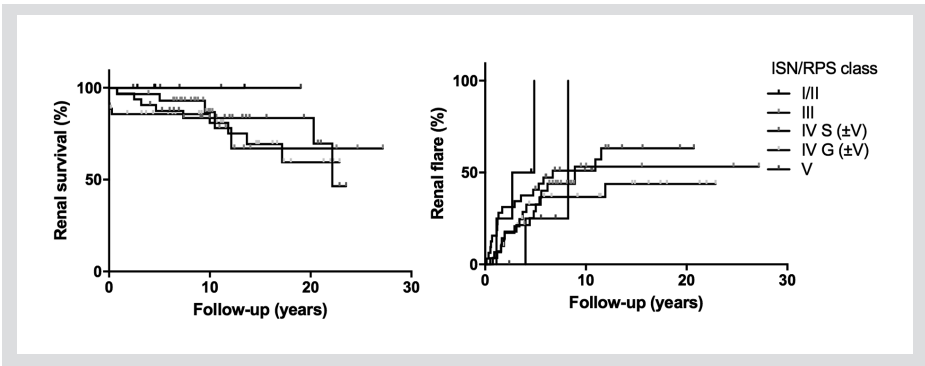
Appendix 5.8. ISN/RPS classes in relation to outcome

Renal flare and ESRD

ISN/RPS classes were not significantly associated with overall renal survival (Log Rank test, $P=0.7$) and renal flare (Log Rank test, $P=0.3$) by Kaplan-Meier analysis (Figures below).

eGFR during follow-up

The ISN/RPS class of LN (either I/II, III, IV-S [$\pm$ V], IV-G [$\pm$ V], or V) was significantly associated with the mean eGFR during follow-up ($P<0.05$). The lowest mean eGFR during follow-up was found in class IV-S LN.



eGFR during follow-up in mL/min/1.73 m² adjusted for ISN/RPS class (Mixed model analysis).

Model	β (95% CI)
(Intercept)	105.1 (76.3; 114.0)
(Time, y)	-0.7 (-1.5; 0.02)
ISN/RPS class	
I/II	-10.2 (-53.4; 33.0)
III	-18.3 (-49.5; 12.9)
IV-S	-36.8 (-67.6; -6.0)
IV-G	-21.9 (-52.9; 9.1)
V*	-

*Class V is the reference category; e.g. eGFR in class IV-S LN is on average 36.8 mL/min/1.73 m² lower than eGFR in class V LN (which is 105.1 mL/min/1.73 m² at the time of biopsy).



Chapter 6

Appendix 6.1. Case histories

Patient 1. NP-SLE, antiphospholipid syndrome, and cerebrovascular disease

This 57-year-old female had a 28-year history of SLE complicated by arthritis, endocarditis, epilepsy, cerebral infarctions, and antiphospholipid syndrome. She suffered an epileptic insult at home and was admitted to the hospital in a confusional state. An MRI scan (1.5-T) two weeks before her death revealed diffuse cortical atrophy, multiple previous cortical infarctions, and diffuse white-matter hyperintensities. The patient developed a myocardial infarction, severe pulmonary embolism, acute renal failure, and multiple cerebral infarctions. Antinuclear, anti-dsDNA, and antiphospholipid antibodies were repeatedly positive. The patient showed no sign of a central nervous system infection. The patient died in a coma from multiorgan failure due to active SLE and diffuse thrombotic complications. Autopsy revealed atrophy of the cerebral cortex, laminar cortical necrosis, old and recent micro-infarctions, macroinfarctions, and diffuse vasculopathy.

Patient 2. NP-SLE, acute neurologic deterioration and vasculitis

This 38-year-old male had a 10-year history of SLE. He was admitted in a sub-comatose condition, a state that had developed the previous night. His SLE was associated with skin lesions, pleuritis, pericarditis, arthritis, and hypocomplementaemia. Antinuclear and anti-dsDNA antibodies were positive, whereas antiphospholipid antibodies were negative. All cerebrospinal fluid cultures at admission were negative. Ante-mortem CT and MRI (1.5-T) scans did not reveal any abnormalities. Upon the clinical diagnosis of NP-SLE, the patient was treated with high-dose immunosuppressive therapy (cyclophosphamide and prednisolone). However, the clinical course was complicated by the development of an opportunistic pulmonary infection with *Klebsiella pneumoniae*, and the patient died from respiratory distress in the intensive care unit. Autopsy revealed venous abnormalities consistent with venous vasculitis (invasion of lymphocytes within the vascular wall and fibrinoid necrosis) and diffuse vasculopathy.

Patient 3. SLE, acute myocardial infarction, no neuropsychiatric symptoms

This 63-year-old female had a 30-year history of SLE complicated by arthritis, glomerulonephritis, pleuritis, and skin lesions. During the course of her disease, she did not develop neuropsychiatric symptoms. The patient was positive for antinuclear antibodies and anti-dsDNA antibodies, but negative for antiphospholipid antibodies. Autopsy revealed a myocardial infarction and no apparent cerebral abnormalities.

Appendix 6.2. Post-mortem neuroimaging and evaluation of the acquired images

Formalin-fixed brains were sectioned into approximately 1-cm-thick coronal sections and stored according to standard protocols. Remnants of the dura and vasculature were removed from the pial surface, and residual formalin was removed by immersion in phosphate-buffered saline for at least one day to partially restore the transverse relaxation parameter.⁶ The brain specimens were placed between polymethyl methacrylate plates (170 mm long; 80 mm wide) and immersed in proton-free fluid (Fomblin LC55, Solvay). Post-mortem MRI scans were acquired using a whole-body 7-Tesla system (Philips Healthcare, Best, the Netherlands) fitted with a Nova Medical transmit coil with a 16-channel receiver array.

Images were acquired as described previously,⁷ with slight modifications; echo times (TE) ranged from 20–40 ms. After visual inspection, a TE time of 35 ms was found to provide the optimum combination of image quality and contrast; this TE was subsequently used for imaging all remaining brain specimens. Scan parameters were as follows: voxel resolution $0.3 \times 0.3 \times 0.3$ mm for a 3D T_2^* -weighted gradient echo sequence, with repetition time/TE/flip angle = 60 ms/35 ms/10°. The number of slices was adjusted to match specimen size and ranged from 60–80 slices, resulting in scan duration of approximately 2.5 hours; seven signal averages were acquired to obtain sufficient image quality.

Appendix 6.3. Relationship between C1q, C4d, and C5b-9 in SLE and NP-SLE

Twenty-five of 34 (74%) patients with SLE and/or NP-SLE had concurrent positive (either focal or diffuse) vascular staining of C1q, C4d, and C5b-9. Twenty-nine of the 30 patients with positive C4d staining (either focal or diffuse) had concurrent C1q deposits and 25 had concurrent C5b-9 deposits (an overlap of 97% and 83%, respectively). Conversely, 29 of the 33 patients with positive C1q staining (either focal or diffuse) had concurrent C4d, and 28 had concurrent C5b-9 staining (an overlap of 89% and 85%, respectively). Of the 28 patients with positive C5b-9 staining, 25 (89%) had concurrent C1q and C4d staining.

C1q staining pattern	C5b-9 staining pattern	C4d staining pattern		
SLE (n=18)		No C4d staining	Focal C4d staining	Diffuse C4d staining
	No C1q staining			
	No C5b-9	0	1	0
	Focal C5b-9	0	0	0
	Diffuse C5b-9	0	0	0
	Focal C1q staining			
	No C5b-9	0	2	0
	Focal C5b-9	2	4	1
	Diffuse C5b-9	0	1	0
NP-SLE (n=16)	Diffuse C1q staining			
	No C5b-9	0	0	0
	Focal C5b-9	0	4	1
	Diffuse C5b-9	0	2	0
		No C4d staining	Focal C4d staining	Diffuse C4d staining
	No C1q staining			
	No C5b-9	0	0	0
	Focal C5b-9	0	0	0
	Diffuse C5b-9	0	0	0
	Focal C1q staining			
	No C5b-9	1	0	0
	Focal C5b-9	1	6	1
	Diffuse C5b-9	0	1	1
	Diffuse C1q staining			
	No C5b-9	0	2	0
	Focal C5b-9	0	1	0
	Diffuse C5b-9	0	1	1

Chapter 8

Appendix 8.1. Primers

Marker name	Position	5' Primer 3'
S01a	F	GGT ACC GGG TCT CCA CAT GA
S01b	F	GTA CCG GGT CTC CAC CAG G
S01a/b	R*	GGG AAA GTC ACT CAC CCA AGG
S03	F	CTT TTG CTT TCT GTT TCT TAA GGG C
S03	R	TCA ATCTTT GGG CAG GTT GAA
S04a/b	F*	CTG GTG CCC ACA GTT ACG CT
S04a	R	AAG GAT GCG TGA CTG CTA TGG
S04b	R	AGG ATG CGT GACTGCTCC TC
S05b	F	AGT TAA AGT AGA CAC GGC CTC CC
S05b	R	CAT CCC CAC ATA CGG AAA AGA
S07b	F	GGT ATT GGC TTT AAA ATA CTC AAC C
S07b	R	CAG CTG CAA CAG TTA TCA ACG TT
S08b	F	GCT GGA TGC CTC ACT GAT GTT
S08b	R	TGG GAA GGA TGC ATATGA TCT G
S09b	F	GGG CAC CCG TGT GAG TTT T
S09b	R	CAG CTT GTCTGC TTT CTG CTG
S10a	F	GCC ACA AGA GACTCA G
S10b	F	TTA GAG CCA CAA GAG ACA ACC AG
S10a/b	R*	TGG CTT CCT TGA GGT GGA AT
S11a	F	TAG GAT TCA ACC CTG GAA GC
S11b	F	CCCTGG ATC GCC GTG AA
S11a/b	R*	CCA GCA TGC ACCTGA CTA ACA
GSTM1	F	GAA CTC CCT GAA AAG CTA AAG CT
GSTM1	R	GTT GGG CTC AAA TAT ACG GTG G
GSTT1	F	TCCTTA CTG GTC CTC ACA TCT C
GSTT1	R	TCC CAG CTC ACC GGA TCAT
RhD	F	GCCTGC ATT TGT ACG TGA GA
RhD	R	CAA AGA GTG GCA GAG AAA GGA
Xq28	F	TGG GTT CCA ACC AGC A
Xq28	R	ACT GAC AAT TAT CAC AGC TT
R271	F	AGA GGA TTG ACT CGG G
R271	R	GTT ACG TCT TAG ATG CCA G
SRY	F	TGG CGA TTA AGT CAA ATT CGC
SRY	R	CCC CCT AGT ACC CTG ACA ATG TAT T

F= forward, R= reverse, *common primer.

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Affiliations

Leiden University Medical Center, Leiden, the Netherlands

Department of Pathology

Mathilde Almekinders

Ingeborg M. Bajema

Hans J. Baelde

Jan A. Bruijn

Daniëlle Cohen

Sjoerd G. van Duinen

Leendert A. van Es

Marlies Penning

Emilie C. Rijnink

Suzanne Wilhelmus

Malu Zandbergen

Department of Nephrology

Tineke Kraaij

Marlies E.J. Reinders

Y.K. Onno Teng

Department of Medical Statistics

Ron Wolterbeek

Department of Rheumatology

Cornelia F. Allaart

Gerda M. Steup-Beekman

Tom W.J. Huizinga

Department of Epidemiology

Olaf M. Dekkers

Department of Radiology

Rob J.A. Nabuurs

Maarten J. Versluis

Bart J. Emmer

Mark A. van Buchem

Department of Gynaecology

Juan D.N. Diaz de Pool

Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, The Netherlands

Department of Paediatric Nephrology

Karliën Cransberg

Radboud University Medical Center, Nijmegen, The Netherlands

Department of Nephrology

Jo H.M. Berden

Meander Medical Center, Amersfoort, The Netherlands

Department of Internal Medicine

Ernst C. Hagen

Martini Hospital, Groningen, The Netherlands

Department of Internal Medicine and Rheumatology

Marc Bijl

Isala Clinics Zwolle

Department of Gynaecology and Obstetrics

Joke Schutte

Curriculum vitae

Emilie Christine Rijnink werd op 25 februari 1992 geboren in Leiden. In 2010 behaalde zij *cum laude* haar gymnasiumdiploma en het International Baccalaureate English diploma (tweetalig onderwijs) aan het Rijnlands Lyceum Oegstgeest. Datzelfde jaar begon zij met de studie Geneeskunde aan de Universiteit Leiden in het Leids Universitair Medisch Centrum. In 2011 werd zij toegelaten tot het Excellente Studententraject van het Honours College van de Universiteit Leiden. Zij startte toen met onderzoek naar chimerisme en lupus naast haar studie Geneeskunde onder begeleiding van dr. Ingeborg Bajema, dr. Onno Teng en prof. dr. Jan Anthonie Bruijn. Naast haar studie was zij ook actief bij haar studentenvereniging L.S.V. Minerva, waar zij lid was van de "Groene Genen" duurzaamheidscommissie van 2013 tot 2014. Na het *cum laude* behalen van haar bachelorsdiploma Geneeskunde en het Honours College certificaat in 2013, verrichtte zij fulltime promotieonderzoek op de afdeling Pathologie van het Leids Universitair Medisch Centrum (hoofd: prof. dr. V.T.H.B.M. Smit). In 2013 ontving zij hiervoor de Kolff studentonderzoeker beurs van de Nierstichting en in 2014 ontving zij een beurs voor een tweejarige aanstelling in het kader van het Excellente Studententraject. De resultaten van dit onderzoek zijn beschreven in dit proefschrift. Tijdens haar onderzoek bezocht Emilie verschillende congressen waar zij haar onderzoek presenteerde, onder andere op de *American Society of Nephrology Kidney Week* in Atlanta (2013), Philadelphia (2014), San Diego (2015) en Chicago (2016). In Philadelphia ontving Emilie de Pirani Award van de *Renal Pathology Society* en in San Diego nam zij deel aan het "Kidney Stars" programma van de *American Society of Nephrology*. Verder nam zij deel aan diverse cursussen, waaronder de "Leiden Oxford Transplantation Summerschool". Haar eerste publicatie over chimerisme tijdens de zwangerschap (dit proefschrift) kreeg internationale media-aandacht, met verhandelingen in onder andere de "New York Times" (Verenigde Staten), "National Geographic" (Spanje), en "El Pais" (Spanje). In September 2016 startte zij met haar co-schappen, waaronder drie co-schappen in het Academisch Ziekenhuis Paramaribo (Suriname). Begin 2019 hoopt zij het artsexamen te behalen.

Emilie Christine Rijnink was born on 25 februari 1992 in Leiden, The Netherlands. In 2010, she graduated from the Rijnlands Lyceum Oegstgeest with honours and received the International Baccalaureate English certificate concluding her bilingual education. The same year, she started medical school at Leiden University at the Leiden University Medical Center. In 2011, she was accepted to participate in the MD/PhD program of the Honours College at Leiden University. At the time, she started doing part-time research on chimerism and lupus next to her medical studies under supervision of dr. Ingeborg Bajema, dr. Onno Teng, and prof. dr. Jan Anthonie Bruijn. Alongside attending her regular curriculum, she was also active at her students' society L.S.V. Minerva, where she was part of the "Green Genes" committee. After completing her Bachelor's degree with honours and receiving the Honours College degree in 2013, she started performing fulltime PhD research at the department of Pathology of the Leiden University Medical Center (head: prof. dr. V.T.H.B.M. Smit). In 2013, she received the Kolff student researcher grant of the Dutch Kidney Foundation and in 2014 she received a PhD grant for 2 years as part of the MD/PhD program of the Leiden University Honours College. The results of the PhD research are described in this thesis. During her PhD research, Emilie visited various international conferences where she presented her work, including the *American Society of Nephrology Kidney Week* in Atlanta (2013), Philadelphia (2014), San Diego (2015), and Chicago (2016). In Philadelphia Emilie received the Pirani Award assigned by the *Renal Pathology Society* and in San Diego she participated in the "Kidney Stars" program of the *American Society of Nephrology*. Furthermore, she attended various courses during her PhD research, including the "Leiden Oxford Transplantation Summerschool". Her first publication on chimerism during pregnancy received worldwide media attention, including exposure in the "New York Times" (United States), "National Geographic" (Spain), and "El Pais" (Spain). In September 2016, she started her clinical rotations, three of which she did in Academic Hospital Paramaribo (Surinam). In the beginning of 2019 she plans to attain her medical degree.

List of publications

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