Systemic lupus erythematosus : from diagnosis to prognosis

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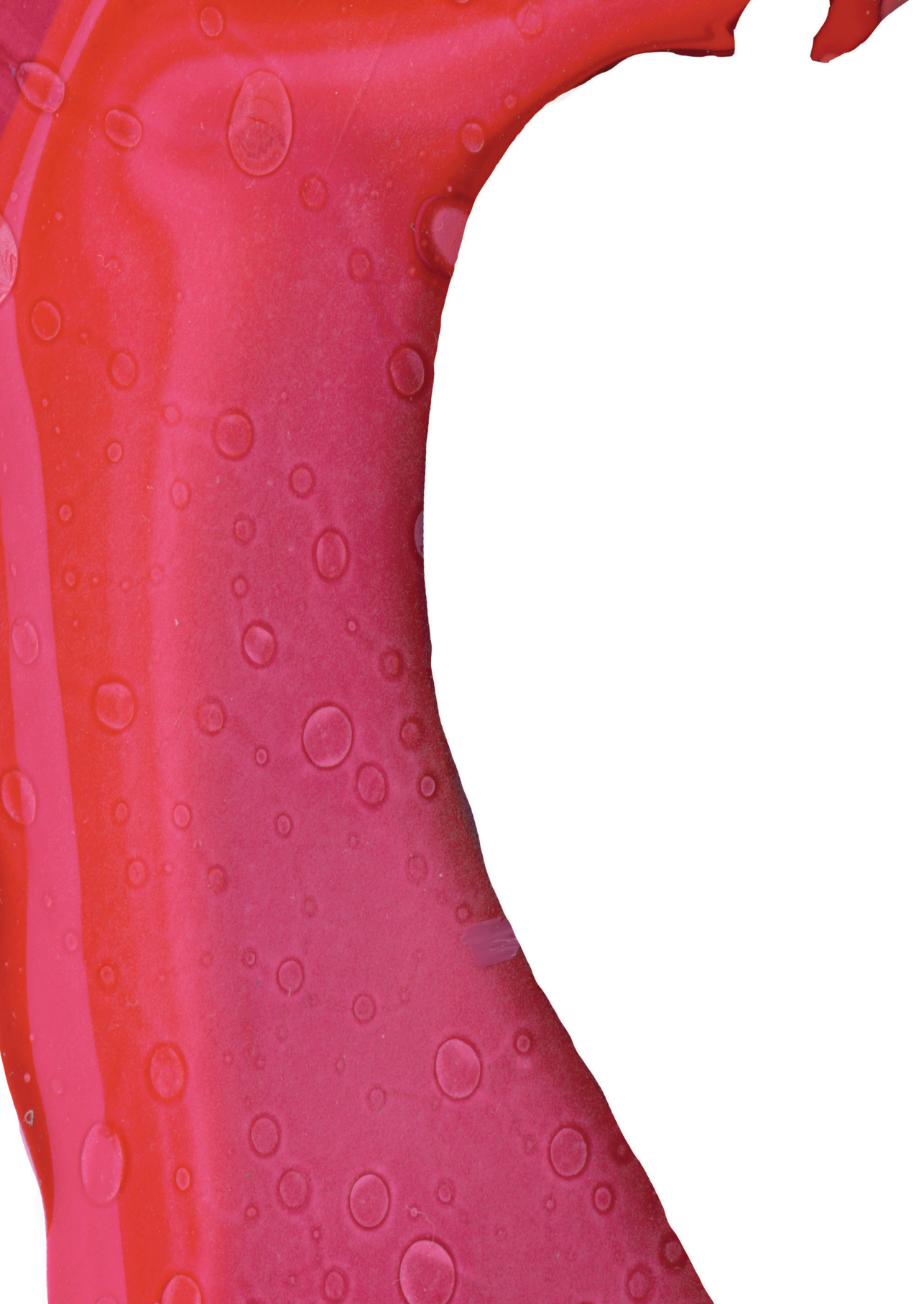

Chapter 9

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 aetiologic 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 nephritis[4] 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 (±V). It is of interest that the evidence for the cut-offs defining patients with class III or IV (±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 (±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 LN[25] 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 hyperintensities[53] in the presence of clinical symptoms, known as the clinicoradiological 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 imagining (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 monocolonal 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 haemoglobinuria[59] 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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