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Clinical epidemiology of Systemic Lupus Erythematosus with emphasis on nephritis and autoantibody production

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If I have seen further it is only by standing on the shoulders of giants.

Sir Isaac Newton (1643 –1727)

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2. PUBLICATIONS INCLUDED

Paper I **The influence of the 1997 updated classification criteria for Systemic Lupus Erythematosus: epidemiology, disease presentation, and patient management.** *J Rheumatol. 2009 Mar;36(3):552-9.*

Paper II **Decreased incidence of lupus nephritis in northern Norway is linked to increased use of antihypertensive and anticoagulant therapy.** *Nephrol Dial Transplant. 2011 Feb;26(2):620-7.*

Paper III **Increased levels of BAFF in patients with Systemic Lupus Erythematosus are associated with acute phase reactants, independent of BAFF genetics: a case control study.** (*Submitted)*

3. ABBREVIATIONS

Ab	Antibodies
ACE-I	angiotensin-converting enzyme inhibitors
aCL	anti-Cardiolipin
ACR	American College of Rheumatology
ACR82	ACR classification criteria for SLE, published in 1982
ACR97	ACR updated classification criteria for SLE, published in 1997
AIR	Annual Incidence Rate
ANA	Antinuclear Antibody
ARA	American Rheumatism Association
ARB	Angiotensin II-receptor blockers
BAFF	B-Cell Activating Factor/BLyS/TNFSF13B
BAFF-R	B-Cell Activating Factor Receptor
BAFF-RQ	BAFF mRNA expression (relative quotient versus β2M)
BCMA	B Cell Maturation
β2M	β2-microglobulin
BAFF-R	BAFF-Receptor
CI	Confidence Interval
CRP	C – Reactive Protein
CTD	Connective Tissue Disease
CYC	Cyclophosphamide
dsDNA	double-stranded DNA
ELISA	Enzyme Linked Immuno Sorbent Assay
ESR	Erythrocyte Sedimentation Rate
ESRD	End stage renal disease
GWAS	Genome-wide association study
HR	Hazard Ratio
Ig	Immunoglobulin
IFN-γ	Interferon-gamma
IL	Interleukin
ISN/RPS	International Society of Nephrology and Renal Pathology Society
IU	International Units
LA	Lupus Anticoagulant
LN	Lupus Nephritis

MHC	Major Histocompatibility Complex
MMF	Mycophenolate mofetil
MRL/lpr	Medical Research Laboratory/lymphoproliferation mice strain
NIH	National Institutes of Health
NSAID	Non-Steroidal Anti-Inflammatory Drug
NZB/W F1	(New Zealand Black x New Zealand White) F1 hybrid mice strain
OR	Odds Ratio
PBMC	Peripheral blood mononuclear cell
pSS	primary Sjögren's Syndrome
PP	point-prevalence
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
RNP	Ribonuclear Protein
RT-PCR	Real time polymerase chain reaction
SD	Standard Deviation of the mean
SDI	SLICC Damage Index/SLICC/ACR-DI
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLICC	Systemic Lupus International Collaborating Clinics
s-BAFF	serum B-Cell Activating Factor/BLyS/TNFSF13B
SMR	Standardized mortality ratio
SNP	single nucleotide polymorphism
Sm	Smith antigen
SSA	Sjögren's Syndrome A antigen/Ro- antigen
SSB	Sjögren's Syndrome B antigen/La- antigen
TACI	Transmembran activator, calcium-modulator and cyclophilin ligand
TNF	Tumor Necrosis Factor
TNFSF	Tumor Necrosis Factor superfamily
UNN	University Hospital of Northern Norway
82acr	SLE patients enrolled during 1978-1995 using the ACR82 criteria
97acr	SLE patients enrolled during 1996-2006 using the ACR97 criteria
82LN+	Patients with LN enrolled during 1978-1995 using the ACR82 criteria
97LN+	Patients with LN enrolled during 1996-2006 using the ACR97 criteria

4. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is one of the most common systemic autoimmune diseases (1;2). SLE is characterized by a highly variable clinical presentation that may range from mild skin involvement to life-threatening multiorgan failure. Currently, no cure exists for the disease, but with appropriate management, SLE is no longer a rapidly fatal disease as it was some decades ago. SLE has become a chronic disease with an unpredictable disease course, generally characterized by alternating periods of quiescence and exacerbations of disease activity.

The basis for virtually all disease manifestations is the occurrence of sterile inflammation that may affect any of the body's organs system and can ultimately lead to tissue scarring and subsequent failure of organ function. The underlying pathological processes in SLE are extremely complex due to the varying severity and longevity of inflammation, and diverse composition of the inflammatory infiltrates. This has led many investigators to believe that SLE represents a common name for a syndrome that comprises various distinct conditions (3). The early beginning of this process is most probably a misled activation of immune cells, resulting in an immune response against self antigens which includes the production of antibodies against self antigens (autoantibodies). This immunological self-intolerance is regarded as an early hallmark of SLE and it has become clear that this is due to a complex process involving a variety of molecules and cells (4). While more than a hundred different types of autoantibodies have now been reported in the serum of SLE patients (5), the evidence for a pathogenic role for many of these autoantibodies is still weak.

Renal involvement affects about 25 – 60 % of patients with SLE, and is one of the more serious manifestations as it can lead to complete renal insufficiency (6-10). Despite decades of research efforts, the pathogenesis of lupus nephritis (LN) is still not fully

understood. However, LN is the most widely studied example of immune complex mediated inflammation. It has become clear that there is an association between the development of LN and the presence of a particular subgroup of autoantibodies against native DNA in serum and renal tissue. These anti-dsDNA antibodies also have a role in the current diagnosis and management of SLE and LN (4).

While some of the pathways in SLE progression have been elucidated, the cause(s) of SLE remain elusive. Technical opportunities for genetic research have increased rapidly in recent years, and studies of how changes in DNA- and RNA affect the structure and function of immunological molecules have become a topic of intense research in various diseases. With regard to SLE, a new hypothesis sustain, that the different clinical phenotypes may be a mirror of genetic variation in one or more of the molecules that are involved in immunological reactions (11). Given the complexities of both immunopathology and the genetic basis of SLE, many questions are yet unsolved and a lot of work is in progress. The ultimate hope is however, that in the future, knowledge of a genetic signature in each individual SLE patient could help to predict and possibly prevent disease and complications.

Background

History of Systemic Lupus Erythematosus

The word lupus is a Latin term which means wolf. "Lupus" has been used since the Middle Ages by the Romans to describe ulcerative lesions in the skin similar to the results of a wolf bite. The first scientific publication that mention these skin lesions emerged in the 1800s, first by Pierre Cazenave in 1838 (12) and 7 years later the butterfly rash that is typical of SLE was described by Ferdinand von Hebra (13). Some years after that Cazenave introduced the term lupus érythèmateux (14) to distinguish the characteristic skin lesions from the more common lupus vulgaris which was the result of tuberculosis. In 1872, Moriz Kaposi recognised the potential dangerous systemic nature of the disease (15) and at turn the of the

century, William Osler described patients with disseminated lupus (16). Histopathological descriptions of disseminated lupus began with the work of Liebman and Sachs in 1924 on verrucucous endocarditis (17) and eleven years later Baehr et al described the characteristic wire loop lesions in the glomeruli (18).

Lupus erythematosus (LE) was recognized as a connective tissue disease of autoimmune nature in 1948 with Hargraves' description of the LE cell, which eventually became the first diagnostic tool for SLE (19). LE cells were subsequently linked to the gamma globulin fraction in the plasma of SLE patients. The discovery of LE cells in combination with a new immunofluorescence technique to confirm antigen localization in tissues, led to the development of antinuclear antibody (ANA) assay. The test's sensitivity for SLE was described by George Friou in 1958 (20) and opened up for a series of investigations of the gamma globulin fraction and the subsequent description of various autoantibodies, including anti-dsDNA. Over time, this has led to the development of more specific diagnostic and prognostic autoantibody assays, which are easier to use than the LE cell test. The implementation of new assays to monitor the disease has led most laboratories discard the LE cell test. These achievements coincided with the discovery of the strong anti-inflammatory properties of corticosteroid drugs and their subsequent introduction in clinical practice. This breakthrough led to the Nobel Prize for rheumatologist Philip Hench in 1950 and was soon also found to be an excellent short term therapy for patients with SLE and especially for LN when used in higher doses (21). Soon thereafter it was observed that the long term administration of steroid was associated with clinical drawbacks, and this has paved the way for the introduction of other immunomodulating/cytotoxic drugs in the treatment of SLE patients. Of note, none of the currently recommended nonspecific immunosuppressive drugs used in SLE treatment have been formally approved by regulatory agencies such as the U.S. Food and Drug Administration. In the present era of targeted biological therapies, is the hope

that specific intervention by monoclonal antibodies against cytokines, cell receptors or inhibition of intracellular signalling pathways, will eventually allow tailored therapy in SLE.

Systemic Lupus Erythematosus (SLE)

Classification

As SLE causes a wide spectrum of clinical symptoms and serological patterns, clinicians must deal with a diagnostic challenge, as they need to distinguish SLE manifestations from infections or other common diseases. In order to facilitate the formal scientific communication about the disease, a subcommittee created by the American Rheumatism Association (ARA), published preliminary criteria for classification of SLE in 1971 (22).The subcommittee revised these criteria in 1982 (ACR82) - after ARA changed its name to the American College of Rheumatology (ACR). The 1982 revision was based on a comparison of findings in a large cohort of SLE patients followed in 18 different US hospitals for a mean period of seven years and a control cohort that included patients with rheumatoid arthritis, osteoarthritis and scleroderma. The final ACR82 criteria (23) were derived from cluster analyses and had high sensitivity and specificity (96 % for both) (Table 1) which was a considerable improvement compared to the 1971 criteria. In ACR82, only eleven items were included, among these a positive test for antinuclear antibodies (ANA) as a separate item and antibodies (Ab) to double-stranded DNA (dsDNA) or to Smith antigen (Ag) as a part of the immunological item. These immunological tests replaced clinical manifestations such as Raynaud's phenomenon and alopecia. The ACR82 criteria were again modified in 1997 (24), when the finding of LE cells was replaced with the presence of antiphospholipid Ab (aPL) encompassing anti-cardiolipin Ab (aCL) or lupus anticoagulant (LA). This update was consensus-based and reflected the fact that most laboratories did no longer perform the LE

cell assay. The updated classification criteria for SLE from 1997 (ACR97) have not yet been formally evaluated, but these criteria aim to be optimally sensitive and specific.

The 82ACR and 97ACR criteria sets require involvement of different organ systems according to strict definitions where at least four of eleven classification criteria have to be fulfilled. The purpose of developing SLE classification criteria was to ensure homogeneity in clinical trials and population studies, but the main drawback of these criteria sets was the exclusion of a considerable number of other relevant disease manifestations. During the first years of the disease, patients often have clinically relevant symptoms excluded from ACR97, e.g. alopecia or Raynaud's phenomenon. This means that patients with a clinical diagnosis of SLE need not always meet the requirements of the ACR97, as the criteria are not well suited for the early stages of the disease. These considerations partly underlie an ongoing international effort to update the SLE classification criteria once more (25).

Epidemiology

SLE has been recognized worldwide and occurs in all ethnic groups, although regional variations in frequency and severity have been reported. The lowest incidence rates are observed in Caucasian populations (26). Studies from Scandinavia show that the average annual incidence rate (AIR) of SLE varies from 1.5 to 4.8 per 100,000 (27-32). Compared to countries with mostly Caucasian population, the incidence of SLE in multi-ethnic countries, such as United Kingdom and the Caribbean Islands is significantly higher (28;33), similar to the disease rate in USA where the reported AIR vary from 1.8 to 7.6 per 100,000 (34-37).

The prevalence of a disease is naturally dependent on its incidence rate and its disease severity in terms of mortality. Epidemiological studies from USA report a wide range in SLE prevalence with rates varying from 14.6 to 122 per 100,000 (34-39). These higher rates exceed prevalence rates in studies from Scandinavia with a reported range from 22.0 to 68.0 per 100,000 (30-32;40). During the last decades, standardized mortality ratios (SMR) have

declined gradually from 10.1 in the 1970s to 2.4 (41;42). Simultaneously, 5 years survival rates have improved from approximately 50 % in the 1950s to over 90 % at present (41;43-45), resulting in increased prevalence of SLE over the last decades.

Traditionally, SLE has been considered as a disease among women of childbearing age (34;39), but nowadays the highest prevalence at 130 per 100,000 is seen in postmenopausal women (Paper I). This change is a consequence of the increased survival of SLE patients in combination with increased life expectancy in the general population. The variability in prevalence and incidence rates of SLE are explained by the effect of ethnicity or study-design, since some studies include only hospitalized SLE patients while others include patients diagnosed by general practitioners or self reported SLE disease (26).

Clinical manifestations

SLE is a pleomorphic disease where many organ systems may be involved either alone or in combination. SLE patients can thus presents combinations of various rashes, arthritis, pleurisy, proteinuria, Raynaud's phenomenon, seizures, or fever of unknown origin. Nonetheless, some manifestations of SLE are more frequent than others, such as rash or arthritis, which is seen in more than two-thirds of patients during the course of the disease. Involvement of the nervous system is also frequent and is seen in 14-90 % of SLE patients depending on the type of CNS involvement studied (46). A common term for affection of the nervous system is "neurolupus" and this involves cognitive, psychiatric, focal and diffuse central and peripheral symptoms. In addition, vasculitis is common in SLE and may involve small and large vessels, resulting in urticaria and sometimes gangrene of a finger or part of a limb. A major complication in SLE is the development of lupus nephritis (LN) and since LN is emphasized in this thesis it will be discussed in a separate paragraph.

Almost per definition, the most frequent cumulative laboratory finding in SLE is a positive ANA test, even though low complement (C3 and/or C4) and positive tests for various antibodies also are common (8;47;48). In addition to these immunologic findings, elevated erythrocyte sedimentation rate (ESR), anemia of chronic disease, leucopenia, lymphocytopenia and thrombocytopenia are regularly seen in patients with active SLE disease.

Assessment of disease activity

Given the pleiotropic nature of the disease, the assessment of disease activity is not easy and several different instruments have been developed to quantify disease activity. These include among others BILAG (49) that rates eight organ systems with scores based on the principles of intention to treat and ECLAM (50-52) that comprise 15 weighted clinical and serological items but exclude the antibody testing. Another instrument is SLE disease activity index (SLEDAI), which was developed at the University of Toronto in Canada and measures disease activity within the 10 last days (53). In 2000 it was updated to SLEDAI-2K that incorporate the presence of some persisting disease features, using a timeframe of the last 10 or 30 days (54). SLEDAI was developed through a model of complete assessment of disease activity by experienced clinicians. Therefore it represents the consensus of a group of experts and has subsequently been validated as a reliable and reproducible measure of disease activity that is sensitive to change over time. The choice for SLEDAI (Table 2) as measurement of disease activity in our registry was based on its validity, sensitivity to change and earlier experiences of its ease of use.

SLEDAI includes 24 clinical and laboratory variables that are weighted differently, where life threatening events such as cerebral manifestations and vasculitis have the highest score (score 8). Disease activity scores may in theory range from 0 to 105, but in practice

rarely exceeds the 40 mark. Four different renal manifestations associated with LN are given a score of 4 each, that leading to a potential SLEDAI score of 16 in patients with LN, which may increase to 20 if also positive anti-dsDNA Ab and low complement are scored with a weight of 2 each (55). Another simple index used in these studies is the visual analogue scale (VAS) that consists of a line of 10 cm along which the patient or the physician draws a perpendicular mark, reflecting their assessment of overall severity of disease activity. The patient VAS gives an overall impression of how patients experience the effects of disease and includes subjective symptoms like fatigue, myalgia, arthralgia and abdominal pain (55). The physician VAS is a reflection of how active the attending doctor considers the disease state to be, especially with regard to the need for intervention. In many ways, physician VAS resembles the old case note summary describing whether patients are doing well, unchanged or poorly.

Assessment of organ damage

As a result of the improved survival for SLE patients, there was a need to develop a system that measures less crude outcomes of the disease. Since the inflammatory process of SLE can result in specific organ damage, the Systemic Lupus International Collaborating Centre Clinics (SLICC) Working Group has developed the SLICC Damage Index (SDI) (56). SDI includes assessment of 12 organ systems and record damage regardless of its cause. Damage may result from previous disease activity resulting in organ failure, such as renal failure or neurocognitive abnormality, or may be the result of side effects of medications. It may also result from intercurrent illness, such as vascular ischemia, diabetes, surgery or cancer.

SDI scores are based on accurate definitions of organ damage resulting in maximum scores of 6 for neuropsychiatric-, cardiovascular-, gastrointestinal- and musculoskeletal

domains, while the maximum score for the renal domain is 3 (renal failure). Since SDI is to be distinguished from disease activity in SLE, the relevant feature must be present continuously for at least six months. Maximum SDI scores can theoretically reach 47, but this is unlikely to be compatible with life (57).

Outcome

Although SLE is mainly a chronic disease, remissions (disease quiescence lasting for at least one year in the absence of any immunosuppressive drug treatment) occur in 2-10 % of the patients (58). The life expectancy for SLE patients has increased during the last decades thanks to a combination of various factors, including increased availability of medical treatment, advances of anti-inflammatory therapy and the development of new cytotoxic drugs (37;41). In addition, the introduction and more widespread application of diagnostic assays leads to earlier diagnosis of SLE patients and subsequent inclusion of milder cases which are also important factors in the improved survival (45). Since cardiovascular disease is a frequent cause of death, the awareness and general advances in preventive therapy for primary and secondary thrombotic complications may have had some impact on the improved life expectancy (59-62).

Infections remain a cause of increased mortality of SLE, even though the types of infections are similar to the general population (63). In periods of high disease activity, intensive immunosuppressive treatment that often includes high dose corticosteroids and cyclophosphamide is frequently required. Such treatment results in a desired impairment of the immune response, but leaves the patient vulnerable to microorganisms that may cause ordinary as well as opportunistic infections (64). In addition, genetic factors like specific variants in the genes encoding mannose-binding lectin and Fcγ receptors may predispose certain SLE patients to develop infections. Thus, an intrinsic risk for infectious complications

that is independent of therapy but related to impaired immune defence exists in these patients (65). Therefore in situations with intensive treatment, clinicians together with patients need to continuously balance the intensity of treatment with the risk of serious and potentially fatal infections.

Lupus nephritis (LN)

Epidemiology of LN

Depending on the ethnicity in the population, about 25 % to 60 % of adults with SLE disease develop LN (defined as renal inflammation caused by SLE) and this happens mainly during the first years of the disease course (6-8). The prevalence of LN is lowest in Caucasian population and highest in Hispanics, Asian, Afro-Caribbean and African-Americans (9;66). Currently, there are indications that kidney involvement is becoming less frequent in SLE (31).

Aggressive immunosuppressive therapy has improved the prognosis of SLE patients with renal disease considerably, however 5-20 % still progress to end-stage renal disease (ESRD) within 10 years following the diagnosis of nephritis (6;67). In addition to an increased morbidity, patients with renal damage have also a decreased 5-years survival compared to the rest of SLE patients (70-80 % vs. 90 %) (68;69).

Diagnosis of LN

LN has a highly variable presentation which can range from no clinical symptoms such as proteinuria, microscopic haematuria, new onset or worsening hypertension to severe nephritic syndrome or acute renal failure. Since LN is often asymptomatic, regular control of serum creatinine, urine dipstick, and if abnormal, microscopy of urine has to be performed.

LN occurs according to SLEDAI definition (53) if any of the four following criteria are met; 1. Urinary casts (Heme-granular or red blood cells casts.) 2. Haematuria (> 5 red blood cells/high power field, excluding stone, infection and other causes). 3. Proteinuria (>0.5 g/24h, regarding new onset or recent increase of >0.5g/24h). 4. Pyuria (> 5 white blood cells/high power field, excluding infection) (Table 2). In our studies, LN is defined according to SLEDAI except for the criterion pyuria because this often turned out to be due to sample contamination.

Histopathology and classification of LN

It is not possible to accurately determine the severity of renal inflammation based on urine sediment findings, amount of proteinuria, glomerular filtration rate or serum parameters like creatinine, complement-levels and autoantibody profiles. Renal biopsy has thus become the preferred method of classifying renal pathology. Renal biopsy was first introduced in the 1951 (70) and has become a customary examination in the work up of renal diseases. Although this procedure has become safer by ultrasound guidance, taking a renal biopsy remains an invasive procedure that leads to life-threatening complications in approximately 0.1 % of the cases (71).

The original World Health Organisation (WHO) classification of glomerulonephritis in SLE patients from 1974 was revised in 1982 and again in 1995 (72). The latter contained 5 different classes of LN. This classification was again modified in 2003 by members of the International Society of Nephrology and Renal Pathology Society (ISN/RPS) to provide a more concise description of various lesions and classes of LN (73) (Table 3). The features of glomerular disease activity (potentially reversible) and sclerosis (irreversible damage) were added to these criteria in each class of LN. This is done by a semi-quantitative analysis (on a scale from 0 to 3+) of specific histological features of activity or sclerosis. Another class was

added to the WHO classification of LN, class VI indicating advanced sclerosis without residual activity (Table 3).

The impact of updating the classification of LN has rendered into a sharper distinction between the six different classes (Table 4). All parameters in the recent ISN/RPS classification of LN are considered important for prognosis, a potential for targeted therapy, and aim to facilitate a higher degree of diagnostic reproducibility of renal biopsy (73). While few studies have reported LN prognosis by ISN/RPS classification, the new criteria still lead to a considerable discordance between renopathologists in classifying renal biopsies in SLE (74). Also, the evaluation of tubulo-interstitial and vascular structures is not well defined and has only received a short recommendation, which still leaves room for the use of NIH (National Institutes of Health) activity - and chronicity scores of biopsies (73). As indicated by the abbreviated ISN/RPS classification of LN (Table 4), class I and II represent milder disease and are associated with a good prognosis. In most studies, severe LN that carries the highest risk for renal failure is defined as class III or IV, the latter observed in approximately 40% of biopsies. However progression from class II to class III/IV occurs in about 20-25% of patients while conversion from class III to class IV occurs in over two-thirds of patients (75). This class switching, in addition to selection bias and a relative new system of classifying LN, makes it difficult to get a clear overview of the frequency of the different classes. Some approximately values are presented in Table 4.

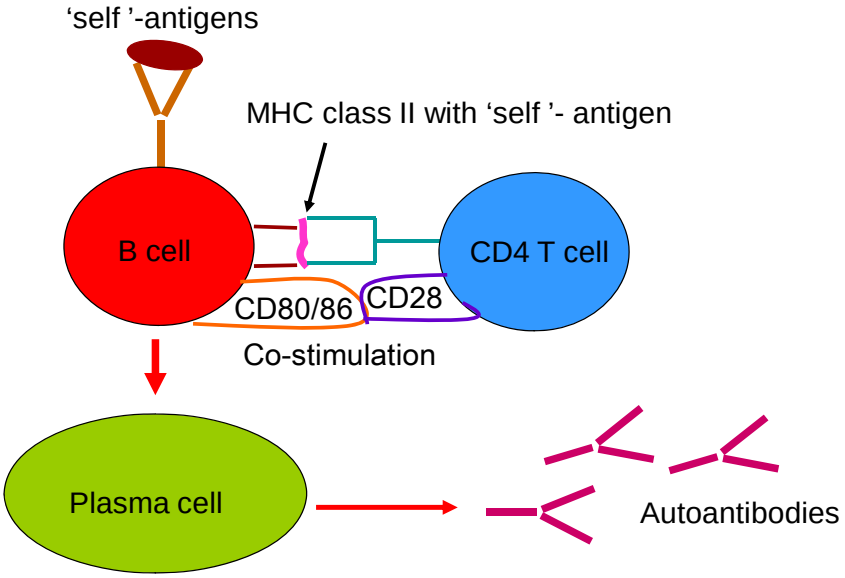
Pathogenetic mechanisms of LN

The induction of renal autoimmunity in LN has been a subject of intense investigation, and given the limited availability of human material these studies are often based on findings in experimental models of lupus prone mice. However, in addition to the difficulties of translating mice data to the human model, the various mice models (NZB/W F1, MRL/lpr,

BXSB) have their own particularities in terms of progression and type of renal pathology and immune abnormalities including autoantibody profile (76).

The immunological self-intolerance in LN involves a range of different cell types, although activated B- and T cells play a major role in this process. B cells express a diverse repertoire of immunoglobulins against a wide array of pathogens, and can function as antigen presenting cells to T lymphocytes. The antigenic specificity of a B cell is determined through the process of gene rearrangement, resulting in antigen-specific cell-surface receptors. These receptors together with MHC class II and co-stimulatory molecules like CD80 and CD86 expressed on B cells makes the antigen-presentation to T cells possible. As illustrated in Figure 1, CD4 T-cells bind to the MHC class II/antigen and co-stimulatory molecules with its antigen-specific cell-surface receptors and co-stimulatory molecules CD28. However, some of these antigen-specific receptors on B cells may develop specificity for self-antigens that for unknown causes escapes the strict selection in the thymus that normally prevents self-intolerance. Upon stimulation by a T cell, which usually occurs in germinal centers in the spleen and lymph nodes, the activated B cell differentiates into more specialized cells and is clonally expanded to plasma cells that produce autoantibodies. These autoantibodies are central in the development of inflammation as they can bind to ubiquitous cells that are carrying Fcγ receptors, and trigger the expression of various cytokines (6;72). While the initial triggering event for self antigen exposure and the exact proceedings remain unclear, the cytokine secretion by B cells (IL-2, IL-6, IL-10, TNF-α as well as the pro-inflammatory cytokine IFN-γ) (77) clearly contributes to the development of LN. In the kidneys, through Fcγ receptors situated on the surface of diverse glomerular cell types, autoantibodies binds and join together with exposed autoantigens from the circulation or in situ autoantigens and form glomerular immune complex depositions which ultimately induce cell and tissue injury (78).

Figure 1



The immunological self-intolerance in lupus nephritis. B cells act as an 'self '-antigen presenting cells to T cells. On the cell surface, B cells express immunoglobulins, MHC class II and co-stimulatory molecules like CD80 and CD86 while CD4 T cells express antigen-specific receptors and the co-stimulatory molecule CD28. This B-T cells interaction, which usually occurs in the spleen and lymph nodes, makes the activated B cell differentiate into more specialized cells like plasma cells that produce large volumes of antibodies.

Nephritogenic autoantibodies

Even though sera from SLE patients often contain multiple autoantibodies, only few have a known nephritogenic potentials, such as high avidity Ab against anti-dsDNA (40 - 90 %), anti-Sm (5 - 50 %), anti-C1q (80 - 100 %) and anti-nucleosome (6;79). In the last decades, anti-dsDNA Ab are the most extensively studied, based on their serological profile in patients with LN and they are enriched in glomerular immune deposits (4;77;80;81).

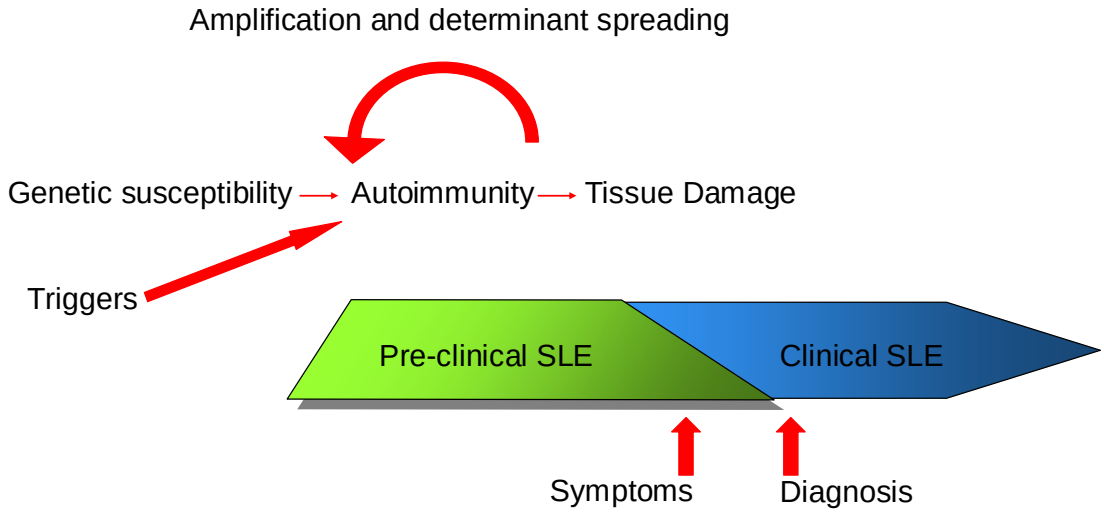
In murine models, immune complexes can be demonstrated as electron dense deposits in the basement membrane in the renal glomeruli (82). These deposits contain oligo-nucleosomes (nucleosomes consist of dsDNA wound around a histone protein core) that are bound to anti-dsDNA Ab. The oligo-nucleosomes are thought to originate through ineffective fragmentation and clearance of apoptotic material (83;84). Although the origin of the

apoptotic material in the renal glomeruli is not clear, it may stem from renal mesangial cells or infiltrating leukocytes. In this scenario, the presence of anti-dsDNA Ab is secondary to abnormal renal apoptosis.

Another study has shown that Ab which are eluted from the kidney of nephritic mice have a higher affinity for DNA compared with serum anti-DNA Ab (85), indicating that these autoantibodies obtained their nephritogenic potential through repeated antigen stimulating cycles, probably through exposure to apoptotic material (80). This theory of affinity maturation of anti-DNA Ab over time is in agreement with the landmark clinical US military study, demonstrating a mean onset of anti-DNA Ab of 2.2 years before SLE diagnosis (86).

A similar time lag has been registered for antibodies against antiphospholipid (aPL) and also these Ab have been shown to be present prior to anti-dsDNA Ab development (87). aPL Ab are seen in 30 - 50 % of patients with LN and represent an additional risk factor for trombotic events including renal and glomerular capillary thrombosis (88;89). This process may be initiated by intraluminar accumulation of fibrin (90). As early as the 1980s, Kant showed that the snake poison “ancrod” decreased fibrin deposition and crescent formation, and improved renal function in LN through decreasing factor VII and von Willebrand factor levels, normalizing platelet hyperaggregation and increasing prostaglandin I2 (91;92). In accordance with this, renal impairment in LN is partly due to an exaggerated synthesis of a thromboxane antagonist (93;94). Thus, we assume that the autoantibodies in SLE follow a predictable course to obtain the nephritogenic potential. The antibodies progressively accumulate prior renal damage, subsequently complex immune deposits are formed which may provoke renal damage and thrombosis as implied in Figure 2.

Figure 2



Stages of lupus pathogenesis.

Genetic factors and environmental triggers act on the immune system to initiate autoimmunity. Autoantibodies and their autoantigens, cytokines and chemokines amplify immune system activation and generate tissue damage. Autoantibody production occurs years prior to the development of clinical signs and symptoms of SLE. Organ damage has likely occurred by the time lupus is diagnosed.

(Self designed following a theory of Crow M, 2009)

Immunomodulating treatment in LN

The goals of therapy in LN are the prevention of renal failure and mortality through early induction and long-term maintenance of remission. For this purpose, several regimes have been studied and represent the one area in SLE research where randomised clinical trials are available to guide management. These studies have in addition been the basis for similar approaches to renal involvement in patients with other types of systemic diseases involving the kidneys, such as the antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (95).

The long running series of NIH studies on LN treatment showed monthly intravenous (i.v.) cyclophosphamide (CYC) (0.5-1 g/m²) to be more efficacious for maintaining life sustaining renal function than oral regimes of azathioprine (96). Thus in almost two decades,

monthly i.v. CYC for 3 months followed by quarterly monthly i.v. CYC for 12 to 24 months was used as a standard treatment for induction and maintenance of remission in LN. However, azathioprine has fewer side effects such as severe infections and amenorrhea compared with CYC and was considered as the standard remission maintenance treatment (97).

Over the last decade several controlled trials have compared various regimens of CYC. The Euro-Lupus Nephritis Trial examined the effect of "low-dose" vs. "high-dose" CYC and concluded that both are equally efficacious, however severe infections were more common in the high dose treated group (98). A similar conclusion was drawn from a Dutch LN study (99) that compared high dose CYC with azathioprine and found them equally effective, although the flare rates were lower after treatment with CYC. In addition, retrospective study of patients with proliferative LN from Northern Norway that compared treatment with azathioprine versus pulse CYC showed similar renal survival rates and patient survival rates (100). As a result, short time i.v. CYC and corticosteroid pulse therapy are currently the commonly accepted standard treatment for induction as high cumulative doses of CYC are associated with significant toxicity, particularly infections, malignancy (bladder and ovarian) and infertility. According to the Euro-Lupus Nephritis Trial, this induction regimen should be followed by azathioprine treatment in the maintenance phase (67).

Despite improvements in LN treatment, failures to induce remission, subsequent relapses and treatment toxicity are remaining clinical challenges hence new alternative treatments have been investigated. Mycophenolate mofetil (MMF) is a relative new oral immunosuppressive drug used extensively in transplant medicine to avoid CYC toxicity and was first given to LN patients' refractory to CYC (101). Later studies have also confirmed that MMF is equally efficient as i.v. CYC as treatment in patients with LN. Treatment with MMF may even be more beneficial over i.v. CYC for remission induction in black patients

(67;101). Furthermore, MMF is preferred over CYC as induction therapy, since MMF does not lead to ovarian toxicity. In maintenance treatment, MMF is equal to azathioprine (67).

Rituximab is an anti-CD20 monoclonal antibody and was the first biological antibody used to specifically target B cells in humans. There were high expectations regarding its use in SLE patients, since B cells are highly involved in SLE/LN. The EXPLORER (The Exploratory Phase II/III SLE Evaluation of Rituximab) and the LUNAR (LUpus Nephritis Assessment with Rituximab) were high-quality randomised controlled trials of Rituximab by the treatment of non renal lupus and proliferative lupus nephritis. Both the EXPLORER and LUNAR trial were unable to detect a large clinical effect in patients with very active disease. There has been much discussion about the disappointing results of these studies, which may have been too strict in their outcome measures. A problem with both trials is that patients entered with very active disease and therefore they had to be treated with moderate- to highdose corticosteroids. Such concomitant therapy makes any benefits from experimental treatment difficulty to detect unless the effects are very strong (102). Case series and registry data indicate a disease modifying role for Rituximab at least in resistant cases (103).

Supportive treatment in LN

In all patients with renal disease, it is important that patients maintain normal blood pressure to avoid deterioration of renal function and prevent cardiovascular disease (104). As proteinuria alone increase the risk of progressing renal disease and may even increase the incidence of hyperlipidemia and thrombosis, blood pressure should be less than 130/80 mm Hg (105). Since treatment with both antihypertensiva as ACE-I and ARB results in lower blood pressure and additionally reduce proteinuria, these drugs should be used, either alone or in combination (67). When nephrotic syndrome (proteinuria > 3 g/L, hypoalbuminemia and

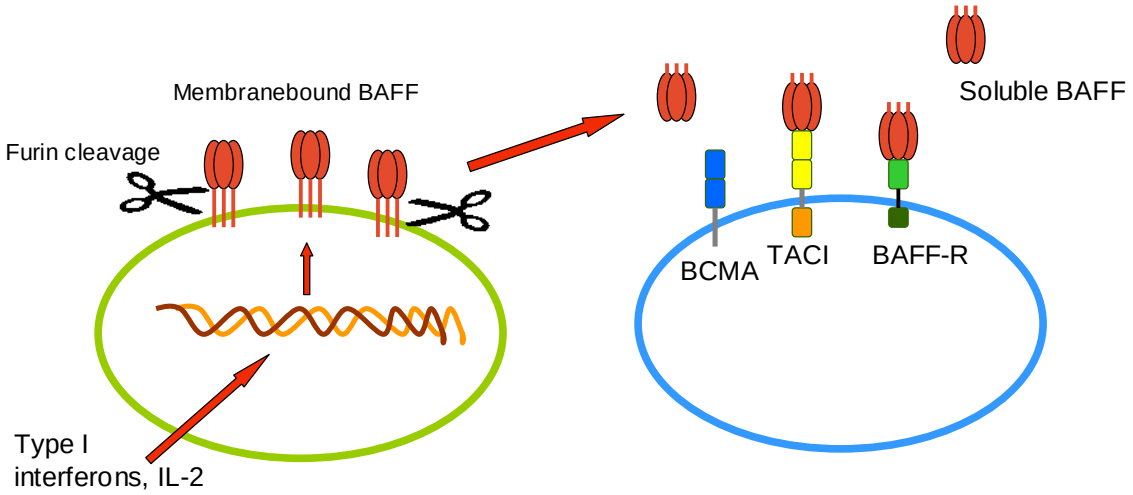
edema) occurs in patients with LN, treatment with diuretics is recommended as long as edema persists (67).

Regular controls of blood lipids are important because all patients with SLE are at increased risk of cardiovascular diseases. Proteinuria as seen in LN, may increase serum lipid levels. If dyslipidaemia is observed in SLE patients, dietary changes and weight reduction should be considered prior to medical treatment. Statins are the preferred drug (with a target LDL cholesterol < 2.6 mmol/l) as they also may have anti-inflammatory effect (106). A coagulation screening including protein C, protein S and ATIII levels is indicated in patients with significant proteinuria , while in patients with aPL aspirin should be considered, especially when vascular disease already is present (105). In addition, hydroxychloroquine should be considered as a basic medication in all SLE /LN patients as it helps to avoid flares in SLE disease, as well as to reduce the risk of LN relapses (107).

B-cell activating factor (BAFF)

B cell activating factor (BAFF, TNFSF13 or BLyS) belongs to the tumor necrosis factor (TNF) superfamily and is an important stimulatory factor for B cell development, B cell homeostasis and immunoglobulin production (108). BAFF is found as a transmembrane protein on a range of immune cells. The biologically active 29 kD subunit from BAFF are proteolytically cleaved at furin consensus sequences and exists as a soluble protein (Figure 3) (109;110). BAFF production can be stimulated by different inflammatory cytokines such as IL-2 and INF-γ (111;112). BAFF is the primary determinant of B-cell longevity and numbers of mature B-cells because it attenuates B-cells apoptosis by interfering with the NF-κB pathway (110;113). Binding of BAFF to the different receptors on mature B cells (BAFF-R, TACI and BCMA), induces either Ig class switching, cell proliferation or increased survival of B cells (110).

Figure 3



BAFF exists in membranebound and soluble forms and bind to three distinct receptors: B-cell maturation antigen (BCMA), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and BAFF receptor (BAFF-R). All receptors are predominantly expressed on B – and T cells.

BAFF in SLE

The above mentioned functions of B cells are all relevant in the pathogenesis of human SLE, when considering the importance of B cells as antigen presenting cells and precursors for autoantibody production.

In lupus prone mice, serum-BAFF (s-BAFF) levels are increased at disease onset and blocking of BAFF-dependent signals with soluble receptor prolongs their survival (114). Transgenic mice over expressing BAFF are developing B cell hyperplasia and hypergammaglobulinemia. In addition, a striking increase in circulating autoantibodies can be measured and an immune complex mediated disease occurs with features of SLE disease (115;116).

In SLE patients, s-BAFF levels are frequently elevated and associated with the presence of anti-dsDNA Ab (117-120). These findings suggest that BAFF is involved in the selective loss of immune tolerance in some the B cell types in human SLE, resulting in autoantibody production. This hypothesis initiated several clinical trials targeting B cells in SLE patients (121).

Great expectations have been related to treatment with various monoclonal antibodies that specifically recognizes and inhibits the biological activity of BAFF. Belimumab, a fully human monoclonal antibody, has been assessed in patients with active SLE. The result of a phase 3, dose-ranging, randomised placebo-controlled trial of belimumab with standard care in patients with SLE is recently published (122). This trial showed efficacy of belimumab in controlling SLE in a broad range of patients, and thus, may be the first targeted biological treatment that is approved specifically for SLE.

Role of TNFSF13b/BAFF gene in SLE

The mechanisms that are responsible for the increased s-BAFF levels in SLE are currently unclear, however genetic predisposition has been postulated to be one of the mechanisms involved (123;124). The B cell hyperactivity in SLE patients could be due to specific mutations/polymorphisms in the *BAFF* gene *(TNFSF 13b),* localized at chromosome 13. Such mutations/polymorphism may influence the expression/stability of the BAFF transcript. However, the only available report on the BAFF genotype was performed in a Japanese SLE patient cohort. In this study no mutations/polymorphisms were found in the coding region of TNFSF-13b. The authors of this study were neither able to find an association between disease susceptibility and single nucleotide polymorphisms (SNPs) in the 5 ' regulatory region of the BAFF gene (13q32-34) (125). However, an association between anti-Ro/La positivity and a specific BAFF haplotype (CTAT) has been shown in Caucasian

patients with primary Sjögren's Syndrome (pSS) (126). Also, c.-871 T allele in the 5' regulatory region of the BAFF gene has been associated with increased s-BAFF levels in patients with pSS and indicating that this SNP may be involved in increased BAFF expression (126).

Increased s-BAFF levels in SLE may be linked to increased BAFF gene expression. Several studies have shown that BAFF gene expression can be increased through interferon type I inducible cytokines (127;128). In a cross sectional study on Chinese SLE patients, BAFF gene expression in peripheral blood mononuclear cells (PBMCs) was shown to be correlated with disease activity and anti-dsDNA Ab levels (129). However, in a longitudinal North American study, BAFF mRNA was not associated with s-BAFF levels in 60% of the investigated patients (118). The discrepancies between these few reports as well as the small number of SLE patients studied make it difficult to draw solid conclusions. Consequently more studies are required to determine the relative contribution of polymorphisms/mutations on the expression of the BAFF gene and its correlation to disease susceptibility of SLE.

5. AIMS OF THE STUDY

Paper I

The aim in paper I was to validate the ACR97 classification criteria (24) and determine to what extent the introduction of aPL antibodies may have influenced the epidemiology, disease presentation and management of SLE in Northern Norway. To achieve this, data on incidence, prevalence, SMR and survival were obtained in a recent inception cohort and then compared with results from a historical inception cohort based on the ACR82 classification criteria (23;30). These findings provided insight into the changes over time in the clinical epidemiology of SLE in Northern Norway.

Paper II

The aim of paper II was to elucidate the reasons behind the remarkable reduction of LN prevalence in the 97acr cohort observed in paper I. We wanted to investigate if and how the inclusion of aPL Ab in the ACR97 criteria had affected the frequency and severity of LN in the context of the increased awareness of cardiovascular morbidity. To this purpose, we evaluated the clinical presentation, laboratory findings, histological severity and management of disease in patients with or without LN in both cohorts. These findings provided insight into the linked changes over time in the clinical presentation and management of LN in Northern Norway.

Paper III

The aim of paper III was to determine whether increased circulating levels of BAFF in patients with SLE can be related to increased gene expression and/or variations in the genetic structure of the promoter region of the BAFF encoding gene. Correlations between four SNP in the regulatory region of the BAFF gene, BAFF gene expression s-BAFF levels, and

different clinical and laboratory findings were investigated. These findings help to improve our understanding of the role of BAFF in the production of autoantibodies and inflammation in SLE.

6. SUMMARY OF RESULTS

Paper I: The influence of the 1997 updated classification criteria for Systemic Lupus Erythematosus: Epidemiology, disease presentation, and patient management.

Two inception cohorts of SLE patients in Northern Norway based 97acr (n=58, enrolled during 1996 -2006 using ACR97/ACR82) and 82acr (n=81, enrolled during 1978-1995 using ACR82) were compared to investigate the possible effects of ACR97 criteria. The mean annual incidence of SLE was slightly higher for cohort 97acr vs. cohort 82 acr, (3.00 vs. 2.63 p=0.5). The crude point prevalence of SLE at January 1, 2007, was 64.1/100,000 overall (109/100,000 in females). In cohort 97acr, significant fewer patients were presented with renal disease (OR 0.28), in contrast to the presence of autoantibodies such as anti-dsDNA (OR 2.57) and aPL (OR 27.9). Also, initial treatment with methylprednisolone (OR 9.23), azathioprine (OR 6.32), and low-dose aspirin (OR 20.9) was more common in cohort 97acr. In addition, five- and ten years survival (95.2 %, 91.9 %) were improved in cohort 97acr compared to 82acr.

This article demonstrates that by use of the ACR97 criteria, the presentation of autoantibodies at disease onset increases while SLE patients are more aggressive treated.

Paper II: Decreased incidence of lupus nephritis in northern Norway is linked to increased use of antihypertensive and anticoagulant therapy.

Using a similar approach as in paper I, reasons for the decreased frequency of LN were sought in two cohorts 97acr (n = 62) and 82acr (n = 87). Between 1978 and 2006, the AIR for LN decreased from 0.7 to 0.45/100 000, while the LN prevalence rose from 7 to 14/100 000. The relative risk reduction in the 97acr for early- and late-onset LN (> 3 months

after SLE diagnosis) was 39 % and 42 %, respectively. During the first 10 years of disease, LN development in all patients (n = 39) was significantly associated with SLEDAI ≥ 10 (HR 6.3), hypertension (HR 3.0) and ESR > 20 (HR 3.0).

Patients who developed LN in the 97acr cohort (97LN+; n = 11) had similar demographics and histological findings by renal biopsy as the 82acr cohort (82LN+; n = 28). However in 97LN+, more often low avidity anti-dsDNA Ab and/or aPL Ab were present at onset of SLE diagnosis, while proteinuria and diastolic blood pressure were lower than in 82LN+. Following onset of LN diagnosis, more 97LN+ patients received pulse corticosteroids (55 % vs. 7 %), anticoagulants (46 % vs. 4 %) and antihypertensive drugs (46 % vs. 11 %). During a 10-year follow-up, three 82LN+ patients (11 %) developed ESRD versus none in 97LN+.

These findings indicate that a strategy including early diagnosis based on low avidity anti-dsDNA- and aPL Ab testing combined with early initiation of treatment can reduce the occurrence and severity of LN. This paper hints at the possibility of LN prevention.

Paper III: Increased levels of BAFF in patients with Systemic Lupus Erythematosus are associated with acute phase reactants, independent of BAFF genetics.

This cross sectional study investigated the role of BAFF in 101 Caucasian SLE patients and 111 healthy controls. We found that genetic variation in the promoter region of the BAFF encoding gene are not associated with SLE susceptibility, *BAFF* gene expression in PBMCs or increased s-BAFF. Increased BAFF mRNA levels were found in SLE patients (RQ 1.8 vs.1.1, $p<0.001$) and BAFF-RQ correlated inversely with CD4+ lymphocytes (β -0.27, $p<0.012$) and IgG levels (β -0.25, $p = 0.023$). S-BAFF was increased in SLE patients (1.73 vs. 0.98 ng/µl, $p<0.001$) and was strongly correlated with acute phase reactants. CRP (β 0.40, $p<0.001$) and inversely with haemoglobin levels (β -0.32, $p<0.001$) and IgA levels (β -0.33,

p=0.001). Also, s-BAFF was increased in SLE patients with anti-dsDNA Ab compared with patients without anti-dsDNA Ab (2.2 ng/μl vs. 1.6, p=0.009).

This paper indicates that increased s-BAFF is the result of local antibody mediated inflammation and not a primary driving factor in the pathogenesis of SLE.

7. METHODS

Ethics

Clinical studies (paper I and II)

Written informed consent was obtained from all patients in the study. The study was approved by the local ethical committee.

Experimental study (paper III)

Experimental protocols and the establishment of a patient biobank were approved by the local ethical committee, the national privacy agency and the Ministry of Health (ref. no 12420).

Study design

Paper I and II are retrospective longitudinal observational studies while paper III is a case control study designed as a cross-sectional study.

Study participation

Clinical studies (paper I and II)

The data for the studies are derived from the Tromsø Lupus Cohort, a longitudinal population-based registry of SLE patients in Northern Norway. The Tromsø Lupus Cohort was established in 1997 by J.C. Nossent (30) and in recent years it has been upgraded several times. All SLE patients in this register meet the classification criteria for SLE disease, either through ACR82 (23) or ACR97 (24) (Table 1). Paper I and II are based on information on SLE patients from all rheumatology outpatient clinics throughout Northern Norway including the Department of Rheumatology at University Hospital in Northern Norway (UNN). The data were recorded during the years 1978-2006 and the SLE patients (> 15 years) were divided in two cohorts based on the year they were diagnosed with SLE disease. The oldest

cohort (82acr) included patients with SLE onset during 1978-1995, using the ACR82 classification criteria, while the youngest cohort (97acr) included patients with SLE onset during 1996-2006 using the ACR97 classification criteria. These two inception cohorts were compared in both paper I and II.

Experimental study (paper III)

The patients in paper III are a selection from Tromsø Lupus Cohort. At the same day, they were extensively clinical examined and blood samples were drawn for analyses used in this study as well as for storage of serum, DNA and RNA. Hundred and one SLE patients (>15 years) were investigated in the period 2006-2008. The patients were mainly (99 %) of Caucasian descent and 87 % were female. The median age was 47 years and median disease duration was 10 years. In the SNP analysis, 111 healthy controls were included; 71% were female and the median age was 48 years. In the studies of BAFF gene expression and s-BAFF levels only 31 healthy controls were included and also these controls had similar gender and age as the SLE cohort.

Data collection

The time of SLE diagnosis was defined as the point of time when the patients cumulatively fulfilled at least four ACR criteria, using either ACR82 or ACR97 (Table 1). Disease duration was recorded as the time interval from SLE diagnosis until the last follow up visit or time of death in paper I and II.

Data for each hospital consultation for each patient were recorded in a database using a predefined data sheet. This included demographics, clinical findings and medication together with results of routine haematology surveys, biochemistry analysis and immunologic tests. In paper III, patients underwent an extensive clinical examination followed by collection

of blood samples for laboratory assays, DNA and mRNA. For every hospital visit the disease activity was calculated using SLEDAI (53) and organ damage development was scored by SDI (57), preformed by an experienced rheumatologist. All information was obtained directly from patients or indirectly from hospital records. When information on clinical items were not available or could not be retrieved from other sources, they were scored as not being present. Medication was assessed at every consultation; oral prednisolone usage was recorded in mg/day, while for other drugs the use of the specific drug for at least three months was required for recording in the clinical studies (paper I and II). In paper III, only the presently used drugs were included.

In paper II, arterial hypertension and results form renal biopsies were central features. Our definition on arterial hypertension followed accepted guidelines and consisted of blood pressure exceeding 140/90 mmHg (135/90 mmHg for persons < 40 years) or the use of antihypertensive drugs for more than 3 months (104). Renal tissue obtained through percutaneous biopsies was re-evaluated independently by two pathologists for the following features classification of LN as defined by ISN/RPS 2003 classification of LN (73;130), Activity and Chronicity indices as defined by the NIH (131) and the presence of vasculitis/vascular thrombi. Histological scores in paper II represent the mean score of the evaluations done by the two independent pathologists.

Laboratory methods

Serology, in paper I, II and III

Routine laboratory investigations reported in all studies were performed in Department of Laboratory Medicine and Immunology at UNN.

ANA-positive sera were routinely tested by enzyme immunoassays (VarELISA Phadia, Freiburg) for the presence of IgG subclass antibodies against double stranded DNA

(anti-dsDNA), Ro (anti-SSA), La (anti-SSB), Smith (anti-Sm), anti-U1 small nuclear ribonucleoparticle (anti-U1-snRNP) and cardiolipin (aCL-G and aCL-M; normal levels < 16 IU/mL). In addition, ANA-positive sera were tested for high avidity anti-dsDNA Ab by Crithidia Lucilliae assay (normal < 1:10) until 2001 and thereafter by enzyme linked immunosorbent assay (ELiA) (Pharmacia, Germany) (normal levels < 15 IU/ml). Lupus anticoagulant (LA) was tested in a three step, phospholipid-dependent coagulation assay (132).

Experimental study, paper III

S-BAFF levels were measured in patients' serum using a Quantikine Human BAFF/BLyS/TNFSF13B Immunoassay (R&D, USA). All measurements were done in duplicates and results were averaged.

In the SNP analysis genomic DNA was extracted from whole blood and purified according to the instructions provided (Puregene Genomic DNA purification Kit, Gentra systems, Minneapolis, Minnesota, USA). The primers and probes were designed using the LightCycler Probe Design Software (Roche Diagnostics, Mannheim, Germany). Primers specific sequences are detailed in paper III (Supplemental Table).

Primers and probes for BAFF gene expression were designed using the BAFF encoding gene *TNFSF13B* (NC_000013.10) and *B2M* gene encoding β2-microglobulin (β2M) (NC_000015.9) as templates. Primers were designed using Enhanced Avian HS RT-PCR software (Sigma-Aldrich, Saint Quentin Fallavier, France) and probes were selected using Universal ProbeLibrary Human Gene Assay (Roche) (Table 1, Paper III).

PBMCs were separated by Lymphoprep™ (Axis-Shield, UK) and the cells were stored frozen as pellets or in RNA later. Total RNA from frozen mononuclear cells was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany), following manufacturer's instructions.

RNA was DNase I treated, and stored at -80ºC until the samples were thawed and used to synthesize cDNA by SuperScript VILO cDNA Synthesis Kit (Invitrogen, CA, USA) according to the protocol provided by manufacturer. Real-time PCR analysis was performed to determine the levels of BAFF mRNA in PBMCs using an ABI PRISM 7900HT, (version 2.3, Applied Biosystems, CA, USA). PCR reactions were done in triplicates. The BAFF transcript quantification was standardized using β2M as internal control, BAFF-RQ was calculated as the ratios of BAFF mRNA to β2M mRNA using the following formula: 2 exp(Ctβ2M – CtBAFF). Cut-off level of BAFF-RQ was determined by the geometric mean + 2 SD of healthy controls (n = 31). More detailed description can be found under the section on methodology in paper III.

Data analysis and statistics

Due to relatively small patient numbers in the cohorts and subgroups in addition to the fact that most data had a skewed distribution reported numbers are median values (unless indicated otherwise) and nonparametric test methods were used in statistical analyses. Continuous data were analyzed by Mann-Whitney U test and dichotomous data by Poisson distribution contingency tables or Fishers' exact test in case of low numbers. Correlations were analyzed by Spearman rank correlation coefficients. Annual incidence rate (AIR) and point-prevalence (PP) are reported per 100,000. Survival rates were estimated by Kaplan-Meyer method and compared by log-rank tests. Standardized mortality rates (SMR) were calculated by randomly assigning each patient 5 controls, born in the same year and month and matched for sex and municipality by area code. Risk factors were analysed by Cox proportional hazard models, and hazard ratio (HR) were reported with 95% confidence intervals (CI).

To determine the potential associations between different variables, all associations with a p-value < 0.2 in univariate analyses were entered into multiple regression models. If appropriate, interdependence was corrected. Statistical analyses were performed with SPSS v 11.0 or 17.0 and Epi Info version 4.1, while genotype and haplotype analyses were performed by the freely available SNPStats software (133).

8. GENERAL DISCUSSION

The investigation of possible changes in the clinico-epidemiological characteristics of SLE in Troms and Finnmark was performed with longitudinal retrospective studies in paper I and II. In paper III, we used a cross sectional case control study design to investigate the genetic and serologic significance of the cytokine BAFF in SLE. There are particular concerns with each of this type of studies that must be taken into account when the results are interpreted.

Paper I

The ACR criteria (22-24) represent the result of extensive statistical modelling to reach the lowest amount of heterogeneity in SLE study cohorts. While some of the included criteria have changed over the years, the basic principle of minimum four criteria to be classified as SLE has not changed. Our data show that the latest change in 1997 lead to a small, but not statistically significant increase in the number of new cases per year, as overall AIR increased from 2.6 in cohort 82acr to 3.0 in cohort 97acr. This trend, we assume is associated with the increased use of assays to detect autoantibodies in cohort 97acr that resulted in diagnosis of SLE at an earlier stage of disease development. This is in accordance with the theory put forward in Figure 2, where autoantibody production occurs years prior to the development of clinical symptoms of SLE and shows that such a using this strategy confers clinical benefits. The fact that neither AIR nor diagnostic delay changes were significantly different is most likely due to the limited number of patients.

SLE remains a clinical syndrome with a diverse phenotype that is also variable over time in each single patient. ACR criteria are often used as the basis for a clinical diagnosis of SLE, even though this practice has several drawbacks. Firstly, the application of ACR criteria leads to selection of patients with a classical SLE presentation and excludes patients not

fulfilling four of the required ACR criteria. In clinical practice, this may lead to a situation where patients with less than four classical ACR criteria but several non-classical manifestations such as alopecia and Raynaud's phenomena, are not appropriately diagnosed and/or managed e.g. by not receiving beneficial treatment such as antimalarial drugs. The importance of considering SLE as a progressive disease is emphasized in paper I and II with regard to early diagnosis and treatment. Early diagnosis may lead to increased survival of SLE patients partly by reducing LN severity. Most likely, the narrow ACR97 criteria should be reconsidered, as shown by the development of the Boston criteria (134). The Boston criteria set reflect the inclusion of patients with objective findings of SLE in a weighted system, highest weight corresponding to presence of renal disorder (especially patients with LN, WHO class III-VI), discoid rash and cytopenias. Accordingly patients with less than 4 ACR criteria can be defined as SLE. The Boston Criteria identifies 7 % patients more compared with the current ACR criteria, while these criteria still retain face validity. Thus, a system like this could minimize selection bias and increase the generalizability of clinical SLE studies. If the currently ACR criteria allow more emphasis on anti-dsDNA- and aPL Ab, renal findings, alopecia, Raynaud's phenomenon and even hypertension, probably more patients would be diagnosed earlier in SLE disease development. Such changes in a new criteria-set can be done by increasing the number of criteria similar as the ARA from 1971 (Table 1) and/or introduce different weighted criteria as the Boston criteria.

In both papers I and II, patients with less than four ACR criteria have been excluded from the studies. In a study from the same region published in 2001, an overview was published of SLE patients and patients with diagnosis that might develop SLE (30). This study demonstrated that as much as 17 % of the patients, with diagnosis such as lupus-like disease (6 %), unclassified and mixed connective tissue disease (4 %), drug induced lupus (2 %) and discoid lupus (5 %), might in theory develop SLE over time. This finding is in

agreement with other studies showing that patients with typical symptoms of SLE without fulfilling four of the ACR criteria will develop sufficient classification criteria over time (32;135;136). These considerations have an implication on the current study presented in paper I and II, since the selection criteria for entry into Tromsø Lupus Cohort was according to the ACR criteria.

It is also important to realize that several clinical manifestations were excluded during the development of the SLE criteria set from 1971 to 1997 (Table 1). Currently, a patient may manifest a multisystem disease consistent with SLE without fulfilling the ACR criteria or even opposite; a patient can fulfil four ACR criteria while the clinical diagnosis of SLE is questionable. The criteria such as photosensitivity and malar rash are highly interrelated and there is also a strong correlation between positive test of anti-dsDNA Ab and ANA (137). In clinical practice, the impact of including patients with interrelated criteria is not of great importance, but a few questionable SLE patients are probably included in most SLE cohort studies, although this does not need to have a major impact on the results. In paper I, we concluded that increased serological surveillance with ELISA-based assays of anti-dsDNA and aPL Ab have contributed to include a number of patients with milder lupus. These findings were based on the use ACR97 criteria, where autoantibodies are more emphasized compared with the ACR82 criteria.

In the future, the increased use of 97ACR criteria as well as the increased serological surveillance, will probably contribute to earlier diagnosis of more SLE patients.

In epidemiological studies, the selection of patients is often a challenge, and inconsistencies can result in large discrepancies that may invalidate results obtained. Therefore, in paper I and II, the methods for selecting SLE patients were especially important to minimize sources of error in epidemiological calculations. In paper I, our goal was to find all SLE patients in Troms and Finnmark (which has an approximately population on 225 000

inhabitants) and include them in Tromsø Lupus Cohort. To enrol patients with SLE, we got access to databases from all departments in hospitals and clinics in this area. The journal of the patients with diagnosis codes that could be related to SLE were reviewed with respect to the classification criteria for SLE was fulfilled. Despite our inclusion criteria, we had no guarantee that SLE patients would not be missed, for example because of limited recognition by general practitioners, patients having quiescent SLE for prolonged periods of time disease or because some patients had recently moved to Troms and Finnmark. By being aware of these sources of errors, we used a capture-recapture analysis of incidence studies. Accordingly, we estimated that the proportion of potentially missed patients is up to 2.4 % (138).

The gold standard for evidence in clinical research comes from prospective, controlled and randomized trials. Such studies allow the quantification of absolute and relative risk associated with a specific condition and/or treatment. Given the low frequency of new onset SLE with only 2-3 cases per 100,000 adults in our study area, an inclusion period of ten years would give a modestly sized cohort of 50-60 incident cases. Furthermore, financial efforts for regional collaboration, that would have increased our cohort size, have so far failed. The current observational study of all SLE patients in Troms and Finnmark within a certain timeframe is therefore a practical compromise that balances the aims of our study with the available resources. As several similar incidence studies exists and average AIR of 2.8 in paper I is in accordance with the reported Scandinavian incidence rates (1.5 – 4.8) (27;29-32), we assume that our inclusion methods are satisfactory and appropriate for further study.

A major challenge in retrospective observational studies as in paper I and II, where potential factors and outcome of interest are recorded, is to draw conclusions that are

sufficiently free from bias. The recorded factors are sometimes not directly responsible for the observed differences in the results, while other unregistered factors actually are related to the observed differences. Furthermore, recorded or unrecorded factors may be correlated with each other and may lead to incorrect conclusions. In addition, as the number of recorded factors increases, the likelihood that at least one of the recorded factors will be highly correlated with the outcome increases simply by chance. Recognizing the limitations of this type of study, guidelines are developed recently by The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). This initiative includes 22 recommendations on what should be included in an accurate and complete report of an observational study (139). While our studies were initiated before these guidelines were available, there is considerable although not complete agreement between the recommendations and the presentation of our results.

The laboratory data reported are based on routinely performed analyses at UNN). The quality of these data is ensured by standardisation of methods including regular quality control checks, as well as participation in a national accreditation process. The immunology laboratory has a similar quality control system for the detection of autoantibodies by ELISA techniques and complement levels measured by standard nephelometry, where the quality of the methods used are ensured by participation in an international accreditation process (UK NEOAS). The laboratory data reported in all the three papers included are considered reliable and representative for clinical practice.

The database of registered SLE patients was established in 1997. The updated database used for paper I and II contained a total of 2671 registered hospital visits with a maximum of 261 variables that were recorded per visit. This large amount of data entailed specific

problems in terms of data management and subsequent newly introduced analyses. Almost all observational research has to deal with missing data. The most common procedure is to omit those cases with missing data and to run analyses on remaining records. However, this method may lead to a loss in statistical power, making it a less attractive option for variables with more than 5 % missing values. Another method use imputation (substitution of values for a missing data point) for which several procedures are available. Imputation was applied in paper I and II, where missing data for potential predictors of survival and LN were substituted with group mean values in order to retain sufficient power for multivariate analyses.

Once the data set was considered as complete as possible after applying the above mentioned corrections, subsequent data analyses was facilitated by the use of statistical computer software (SPSS). The use of nonparametric methods in the evaluation of group differences resulted in stringent conditions of the reported statistical analyses. This was done to avoid type I errors (false positive results), although this increased the risk of type II errors (false negative results). Type II errors may exist in all papers in this study, where subgroups with low numbers resulted in limited statistical power and accordingly increases the risk of not detecting a real existing difference.

Paper II

The methods applied in paper II were to a large extent similar as paper I and the arguments in the discussion above are also valid for paper II. However, the specificities of renal disease and its implications merit further discussion.

Main findings in paper II were that early detection of autoantibodies with sensitive assays in combination with early treatment, including administering antihypertensives and

anticoagulants, can contribute to reduce the prevalence and severity of LN, with subsequent improved renal survival. Therefore, it seems important to diagnose and treat SLE patients as early as possible, and preferably before manifestation of several renal symptoms occurs, because once these are observed, the risk for organ damage increases sharply. This treatment strategy is in accordance to the current paradigm in the management of inflammatory joint diseases, where early diagnosis and treatment is instituted to avoid the development of erosive bone lesions. In our study, no significant differences were seen between the cohorts regarding the various ISN/RPS classes or NIH Activity or Chronicity Indices scores. The histological findings at LN diagnosis in patients with SLE showed a majority of proliferative lesions (ISN/RPS class III and IV), and the overall percent for these lesions in our cohorts is in agreement with a review report of Tumlin (Table 4) (140). Currently, no patients with LN in the 97acr cohort have developed ESRD or advanced renal damage (as ISN/RPS class V or VI) by renal biopsy findings. This is in contrast to the above mentioned report showing that 10 - 20% of renal biopsy from patients with LN was evaluated as ISN/RPS class V. All together, this may indicate that renal damage in the last cohort included (97acr) in our study has become less severe.

Concerning the initial development of autoreactive Ab in SLE/LN patients, this can be due to molecular mimicry of viral or bacterial antigens with self-determinants that may succeed in epitope spreading (141). Epitope (the binding-site of Ab with antigen) spreading involves the recognition of new epitopes within the same antigen. The term also covers epitopes residing in proteins that are associated in the same macromolecular complex like the nucleosome, that in fact is the main autoantigen circulation in patients with SLE (142). This initial binding, as seen in Figure 1, implies further the progression of an autoimmune response to a chronic state involving increased targeting of autoantigens by T cells and Ab. Once

immune tolerance to one component is abrogated, B- and T-cell responses can diversify to other components of the macromolecule with the recognition of other epitopes in the intact particle. Over time, most likely epitope spreading results in amplification of autoimmunity that includes production of autoantibodies as shown in Figure 2.

The above mentioned theory is strengthened based on results of LN in mouse experiments. In mice, IgG autoantibodies against dsDNA or nucleosomes are detected in serum before clinical findings like proteinuria occur, indicating the fact that nephritogenic autoantibodies play a central role in LN (82). Two main hypotheses exist explaining how antibodies are involved in the development of LN. 1) Antibodies cross-react with non-nucleosomal glomerular antigens, or 2) they recognise exposed chromatin fragments associated with glomerular basement membranes (82;143;144). Regardless to which theory account, this autoimmune development of LN over time is in line with our results in paper II, where the incidence of LN decreased simultaneously with increased detection of low avidity autoantibodies, which suggesting that SLE patients were identified earlier in the disease course.

Both general practitioners and rheumatologist should however emphasize the detection of autoantibodies to a greater extent because this is an early warning that these patients probably will develop SLE. Studies have shown that detection of anti-DNA-, anti-nucleosome and aPL Ab occurs several years before onset of SLE disease (86;87). In a recent study from Northern Sweden, it was shown that the interval between a positive test for anti-dsDNA Ab and diagnosis of SLE was as long as 6.6 years (145). These results are in agreement with paper I, where the SLE patients in the cohort 97acr had more detected autoantibodies as criterion of SLE and less clinical manifestations including renal disease.

Even when patients do not meet the four ACR criteria, but rather have diffuse symptoms that may be associated with SLE including these autoantibodies; they should be

regarded as having an as yet unclassified autoimmune disease that may require intervention. Such patients will therefore need to be evaluated by a consultant in Rheumatology and follow-up needs to be scheduled. Urinalysis is easy to perform and provides much information about renal involvement in SLE patients by detection of proteinuria and/or erythrocytes. This basic investigation should be done regularly in general practice, and if abnormalities are detected, also urinary microscopy should be performed before the examination at the rheumatologic outpatient clinic. Hypertension is associated with renal impairment, hypercholesterolaemia and increased risk of coronary heart disease in patients with SLE. Therefore, blood pressure should be monitored closely and if systolic blood pressure > 130mm Hg and diastolic > 80mm Hg are measured, patients should be treated with ACE-I and/or ARB which also reduce proteinuria (105). Anticoagulation should be considered for patients with aPL and/or increased risk of cardiovascular disease.

Implementing the outlined strategy for earlier diagnosis, referral and intervention in clinical practice will be a major challenge, as doctors need to rethink their tendency to diagnose SLE patients mainly based on clinical symptoms according to the ACR criteria. Given it relative ease and health benefits for each patient, this message should be clearly brought forward to the general practitioners.

Paper III

We investigated the importance of BAFF in patients with SLE and applied a cross sectional, case control study design. We are aware of the limitations of this study as Stohl and his colleagues who conducted a longitudinal observational study of SLE patients and showed that levels of s-BAFF and BAFF mRNA in PBMCs varies over time. In addition to a marked heterogeneity of the persistent s-BAFF and BAFF mRNA levels, intermittent elevated levels were registered (118). Since BAFF is a signal molecule and the levels present may fluctuate in blood in single individuals, the study design that we chose has an intrinsic weakness.

Ideally, a longitudinal study to investigate the temporal relationship between s-BAFF and BAFF gene expression should have been preformed to complement our data.

Assays for measuring s-BAFF levels were performed at the Rheumatology Research Laboratory at the University of Tromsø. A longstanding experience with ELISA based determination of antibodies, along with the regularly carried out quality control check, ensured the robustness of our analysis. S-BAFF analyses were performed with commercially available kits based on ELISA techniques that have undergone pre-marketing quality controls. Since BAFF is a short-lived cytokine involved in paracrine cell communication, it was important to minimize the time from blood samples were taken to the serum was frozen. Accordingly, blood samples were processed within two hours after the samples were retrieved, even though during this time various molecules in serum might be degraded. Also, the freezing of serum samples prior to analysis could have caused a bias, because cytokines are sensitive to freeze-thawing procedures. However, all measurements were performed on sera that had been frozen only once, in accordance with other studies on serum BAFF (118).

Currently, 366 SNPs are recognized in the BAFF gene (www.ncbi.nlm.nih.gov/snp). Four of the examined SNPs in the5' promoter region of the BAFF gene were selected based on findings from other articles (125;126;146). Whereas c.-514 (A>G) was chosen because it might be associated to the NF-κB pathway and thereby inhibiting apoptosis (113). The haplotypes, associated with the SNPs located within the promoter were in strong linkage disequilibrium ($p<0.00001$). Our study was a genetic pilot study to see if any obvious associations between the examined SNPs and SLE susceptibility existed. Given the small differences we noted, the cohort size need to increase by at least a factor ten to reach sufficient statistical power. While the protocol for this BAFF study was planned years before the results of genome wide studies were available, the lack of association in our study is nonetheless consistent with current findings in GWAS (147).

The assays used for the SNPs analysis were designed and performed in Laboratory Medicine Department, whereas BAFF gene expression was investigated in Medical Genetic Department, UNN. These departments have long experience with similar assays, including quality control tests. Regarding to BAFF gene expression assays, cDNA was synthesized from total RNA at the Rheumatology Research Laboratory at the University of Tromsø. Since no correlation with BAFF gene expression and s-BAFF levels were observed, we speculate in paper III that a negative feedback mechanism may exist. In agreement with a previous Chinese study (129), we found increased BAFF mRNA in SLE patients compared to controls. In contrast to our findings, Ju and colleagues found increased BAFF mRNA correlated with anti-dsDNA Ab levels. However, correlation between BAFF mRNA and s-BAFF levels was not examined in this Chinese study, where only 37 SLE patients were included.

We did find a strong inverse correlation of BAFF gene expression with numbers of CD4+ T-cells, in disagreement with Morimoto and colleagues who suggested that autoantibody production is driven by BAFF produced by T cells and may accordingly play a pathological role in SLE (148). Their results are based on the expression of BAFF mRNA in isolated cultured T cells of SLE patients. Since neither s-BAFF levels nor their relation with BAFF gene expression were reported in that study, it is not possible to determine in which extent BAFF production by CD4+ cells is affecting the s-BAFF levels. The discrepancy between the BAFF gene expression and s-BAFF levels, have also been reported elsewhere (118) and suggest that the origin of s-BAFF is more complex. Therefore, our data support a prior suggested hypothesis that a negative regulatory feedback mechanism may exist between s-BAFF levels and BAFF mRNA expression in PBMCs (146).

In paper III, increased s-BAFF was associated with the acute phase reactants CRP and hemoglobin, but not with ESR, autoantibodies and hypocomplementemia which usually reflect SLE disease activity. CRP levels on the other hand do not reflect disease activity in

SLE very well, except for that very high levels of CRP are seen in SLE patients with polyarthritis, serositis or a bacterial infection. Therefore, the correlation between s-BAFF and CRP in our study may reflect cytokine signalling to hepatocytes, related to production of CRP. Increased production of CRP may be related to the presence of antibodies against CRP or the tissue binding of CRP on immune complexes that reduces serum CRP availability (149). In response to infection or injury, local inflammatory cells (neutrophils, granulocytes and macrophages) secrete a number of proinflammatory cytokines into serum, most notable of which are the interleukins IL-1, IL-6 and IL-8, and TNF-α, which further stimulates hepatocytes to produce CRP.

Anemia of chronic disease is a common manifestation in rheumatic diseases, and is related the above mentioned cytokines included CRP (150). Since s-BAFF was associated to both anemia and CRP it would be interesting to conduct a new study to see if there are any correlation between s-BAFF and any of the proinflammatory cytokines in SLE patients. S-BAFF was also associated with the presence of anti-dsDNA Ab, although it did not correlate with the levels of autoantibodies. Secretory IgA in the mucosa is important to protect individuals against microorganisms. In paper III, a significant inverse correlation between s-BAFF and IgA was seen, which can either be caused by impaired production of IgA or possibly a negative feedback between s-BAFF and IgA.

9. MAIN CONCLUSIONS

1. The use of the 1997 update of the ACR classification criteria of SLE, did not lead to significant changes in SLE incidence or demographics when compared to the 1982 criteria (Paper I).
2. The mean AIR of LN in Northern Norway decreased from 0.7 during 1978-1995 to 0.45 in during 1996-2006, with a relative risk reduction of developing LN of about 40% (Paper I and II).
3. Increased use of sensitive assays for anti-dsDNA- and aPL Ab during 1996-2006 at the onset of both SLE and LN seems to have contributed to earlier identification of patients at risk for severe disease (Paper I and II).
4. This early detection by sensitive assays for anti-dsDNA- and aPL Ab together with an early and more aggressive therapeutic approach, seems to have contributed to a milder disease course and subsequent improvements in survival (Paper I).
5. Early detection of low avidity assays for anti-dsDNA and aPL Ab, probably in combination with early treatment that includes immunosuppressive and vasoprotective drugs have contribute to reduced incidence of LN and improved renal survival (Paper II).
6. Polymorphisms in the BAFF promoter do not increase the susceptibility for SLE in a Nordic population (Paper III).
7. In SLE patients, s-BAFF is at increased levels independent of BAFF promoter polymorphisms or BAFF mRNA expression in PBMC (Paper III).
8. Increased levels of s-BAFF in SLE patients are not correlated with overall measures of disease activity, but with positive test of anti-dsDNA Ab (Paper III).
9. Increased levels of s-BAFF in SLE patients are associated with acute phase proteins (Paper III).

10. S-BAFF production most likely occurs at specific sites where anti-dsDNA Ab is involved in the inflammatory process (Paper III).
11. To solve all these challenges has been a joyful experience.

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11. TABLES

Table 1 Changes in the classification criteria of SLE. 1971 preliminary criteria for SLE, ACR82: American College of Rheumatology (ACR) - revised classification criteria for SLE, published in 1982 and ACR97: updated classification criteria for SLE, published in 1997. For the diagnosis of SLE four or more positive criteria in all three groups are required.

	ARC97	**ARC82**	**1971 criteria**		
r.		Tot. sensitivity 96 % Tot. specificity 96 %	Tot. sensitivity 78 % Tot. specificity 87 %	Sensitivity %	Specificity %
1.	Malar rash	Malar rash	Malar rash	57	98
2.	Discoid lupus	Discoid lupus	Discoid lupus	18	99
3.	Photosensitivity	Photosensitivity	Photosensitivity	43	96
4.	Oral ulcers	Oral ulcers	Oral ulcers	27	96
5.	Arthritis	Arthritis	Arthritis	86	37
6.	Serositis	Serositis	Serositis	56	86
7.a)	Proteinuria >0.5 g/d	Proteinuria >0.5 g/d	Proteinuria >3.5 g/d	51	94
7.b)	Urinary cellular casts	Urinary cellular casts	Urinary cellular casts	36	97
8.	Neurologic disorders	Neurologic disorders	Neurologic disorders	20	98
9.	Haemolytic anemia or leucopenia* or thrombocytopenia or lymphopenia*	Haemolytic anemia or leucopenia* or thrombocytopenia or lymphopenia*	Haemolytic anemia or leucopenia* or thrombocytopenia	59	89
10.a)	Pos. aCL/ LA Ab	-	-	50	85
10.b)	Pos. anti-dsDNA Ab	Pos. anti-dsDNA Ab	-	67	92
10.c)	Pos. anti-Sm Ab	Pos. anti-Sm Ab	-	30	98
10.d)	Chronic false-positive serologic test for syphilis	Chronic false-positive serologic test for syphilis	Chronic false-positive serologic test for syphilis	15	99
11.	Pos. ANA	Pos. ANA	-	99	49
	-	Positive LE cells	Positive LE cells	73	96
	-	-	Raynaud's phenomenon	29	81
	-	-	Alopecia	56	88

* Requires two or more occasions

Table 2 SLEDAI, data collection sheet.

SLEDAI

(Enter weight in SLEDAI. Score column if descriptor is present at the time of the visit or in the preceding 10 days)

Study No.: **Patient name:** **Visit date:** day month year

Weight	SCORE	Descriptor	Definition
8		Seizure	Recent onset, exclude metabolic, infectious, or drug causes.
8		Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked illogical thinking, bizarre, disorganized, or catatonic behaviour. Exclude uraemia and drug causes.
8		Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increase or decrease psychomotor activity. Exclude metabolic, infectious or drug causes.
8		Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudates or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection, or drug causes
8		Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8		Lupus headache	Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.
8		CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8		Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4		Arthritis	$\geq$ 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4		Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4		Urinary casts	Heme-granular or red blood cell casts.
4		Hematuria	> 5 red blood cells/high power field. Exclude stone, infection or other cause.
4		Proteinuria	>0.5 gram/24 hours
4		Pyuria	>5 with blood cells/high power field. Exclude infection.
2		Rash	Inflammatory type rash.
2		Alopecia	Abnormal, patchy or diffuse loss of hair.
2		Mucosal ulcers	Oral or nasal ulcerations.
2		Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2		Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2		Low complement	Decrease in CH50, C3 or C4 below the lower limit of normal for testing laboratory.
2		Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1		Fever	>38° C. Exclude infectious cause.
1		Thrombocytopenia	<100,000 platelets / $x10^9$ /L, exclude drug causes.
1		Leukopenia	<3,000 white blood cells / $x10^9$ /L, exclude drug causes.

TOTAL SCORE

Table 3. The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of lupus nephritis.

Class I	**Minimal mesangial lupus nephritis** Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative lupus nephritis** Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits May be a few isolated subepithelial or subendothelial deposits visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal lupus nephritis**[a] 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 III (A)	Active lesions: focal proliferative lupus nephritis
Class III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
Class III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	**Diffuse lupus nephritis**[b] 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) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis 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 IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
Class IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
Class IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
Class IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
Class IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	**Membranous lupus nephritis** 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 lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed Class V lupus nephritis show advanced sclerosis
Class VI	**Advanced sclerosis lupus nephritis** ≥90% of glomeruli globally sclerosed without residual activity

[a] Indicate the proportion of glomeruli with active and with sclerotic lesions.
[b] Indicate the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents.
Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis or other vascular lesions.

(Weening et al 2004)

Table 4. Abbreviated International Society of Nephrology/Renal Pathology Society Classification of Lupus Nephritis (2003)*

WHO class		Prevalence	Treatment options
Class I	Minimal mesangial lupus nephritis		No specific therapy
Class II	Mesangial proliferative lupus nephritis		No specific therapy**
Class III	Focal lupus nephritis†	25 – 30 %	Mild: As for class II or steroids Moderate: Steroides ± MMF/AZA Severe: See treatment for class IV
Class IV	Diffuse segmental (IV-S) or global (IV-G) lupus nephritis‡	40 %	Induction (6 months): CYC or MMF Maintenance: MMF or AZA or quarterly CYC
Class V	Membranous lupus nephritis§	10 – 20 %	Steroids ± Ciclosporin, AZA, MMF, CYC
Class VI	Advanced sclerosing lupus nephritis		No specific therapy

Additional comments

* Indicate and grade (mild, moderate, and severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis, or other vascular lesions (73).

† Indicate the proportion of glomeruli with active and with sclerotic lesions.

‡ Indicate the proportion of glomeruli with fibrinoid necrosis and cellular crescents.

§ Class V may occur in combination with class III or IV, in which case both will be diagnosed.

**Angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers are recommended as adjunct therapy for proteinuria in all classes (151).
CYC; Cyclophosphamide intravenous, MMF; Mycophenolate mofetil, AZA; Azathioprine;

12. PAPERS I-III

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