FROM THE DEPARTMENT OF MEDICINE,

Karolinska Institutet, Stockholm, Sweden

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND CANCER. EPIDEMIOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON PATIENTS WITH SLE AND MALIGNANT LYMPHOMA OR MYELOID LEUKAEMIA

Björn Lövström

Stockholm 2009

Illustrations: (front cover) “the problem”:Olov Lövström, (back cover) “the solution”:Ludvig Lövström, (paper IV) Lymphoma tissue images: Christer Sundström

Published by Karolinska Institutet. Printed by US-AB

ISBN 978-91-7409-652-1

ABSTRACT

Malignancy as a cause of death was reported in occasional SLE patients, but the question whether patients with SLE have an increased risk of developing cancer compared to the general population has remained unanswered. To address this question, we created ***a national Swedish SLE cohort*** from the Hospital Discharge Register where all patients with an SLE diagnosis between 1964 and 1994 were included. The number of observed cancer cases in this cohort was identified by register linkage with the Cancer Register 1964-1995 and was compared with the expected numbers in the general population. ***We found a 25% overall increased risk of cancer in SLE***. ***Haematological malignancies constituted the major excess risk***. A doubled increased risk of respiratory cancer and a tripled of squamous cell skin cancer - most pronounced after 15 years of follow-up – were also observed.

Non-Hodgkin´s lymphoma (NHL) represented the most outstanding (a tripled) ***cancer risk.*** To investigate the lymphoma subtype and to identify risk factors we performed a nested case control study comparing SLE patients who developed NHL during the observation period with those SLE patients without malignancy. Lymphoma tissues were stained with new classification markers and reclassified. ***The NHL subtype diffuse large B cell lymphoma (DLBCL) dominated*** - 10 out of total 16 cases. Two of these were subtyped into germinal centre (GC) (better prognosis) and eight into non-GC. ***There were no indications of treatment-induced lymphomas, but lymphoma risk was elevated if haematological or sicca symptoms, or pulmonary involvement were present in the SLE disease.***

For myeloid leukaemia, another haematological malignancy, ***the SLE patients had a doubled risk.*** In a nested case–control study eight SLE patients in our cohort developed acute or chronic myeloid leukaemia. ***Leucopenia was a risk factor for leukaemia development whereas low-dose chemotherapy was not a major cause in our cohort*** - or in the reported cases we found in a Medline search - but a preceding myelodysplastic syndrome was frequently seen.

Finally, with the hypothesis that some factors related to rheumatic disease may contribute to the risk to develop lymphoma we investigated the presence of a co stimulator for B-cell activation, A PRoliferating-Inducing Ligand (APRIL), in lymphoma tissue of patients with SLE, rheumatoid arthritis (RA), and patients without a chronic inflammatory disease and correlated to clinical variables. We found an overexpression of APRIL mainly in lymphomas of the DLBCL type. Moreover, ***APRIL was higher up regulated in the DLBCLs of the SLE patients, and in the RA subset with high cumulative RA disease activity*** suggesting a particular importance for the DLBCL development in these patient groups but possibly also reflecting the APRIL dysregulation per se seen in these diseases.

In conclusion; patents with SLE have an increased risk to develop malignancies, particularly haematological types. This could be related to disease specific risk factors such as chronic activation of the immune system.

LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to by their Roman numerals:

I. Björnådal L, Löfström B, Yin L, Lundberg IE, Ekbom A.
Increased cancer incidence in a Swedish cohort of patients with systemic lupus erythematosus.
Scand J Rheumatol 2002;31:66-71.

II. Löfström B, Backlin C, Sundström C, Ekbom A, Lundberg IE.
A closer look at non-Hodgkin´s lymphoma in a national Swedish systemic lupus erythematosus cohort - a nested case-control study.
Ann Rheum Dis 2007;66:1627-32

III. Löfström B, Backlin C, Sundström C, Hellström-Lindberg E, Ekbom A, Lundberg IE.
Myeloid leukaemia in systemic lupus erythematosus – a nested case-control study based on Swedish registers.
Rheumatol 2009;48:1222-6

IV. Löfström B, Backlin C, Sundström C, Pettersson T, Ekbom A, Lundberg IE, Baecklund E. Comparison of APRIL expression in diffuse large B cell lymphomas in patients with Systemic Lupus Erythematosus, Rheumatoid Arthritis and patients without any concomitant chronic inflammatory disease.
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
AML	acute myeloid leukaemia
ANA	antinuclear antibodies
anti ds-DNA	antibodies to double stranded DNA
anti-Sm	antibodies to Smith antigen
APRIL	a proliferating inducing ligand
AZA	azathioprine
BAFF	B cell activating factor belonging to the TNF family
BAFF-R	BAFF receptor
BCMA	B cell maturation antigen
CI	confidence interval
CNS	central nervous system
CTX	cyclophosphamide
DLBCL	diffuse large B cell lymphoma
EBV	Epstein-Barr virus
FAB	French American British
GC	germinal centre
HDR	hospital discharge register
HL	Hodgkin´s lymphoma
ICD	international classification of diseases
IL	interleukin
IPI	international prognostic index
MDS	myelodysplastic syndrome
NHL	non Hodgkin´s lymphoma
NRN	national registration number
NSAID	non steroid anti inflammatory drug
OR	odds ratio
pSS	primary Sjögren syndrome
RA	rheumatoid arthritis
RR	relative risk
SCR	the national Swedish cancer register
SIR	standardised incidence ratio
SLE	systemic lupus erythematosus
TACI	transmembane activator and CAML interactor
TNF	tumour necrosis factor
WHO	World Health Organisation

1 INTRODUCTION

In the mid nineties, about the time when I became a specialist in Rheumatology, I took my first few steps in research. At my out-patient clinic I identified and characterized all Systemic Lupus Erythematosus (SLE) patients, sent them to the laboratory for blood tests and provided all information and blood samples to a large project concerning SLE and genetics as my initial contribution to science.

I was struck by how heterogeneous these patients were and my fascination for this rheumatologic diagnosis was raised. Today, almost 15 years later quite a few of these patients from "my" cohort, mostly women, have passed away without reaching what we call average length of life in Sweden. Furthermore, a considerable proportion have experienced a cancer diagnosis with different outcomes. So, much of what this thesis is about I have experienced "on the quiet", on "home ground".

The medical development during the 20th century has had an enormous impact on the panorama of morbidity and mortality in mankind. In the western world, thanks to antibiotics, life expectancy of newborns has increased about 30 years and nowadays man struggles more against cardiovascular disease, cancer and other chronic diseases, among them the rheumatic diseases.

SLE was a dreaded disease fifty years ago with high mortality due to the life threatening organ manifestations of the disease. These can now, most often, be handled medically and therefore the heaviest burden of morbidity and mortality in SLE today is from cardiovascular disease. However, frequent observations of cancer in patients with rheumatic diseases, among them SLE, have raised the question of an association.

Epidemiology is based on two assumptions. One of them is that human disease has causal as well as preventive factors and that these can be investigated. By looking retrospectively and analyzing systematically hundreds of lives of SLE patients, this work has been an attempt to shed light upon possible causative factors of cancer in SLE. Hopefully, it is a valuable piece of a puzzle in the effort to prevent the cancer complication in an already bothersome disease.

2 BACKGROUND

2.1 SYSTEMIC LUPUS ERYTHEMATOSUS

2.1.1 The disease, the diagnosis, differential diagnoses

SLE is a chronic inflammatory multi-organ disease where autoimmune features are present and where practically any body organ could be affected. Therefore these patients could be cared for in several different disciplines. The high frequency of arthritis and joint complaints – the most common clinical manifestation of the disease [1] - the accompanying inflammation se and the need for a physician with a comprehensive view, might lead many SLE patients to a rheumatologist for consultation. However, some SLE patients are cared for by other specialists such as nephrologists, dermatologists or internists reflecting the heterogeneity of this disease.

The typical SLE patient is a woman – about 85-90% of SLE patients are women [2] – of childbearing age (a period where the female: male incidence ratio is even more distorted [3,4]) with arthralgia or arthritis, fatigue, malaise, recurrent fever episodes and skin manifestations along with photosensitivity. Arthritis is not just the most frequent clinical manifestation; it's also the most common initial manifestation.[1] Immunological markers are almost always present at time of diagnosis, above all the antinuclear antibodies (ANA). Although antibodies to double-stranded DNA (anti-dsDNA) and anti-Smith antibodies are regarded as specific for SLE,[5,6] there is no single test or symptom on which the diagnosis can be confirmed. Rather, the diagnosis is made upon a constellation of signs and symptoms together with laboratory tests.[7] The disease course is typically relapsing and remitting and there is a huge heterogeneity in the way the disease presents itself during the years. Several organ systems can be affected. Most common are the joints, the skin and the serous membranes (pleura, pericardium).

According to the American College of Rheumatology (ACR) criteria for the classification of SLE, which were revised 1997 [8] a patient must fulfil at least four of the eleven settled criteria (table 1). These are actually classification criteria, not diagnostic criteria, primarily developed for research purposes. No established diagnostic criteria exist but as a guess in clinical practice physicians consider these classification criteria.

The diagnosis is not always obvious, especially at the time of onset of the disease. Depending on the clinical picture, differential diagnoses towards other rheumatic conditions like rheumatoid arthritis (RA) and primary Sjögren´s syndrome (pSS) are common as well as towards other inflammatory conditions such as "fever of unknown origin" or sarcoidosis.[9] The non-erosive, but still sometimes deforming, polyarthritis separates SLE from RA. Because so many symptoms and autoimmune laboratory findings in pSS and SLE are similar experienced rheumatologists talk about that the diseases overlap but also that in some cases the diseases coexists.[10]

Table 1. The 1997 ACR revised criteria for the classification of SLE [8]

1.	Malar rash	Fixed erythema, flat or raised, over the malar eminences
2.	Discoid rash	Erythematosus circular raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur
3.	Photosensitivity	Exposure to ultraviolet light causes rash
4.	Oral ulcers	Includes oral or nasopharyngeal ulcers, observed by physician
5.	Arthritis	Non-erosive arthritis of two or more peripheral joints, with tenderness, swelling or effusion
6.	Serositis	Pleuritis or pericarditis documented by ECG or rub or evidence of effusion
7.	Renal disorder	Proteinuria>0.5 g/d or 3+, or cellular casts
8.	Neurological disorder	Seizures or psychosis without other causes
9.	Haematological disorder	Haemolytic anaemia or leucopenia (<4000/L) or lymphopenia (<1500/L) or thrombocytopenia (<100,000) in the absence of offending drugs
10.	Immunologic disorder	Anti-dsDNA, anti-Sm, and/or anti-phospholipid
11.	Antinuclear antibodies	An abnormal titer of ANA by immunofluorescense or an equivalent assay at any point in time in the absence of drugs known to induce ANAs

2.1.2 Aetiology and pathogenesis

The complexity and multiplicity of all currently known disturbances and abnormalities of the immune system in the SLE disease are too extensive to be addressed here and are described in text books [2]. However, an effort to make a very brief introductory summary follows.

Characteristic features are the loss of the immune system to recognize self and the development of an autoreactive, autoimmune state. Both T- and B-lymphocytes play important roles in the pathogenesis. The B-cells are hyperactive and produce polyclonal hypergammaglobulinemia, and an overproduction of autoantibodies which can lead to immune complex formation. Immune complexes may deposit in tissues, activate complement and subsequently cause tissue damage after attracting cells with inflammatory and tissue destroying qualities. More than one hundred different autoantibodies have been demonstrated in SLE including the ones described above used for diagnostic purposes.

The reason for the hyper reactive state in the B-cells may rise from the interplay with T-cells or dendritic, antigen presenting cells. Also aberrations in the act of apoptosis (programmed cell death) may play an important role since, for instance, impairment in phagocytosis of apoptotic cell material by macrophages may result in exposure of this material to autoreactive lymphocytes and the formation of antibodies to self antigens.

Genetics are also important, which has been substantiated in twin studies, although no specific gene has been found to be entirely responsible. Certain extremely rare genetic complement defects, however, have almost always an SLE development. About 10 % of SLE patients have a first-degree relative with the disease. [11]

Among environmental factors UV-B light is known to cause exacerbations as well as début of the disease. Cigarette smoking is associated with elevated risk of SLE development. [12] Certain drugs may induce SLE or lupus-like syndromes including older cardiological therapeutics such as hydralazine, procainamide, quinidine as well as antirheumatic drugs such as penicillamine, gold salts and sulphasalazine. Removal of the drug usually results in a resolution of the clinical manifestations, but persistent SLE has been reported with sulphasalazine use.[13]

2.1.3 Mortality and causes of death

During the 1950s, SLE patients with a serious organ manifestation like nephritis or CNS vasculitis had an extremely poor prognosis. In an SLE cohort from California from the 1950s more than 50 % were dead at follow-up 10-20 years later [14]. Of these about 40 % of the deaths were related to uraemia or central nervous system damage. Another important cause of death was infection. The cause of death in this SLE cohort during a 23 year period showed a changing pattern, deaths "not related to SLE", like myocardial infarction and malignant neoplasm, increasing over time.[14] Another 25 year follow up, from the Toronto SLE cohort between 1970 and 1994, showed significantly decreased mortality over time with only 19 % of the patients having died by the end of the observation period and also active SLE-related death more common in early disease (<5 years from diagnosis). Cardiovascular events that in earlier reports were totally absent, now were the cause of death in 15% of the patients and malignancy in 6.5 %.[15,16]

The mortality in the Lund-Orup SLE cohort between 1981 and 1991 was generally low and longer disease duration (>10 years) was associated with slightly reduced survival compared to an age- and sex- matched population. Cardiovascular disease was the dominating cause of death (76%) [17] That was also the case in a national register-based Swedish SLE cohort study with patients followed between 1964 and 1995. These patients had a 3-folded risk of cardiovascular death compared to the general population. Cardiovascular events were responsible for 42% of the deaths among the SLE patients and malignancy was responsible for 12 %. [18]

2.1.4 Epidemiology in SLE

2.1.4.1 Incidence

SLE is an uncommon, even rare disease. The overall incidence rate for people in Western Europe and the USA over the last decades showed about 5 new cases per 100,000 and year [17,19,20] with a tendency for a slight increase during the last decades [17,19] but a more pronounced increase compared with the 1950s [20]. A meta analysis of 32 studies of incidence in SLE including ethnicities from a greater part of the world reported a considerable variation between 1.4 and 21.9, the highest observed in an Afro-Caribbean cohort. [21] Although, there is a possibility that

differences in methodology and selection of patients could influence this variation, differences between ethnic groups are likely. Age-specific incidence rates also differ with a peak exceeding 20 per 100.000 per year in the 25-34 age group among Black females in Baltimore, whereas the estimated incidence in Southern Sweden is quite the opposite with a peak incidence in the 65-74 year group both among women and men (14.1 and 3.2/100.000/year respectively.[17,22]

2.1.4.2 Prevalence

With reports of a slightly increasing incidence of SLE, probably due to milder disease forms being recognized and steadily improving medical care and treatment, the prevalence of SLE is likely to increase. For example, in 1991 the prevalence of SLE in a Swedish study was estimated to 68/100.000. In the same population the prevalence five years earlier was estimated to 42/100.000. [17] In 1993, the age- and sex-adjusted prevalence was 122/100.000 in an SLE cohort in USA.[20] Worldwide and between different ethnicities, a meta analysis show huge variation between 7.4 and 159.4/100.000 but again, methodologic and selection issues must be considered. [21]

2.1.5 Pharmacological treatment of SLE

2.1.5.1 Glucocorticoids

The first drug to make a real difference for moderate to severe SLE was glucocorticoids, which became available in the 1950s. Some decades later the obvious impact on morbidity and mortality was described. [14] Even today, most patients with SLE will be exposed to treatment with glucocorticoids in some form at some time in their disease course. There are preparations for local treatment of cutaneous manifestations and intraarticular injections can be used for arthritis. For treating constitutional symptoms as well as moderate to severe organ manifestations like polyarthritis, widespread lupus lesions in the skin and serositis, short or long – term prescriptions of oral glucocorticoids is common.

Severe, life-threatening lupus manifestations like glomerulonephritis and central nervous system vasculitis have been treated with glucocorticoids in high-dose regimes with efficacy. Side effects of long-term glucocorticoid therapy, especially if more than “low-dose” (5-10 mg/day) is needed, has propelled complementary, steroid-sparing drug regimes, of which the cytotoxic drugs azathioprine(AZA) and cyclophosphamide(CTX) have perhaps been the most frequently used to date.[23,24]

2.1.5.2 Cytotoxic drugs

Azathioprine

AZA is a purin analogue, which has a checking effect on nucleic-acid synthesis and has an effect by modulating both cellular and humoral immune function. AZA has been widely used in the management of SLE, for instance in lupus nephritis treatment. [25] Nowadays, however, its effect is mostly not considered as sufficient for induction treatment of lupus nephritis but still has approval to maintain long-term remission.[26] Perhaps the greatest area of use in SLE

treatment has been as a steroid-sparing drug over many years. Whether this long-term treatment is safe or whether long-term usage of this drug with an effect on nucleic acid synthesis might give rise to haematological malignancies in SLE patients has not been carefully studied.

Cyclophosphamide

CTX is an alkylating cytotoxic drug. It creates double bindings and breakages in DNA in cells that undergo mitosis and has a long tradition as a cancer therapy. It has for many years – in combination with glucocorticoids – been the drug for treating severe lupus manifestations: nephritis, CNS disease, interstitial inflammatory pulmonary disease and, paradoxically, cytopenias due to its demonstrated superiority in the long term beneficial effects in lupus nephritis compared to glucocorticoids alone [27]. Side effects, not only the obvious risk of a future malignancy, but infections and gonadal toxicity have propelled procedures to minimize the cumulative doses and a search for alternative drugs.[28,29] Due to the concern of potential risks of malignancies as well as other complications, daily oral cyclophosphamide may be replaced by monthly pulse regimens with similar beneficial effect but with lower cumulative dose. In patients with Wegener´s granulomatosis an increased risk of secondary malignancies that was associated with the use of cyclophosphamide was reported.[30,31] However, the role of cyclophosphamide as a risk factor for development of secondary malignancies in SLE patients has not been investigated in epidemiological settings.

Methotrexate

In contrast to RA, methotrexate has not been a major drug in treating SLE patients. However, in doses of 15-20 mg/week it has proved effective for skin and joint manifestations as well as a steroid-sparing agent.[32]

2.1.5.3 Cyclosporine A

The great importance of Cyclosporine A has been to treat organ transplant recipients. In SLE it has also been an alternative regimen in treating lupus nephritis. Nowadays it is less often used in the shade of CTX and mycophenolate mofetile (MMF). The best effect is shown in membranous nephritis (WHO class V). [33]

2.1.5.4 Mycophenolate Mofetile

MMF is another drug that was initially used in transplantation to reduce the risk of rejection of organ transplants by inhibiting T-cell function. During the last decade it has been used in lupus primarily for nephritis treatment. Studies have shown efficacy well in line with – or even better than - CTX as induction therapy [26]. Also compared with to CTX, MMF has a more favourable toxicity profile, making it a conceivable option remission therapy, that is, long term therapy. [34]

2.1.5.5 Antimalarials

No enumeration of medical treatment in SLE is complete without mentioning the antimalarials; (in Sweden) chloroquine and hydroxychloroquine. They have several

mechanisms of action that make them very suitable for treating mild to moderate SLE manifestations such as musculoskeletal and cutaneous manifestations. Antimalarials should most often be considered as at least a background medication, flare reducing, lessening the need for symptomatic treatment with non steroid anti inflammatory drugs (NSAID) or glucocorticoids.

2.2 RHEUMATOID ARTHRITIS

Since a large amount of NHL in rheumatoid arthritis (RA) patients is part of paper IV for the sake of completeness a short presentation of this rheumatic disease follows.

2.2.1 The disease, the diagnosis, epidemiology

RA is a chronic, symmetric, inflammatory, erosive-destructive polyarthritis. About 70 % of the patients are either seropositive, (Rheumatoid factor positive in blood tests), anti CCP positive (antibodies to cyclic citrullinated peptides demonstrable in serologic blood tests) or both.[35] The course of the disease is variable, usually slowly progressing, sometimes periodic with a remitting and relapsing course. Untreated, the inflammatory, erosive-destructive properties usually lead to deformation and destruction of joints and increasing disability. Early disease features associated with unfavourable prognosis include widespread arthritis, marked elevations of inflammatory laboratory parameters like C-reactive protein autoantibodies (RF,CCP) and early radiologic erosive changes. Many patients do not only experience symptoms from the locomotor system but are also affected by the systemic inflammatory properties of RA, including serositis, interstitial lung disease, development of rheumatoid nodules and vasculitis.

The 1987 ACR classification criteria for RA (in clinical practise often used as diagnostic criteria) are: 1. Morning stiffness, 2. Arthritis in three ore more joint areas, 3. Arthritis of hand joints, 4. Symmetric arthritis, 5. Rheumatoid nodules, 6. Rheumatoid factor and 7. Radiographic changes. For a RA diagnosis patients have to fulfil at least 4 of 7 criteria and the symptom duration of the criteria 1-4 has to be at least six weeks.[36]

The incidence rate in Sweden is between 20-30 new cases/100,000 person years. The prevalence is somewhat over 0.5%. There is a female predominance with about 65-75% women but the gender differences in incidence decreases with increasing age.[35]

2.2.2 Pharmacological treatment of RA

Symptom relieving analgesics (paracetamol) and NSAIDs are often used, continually or as needed. A prompt relief of joint swelling and pain with intraarticular corticosteroid injections is another traditional cornerstone in the treatment of patients with RA. Often the glucocorticoids are also used systemically with low-dose oral regimens. The reputation of the glucocorticoids among physicians/rheumatologists has varied widely through the years partly due to the long-term side effects such as osteoporosis and diabetes. The positive effects of the

drug such as delaying radiologic changes make the issue complex. The number of anti-rheumatic or disease modifying anti-rheumatic drugs (DMARD) has increased since the 1930s and the incidental discovery of intramuscular gold salt given to tuberculosis patients relieving arthritis in those patients that had a concomitant rheumatic disease. The original purpose of the gold therapy -the granulomatous lung disease - did, on the contrary, not respond.

Today, structured treatment with weekly doses of the methotrexate and treatment early in the disease has meant a lot to reduce the ravaging of rheumatism as well as the insight of combination DMARD therapy for those who failed on single therapy. With the exception of the first cortisone treatment, the most important event in the pharmacological history of RA may be the introduction of the "biologic drugs", specifically the tumour necrosis factor (TNF)-alfa- neutralizing drugs. After one decade of experience with these agents, rheumatologists have been able to improve treatment ad medical care for those who failed to achieve complete remission even on combination DMARD therapy.

2.2.3 RA and lymphomas

Already in the 1970s, Isomäki et al in a large Finnish register-based study reported an increased risk of haematological malignancies in RA, estimating the relative risk (RR) for lymphoma as 2.7.[37] Following studies from different countries and different kind of RA cohorts showed, with few exceptions, a RR of about 2.[38-40] Using Swedish national register data, an increased lymphoma risk of SIR 1.98 (CI 95: 1.5-2.6) was found in 1993 in RA. Ten years later, with a longer time of follow-up, results were essentially unchanged SIR 2.00 (CI 95: 1.83-2.17). The latter study also investigated if the lymphoma risk of RA could come from genetic or environmental risk factors, by assessing the risk of lymphoma in first-degree relatives of the RA patients, but did not find any grounds for that.[41,42] RA-lymphoma studies from Baecklund et al showed that the risk of lymphoma was dramatically elevated in the subgroup of RA patients with the highest cumulative inflammatory activity. Moreover RA-lymphoma subtyping demonstrated an increased risk of the aggressive subtype diffuse large B cell lymphoma (DLBCL).[43,44]

2.3 CANCER

Since man´s life is not eternal, for those lucky people who during life can avoid significant atherosclerosis, severe life-threatening infections and traumas the issue of cancer may unfortunately become a real threat. In Sweden the probability of developing any type of malignant tumour before the age of 75 is 30% for men (women 27%). The slope of the curve of the age-specific cancer incidence rates steepens before the age of 60. [45]

2.3.1 Malignant lymphoma including NHL

A malignant lymphoma is a solid tumour of malignant transformed cells of the reticuloendothelial/lymphatic system.

2.3.1.1 Classification

The ability to classify malignant tumours in the lymphoid system has changed and improved enormously during the second half of the 20th century along with advances in technology, genetics and immunohistochemistry. The new classification system has prognostic implications. Further subtyping and lymphoma dividing is also likely to occur. Before the present WHO classification of tumours of haematopoietic and lymphoid tissues was established in 2001 there have been several classification systems. The Willis classification from 1948 relied solely on histological appearance. The Rappaport classification from 1966, the Kiel and Lukes-Collins classification from 1974, the Working formulation from 1982 and the Revised European-American lymphoma classification system (REAL) from 1994 followed. They are all from the time interval when the lymphomas of the included in this thesis were diagnosed. In the current WHO classification about 40 different lymphomas are defined after a valuation of histological appearance, immunophenotype, genetic abnormalities and clinical features. For every lymphoma that is classified a cell of origin is pointed out. There are three main categories: the B-cell neoplasms, The T- and NK-cell neoplasms and Hodgkin lymphoma (HL).[46]

2.3.1.2 Epidemiology

During 2007 malignant lymphomas (non Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL) and chronic lymphatic leukaemia (CLL) accounted for 4.1% – or 2065 new cases -of all malignancies in Sweden. For NHL the corresponding figures were 1372 and 2.7%.[45] NHL incidence is about 50% higher in men,18/100.000 (women 11.7), it increases with age and the trend for the last 20 years is stable if not slightly falling.[47] Worldwide, developed countries like the USA, Australia, New Zealand and Europe report the highest incidence. The most common subtypes of NHL are diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. Together they constitute more than 50% of the cases. [46] A large Scandinavian lymphoma study (SCALE) of incident lymphoma in Sweden and Denmark between 1999 and 2002 showed that, when CLL was excluded, that 34% of the lymphomas were DLBCL and 25% were follicular.[48] The distribution of the lymphoid neoplasms according to cell of origin showed that approximately 80 % were B-cell neoplasms. About 10 % was of T/NK cell origin and 10% was HL.

2.3.1.3 Risk factors for Lymphoma

Insistent observations have led to identification of some risk factors for lymphomas. Still, the cause is most often unknown.

The most important risk factors for lymphomas are immunodeficiency or autoimmune disease. [46] Acquired immunodeficiency syndrome (AIDS) has been associated with the most pronounced risk of NHL development, for high grade NHL like DLBCL a RR of 400 was reported.[49] Solid organ or stem cell transplanted individuals run the risk of acquiring a post-transplant lymphoproliferative disorder (PTLD) which includes stages from benign hyperplasia to malignant lymphoma. Epstein-Barr virus (EBV) driven tumour formation in B cells is most often associated with this condition.[50] Besides HIV and EBV a few infectious agents

have been associated with certain lymphomas, for instance the gastric ulcer inducing bacteria Helicobacter pylori and gastric MALT lymphoma. Interestingly, treatment of the bacterial infection often leads to lymphoma regression.[51] Genetics or family history of haematopoietic malignancy also implies an increased NHL risk of about 50 % according to a pooled analysis from the International Lymphoma Epidemiology Consortium.[52] Last but not least lymphoma development has been associated with many autoimmune or chronic inflammatory diseases, including: RA [37], pSS [53], SLE [54], celiac disease [55], inflammatory bowel disease [56] and sarcoidosis [57].

2.3.1.4 Lymphoma staging, prognosis and survival

At the time of lymphoma diagnosis examinations are customarily performed to establish the degree to which the lymphoma has spread in the body. Ann Arbor staging classification of lymphomas divides lymphoma into four stages, where I and II are regarded as localized disease and the stages III and IV as widespread (table 2).[58]

Table 2. Ann Arbor staging classification of Hodgkin's disease and non-Hodgkin's lymphoma

Stage*	Criteria
I	In one lymph node only
II	In two or more lymph nodes on the same side of the diaphragm
III	In the lymph nodes, spleen, or both and on both side of the diaphragm
1.	Above the renal vessels
2.	In the lower abdomen
IV	Extranodal involvement (e g bone marrow, lung, liver)

*Subclassification E indicates extranodal involvement adjacent to an involved lymph node. Stages can be further classified by A to indicate the absence or B to indicate the presence of constitutional symptoms (weight loss, fever, or night sweats). B symptom generally occur with stages III and IV

Based on a number of factors associated with poor prognosis, a tool has been developed to predict survival in lymphoma patients. The International Prognostic Index (IPI) score takes five factors into account: the pre-treatment serum level of lactate dehydrogenase (LDH), patient age at presentation, Ann Arbor Stage, number of extranodal sites and a performance status. [59]

The most common NHL subtype, the aggressive DLBCL is a heterogeneous malignancy where a need for better prognosis making, to complete the IPI, has fueled a further subtyping into three groups by using a cDNA microarray.[60,61] The germinal centre B-cell-like (GC) has a better prognosis than the activated B-cell-like (ABC) and the type 3 gene expression. The latter two are also designated as non-GC. Immunohistochemical stainings of lymphoma tissues using antibodies to CD-10, bcl-6 and MUM1 (IR-4) do also enable a DLBCL subclassification into GC/non GC. [62]

Since the introduction of combination chemotherapy during the 1970s, and the pharmacological progress thereafter a previously extremely bad prognosis has

slowly but steadily improved. Today the five-year survival from lymphoma varies a lot between lymphoma subtypes. For an aggressive high-grade lymphoma like DLBCL it is just about 50% (Sweden 2000-2005) but for the same diagnosis with an IPI =0 at presentation it is near 80%. [47]

2.3.2 Acute myeloid leukaemia

Acute myeloid leukaemia (AML) is a consequence of a malignant transformation of a haematopoietic stem cell leading to a rapid replacement of normal bone marrow with myeloid blast cells arising from that transformed cell.

2.3.2.1 Classification

Just like the malignant lymphomas, AML is a heterogeneous group and the grounds for classification are steadily changing due to technological advances in genetic analysis. The French-American-British (FAB) morphologic classification – which divides the leukaemias into eight groups, M0-M7 – has been accepted and used for many years. The current WHO classification from 2001 [46] recognizes four major groups:

I. AML with recurrent cytogenetic abnormalities
   - AML with t(8;21)(q22;q22); (AML1/ETO)
   - AML with abnormal bone marrow eosinophils inv(16)(p13q22) or t(16;16)(p13;q22); (CBFβ/MYH11)
   - Acute promyelocytic leuk. with t(15;17)(q22;q12) (PML/RARα) and variants
   - AML with 11q23 (MLL) abnormalities

II. AML with myelodysplasia-related features
   - Following a MDS or MDS/myeloproliferative disorder
   - Without antecedent MDS

III. Therapy related AML and MDS
   - Alkylating agent-related
   - Topoisomerase type II inhibitor-related (some may be lymphoid)
   - Other types

IV. AML not otherwise categorized:

AML min. differentiated	AML without maturation
AML with maturation	Acute myelomonocytic leukaemia
Acute monoblastic and monocytic leukaemia	Acute erythroid leukaemia
Acute megakaryoblastic leukaemia	Acute basophilic leukaemia
Acute panmyelosis with myelofibrosis	Myeloid Sarcoma

2.3.2.2 Epidemiology

Acute leukaemia (myeloid and lymphocytic) is a very rare haematological malignancy with an age standardised incidence rate of 4,5 cases /100.000 person years, with >80 % of these being AML. The male: female ratio is approximately

1,25:1 (Sweden,2000).[63] Only 416 cases of myeloid leukaemia were diagnosed in Sweden during 2007 corresponding to 0.8 % of total cancer incidence. [45]

Alkylating drugs, like many other chemotherapeutic drugs, constitute one of the relatively few known etiologic risk factors for leukaemia, besides ionizing radiation, benzene and viral infections. [46]

2.3.2.3 Prognosis

Chemotherapy has been the cornerstone of treatment of AML. In recent years stem cell transplantation has been added to the therapy arsenal. The prognosis depends on a lot of factors. Older age, certain AML subtypes and if the leukaemia is secondary to a myelodysplasia or chemotherapy for another cancer are unfavourable prognostic factors. The five year survival in AML patients (all subtypes taken together) <55 years old is approximately 50% in Sweden 2007.[64]

2.3.3 Myelodysplastic Syndrome

Myelodysplastic Syndrome (MDS) is not *a* syndrome, rather it is a group of haematological disorders which is associated with ineffective and abnormal myelopoiesis. The name preleukaemia has sometimes been applied since an increased risk of developing AML exists. MDS patients present clinically with weakness, fatigue, anaemia, haemorrhage, and fever-infection. To what extent these symptoms appear depends on the MDS-subtype and the degree of haematological disorder. Anaemia is the most common clinical finding.[65]

The FAB classification from 1982 [66] divides MDS into five subtypes:

Classification	Criteria
Refractory anaemia(RA)	Anaemia with reticulocytopenia, normal or hypercellular marrow with erythroid hyperplasia and duyserythropoiesis; blasts≤ 5%
RA with sideroblasts (RARS)	As above and ringed sideroblasts > 15% of nucleated marrow cells
RA with excess blasts (RAEB)	Cytopenia of two or more cell lines with morphologic abnormalities of blood cells; hypercellular marrow with dyserythropoiesis and dysgranulopoiesis; blasts = 5-20% of nucleated marrow cells
RAEB in transformation	RAEB and one or more of the following:≥ 5 % blasts in blood, 20-30% blasts in marrow, Auer rods in granulocyte precursors
Chronic myelomonocytic leukaemia (CMML)	Same as RAEB with absolute monocytosis in blood, < 5% blasts in blood and < 20% bone marrow blasts

In the WHO classification (2001) [46] further clinical and morphological insights and the issue of better prognosis assignment and eight groups were identified and separated CMML into the myelodysplastic/myeloproliferative diseases:

1. RA 2. RARS 3. Refractory cytopenias with multilineage dysplasia (RCMD), 4. RCMD and ringed sideroblasts (RCMD-RS) 5. RAEB-1 6. RAEB-2 7. Myelodysplastic syndrome –unclassified (MDS-U) 8. MDS assoc. with isolated del(5q)

The risk of AML development and survival differs a lot among the groups. This fact and the possibility of pharmacological therapy/bone marrow transplantation have driven the development of different prognosis systems. In 1997 an International MDS risk analysis workshop was convened, which resulted in the International Prognostic Scoring System (IPSS). [67] Hereafter further attempts have been made for better prognosis making. Recently the WHO-classification-based prognostic scoring system (WPSS) was presented where they integrate the karyotype (chromosomal abnormalities), the WHO subgroup and the Red blood cell transfusion requirement.[68]

2.3.4 Female cancer

SLE is to a great extent a disease of women. Therefore I will briefly mention the typical female cancer sites.

Breast cancer is by far the most common cancer among women with just over 7000 new cases (and actually, some forty male cases) annually in Sweden or almost 30 % of all cancers in females. Its incidence is steadily increasing, while the mortality is not. A national screening programme with mammography on women 50-69 years old (nowadays 40-69) started 1985.

There are about 1500 ovarian tumours every year, nearly as many in the uterus and just below 500 in the uterus neck. For the uterus the incidence trend is, like breast cancer, steadily slightly increasing, whereas for the ovary and the uterus neck it's the opposite. [45] Regular testing of cervical smears is another national cancer screening programme for women in Sweden

2.4 SYSTEMIC LUPUS ERYTHEMATOSUS AND CANCER

2.4.1 Introduction

Half a century ago, two unusual diseases like SLE and cancer very seldom occurred in the same patient due to the grim fact that the SLE patients succumbed to their disease or a disease related complication long before any malignancy development. Gradually, the survival of the SLE patients improved, and SLE patients got cancer now and then, leading to speculations of an association.[69-71] Further case reports of SLE and cancer came. An association between organ transplanted patients and malignancy, first and foremost lymphomas, was observed.[72] A suspicion that immunologic disturbance in SLE might also imply a risk of malignancy grew. Observations from single rheumatology centres began to report on the extent of malignancy development in their cohorts.[73,74] Experimental models of SLE from the 1960s with NZB mice also resulted in a number of lymphomas, a number that could be increased by administration of AZA[75,76] In the 1970s immunosuppressive treatment had been used for more than a decade and, reports of

leukaemia among patient groups developing leukaemia that had been treated with cytotoxic drugs for "non-neoplastic disorders", like rheumatic diseases followed.[77] It became important to decide to what extent a malignancy in a patient with rheumatic disease could originate from the disease itself and to what extent the cancer diagnosis was iatrogenic and possibly a consequence of immunosuppressive treatment.

2.4.2 SLE cohorts and Cancer

In 1992 T Pettersson found that the SLE patients had a doubled risk to develop cancer compared to the Finnish population when he compared a Helsinki SLE cohort combined with the Finnish Cancer Registry [78]. Due to the fact that 4 of the 15 cancer cases in the cohort were NHL, the relative risk of NHL in SLE was quite pronounced (RR=44). Coming observations from other clinical units modified the size of the risk figures to between five and ten.[79-81]. However, a case control study with the aim to evaluate a possible association with exposure to cytotoxic drugs turned out that there was no association.[78]

More reports from local hospital-based SLE cohorts with varying numbers of SLE patients, cancer cases and follow up time are presented in Table 2. In common is a *relative risk >1*, even if some of the observations were not statistically significant as is evidenced by the confidence intervals included the null value of 1.0. In most of the studies the number of SLE patients is fairly low and the follow up time is in general short in terms of cancer origin (the median follow-up time is about half of the maximum that is stated in table 3).This makes it harder to rule out chance and difficult to gain statistical power enough to support an association between two unusual diseases as SLE and cancer. Another limitation of hospital-based cohort studies is the issue of selection bias towards more severe forms of SLE since most studies are from SLE referral centres.

Table 3 Studies of cancer risk in patients with Systemic Lupus Erythematosus

Author, year [ref]	SLE patients (number)	Follow up (in years)	Cancer cases (number)	SIR (95% CI)
Lewis,1976 [74]	484	up to 19	18	-
Pettersson,1992 [78]	205	up to 20	15	2.6 (1.5-4.4)
Sweeney,1995 [79]	219	up to 10	6	1.4 (0.5-3.0)
Abu-Shakra,1996 [80]	724	up to 24	23	1.1 (0.7-1.6)
Mellemkjaer,1997 [81]	1585	up to 15	102	1.3 (1.1-1.6)
Ramsey-Goldman,1998 [82]	616	up to 10	30	2.0 (1.4-2.9)
Sultan,2000 [83]	276	up to 21	15	1.2 (0.6-2.1)
Cibere,2001 [84]	297	up to 20	27	1.6 (1.1-2.3)

Abbreviations: SIR= standardised incidence ratio, CI= confidence interval

In Denmark Dr Mellemkjaer used not only the national cancer register but also the nationwide Danish Hospital Discharge Register to create a national SLE cohort and investigate their cancer risk [81]. This register study consisted of more than 10.000 patient-years at risk. The two most important findings with this study were; first *the*

slightly, but still statistically significant, elevated overall cancer risk in SLE and second that *a great part of that increased risk was for haematological malignancies, especially NHL*. By creating a national SLE cohort the selection bias phenomenon was diminished with the possibility that more SLE phenotypes were included but still the hospitalization might exclude patients with milder forms of the disease. The methodology assumes that the accuracy of the SLE diagnosis is high in all medical departments all over the country. By retrieving medical records, the SLE diagnoses were checked from two subgroups: the eight patients who developed NHL and the 15 with a coincident lung cancer. In principle all eight NHL cases had SLE according to the ACR criteria but among the lung cancer cases the SLE diagnosis could only be confirmed in 6 (40%).[81]

2.4.3 SLE and haematological cancer

Through the years a great many of the case reports of SLE and cancer have dealt with lymphoma and leukaemia. Besides the NHL risk figures from Finland and Denmark, some of the SLE cohort studies that did not find an overall increased risk of cancer did on the other hand show an increased risk of NHL in SLE with some statistical significance (table 4).[80,85] This implies that the risk of NHL is the largest of all cancers in SLE, but the risk factors or the mechanisms that could explain this risk are not known.

Table 4 *Studies of (non-Hodgkin's) lymphoma risk in Systemic Lupus Erythematosus*

Author, year [ref]	SLE patients	Follow up (in years)	NHL, number of patients	SIR (95% CI)
Pettersson,1992 [78]	205	up to 20	4	44 (12-111)
Sweeney,1995 [79]	219	up to 10	1	10 (0.2-56)
Abu-Shakra,1996 [80]	724	up to 24	3	5.4 (1.1-16)
Mellemkjaer,1997 [81]	1585	up to 15	8	5.2 (2.2-10)
Sultan,2000 [83]	276	up to 21	1	18 (0.5-99)
Cibere,2001 [84]	297	up to 20	4	7.0 (1.9-18)
Nived,2001 [85]	116	up to 16	2	12 (1.4-42)

The two studies with the largest number of SLE cases (Table 4) also showed a relative (but not statistically significant) risk of leukaemia of 2 and 3 respectively.[80,81] Consequently, establishing an association with this haematological cancer – 5 times rarer than NHL – demands even larger studies with more patient-years at risk.

Regarding Hodgkin's lymphoma (HL) the detecting an association is even more difficult since HL constitutes only about 10 % of all lymphomas. Theoretically, the similarities with NHL, might also suggest an excess risk of HL in SLE. Occasional cases of HL were reported in three of the cohort studies mentioned above, immediately bringing elevated relative risks, but without statistical significance.[81,83,84]

2.4.4 SLE and other Cancer sites

Of the eighteen neoplasms among SLE patients in a report by Lewis from1976, 6 (33%) were carcinoma of the cervix or the uterus.[74] A few years later an increased frequency of atypical smears was found in SLE women when compared to age-matched women and the increase was also associated with AZA usage.[86] Significantly increased frequency of cervical atypia/dysplasia was also reported in three later studies, one not dealing with the cytotoxic treatment issue and the other two with conflicting results regarding an association between cervical intraepithelial neoplasia and treatment with intravenous CTX.[87-89] Although cancer of the cervix uteri only constitutes a small proportion of all female cancers, almost all cohort studies of SLE contained cases of this type of malignancy, but with conflicting relative risks (Table 5). The high frequency of breast cancer in the general population makes it less surprising with observations of breast cancer in SLE in the cohort studies. The largest studies showed no increased risk of breast cancer in SLE. [80,81] However, most of these studies are cohort studies with a limited number of SLE patients and a limited follow up time making the results uncertain.

Table 5. *Observations of carcinoma of the cervix uteri and of breast cancer in SLE*

Author, year [ref]	Cervix cancer Nr of patients	SIR (95% CI)	Breast cancer Nr of patients	SIR (95% CI)
Lewis,1976 [74]	6 *	not stated	2	not stated
Pettersson,1992 [78]	1	not stated	4	2.7 (0.7-6.8)
Sweeney, 1995 [79]	-	-	3	2.0 (0.4-6.0)
Abu-Shakra,1996 [80]	1	not stated	4	0.7 (0.2-1.8)
Mellemkjaer, 1997 [81]	2	0.7 (0.1-2.5)	14	1.0 (0.5-1.7)
Ramsey-Goldman,1998 [82]	4	1.5 (0.6-3.9)	8	1.7 (0.9-3.3)
Sultan, 2000 [83]	1	4.2 (0.1-25)	3	1.1 (0.2-5.9)
Cibere, 2001 [84]	3	8.1 (1.6-24)	4	1.1 (0.3-3.0)

*Carcinoma of cervix and uteri

A considerable portion of the SLE patients have renal involvement with nephritis and in many of these there is a need of potent immunosupression. As stated above CTX (together with glucocorticoids) has for many years been regarded as the best option to achieve remission and to avoid end-stage renal failure. However, this notion is going through a revision.[29,90] Since the 1960s, CTX was given orally. Gradually, side effects such as hemorrhagic cystitis and carcinoma of the bladder were observed.[91] The causative factor for this was identified as acrolein, a CTX metabolite.[92] Adding the protective properties of compounds like mesna administered after CTX [93] along with rigorous hydration were measures taken to minimize these complications. Moreover, an effort to lessen the cumulative doses of CTX by using a monthly intravenous regime instead of daily oral treatment proved

to be just as effective in nephritis treatment.[27,90] Studies of bladder cancer in patients treated with cyclophosphamide for neoplastic (NHL) and non-neoplastic causes (RA and Wegener's granulomatosis) show substantially increased relative risks of bladder cancer when the cumulative doses of CTX are high.[31,94] Occasional case-reports of urinary bladder cancer in SLE patients treated with CTX have emerged. [95-97] However, there were no reported cases in the SLE cohorts described above with the exception of five cases (RR 1.6 CI 95 0.5-3.7) by Mellemkjaer et al.[81] Cancers in urinary organs (except kidney) are not very rare, constituting about 5% of all cancer in Sweden in 2007. Thus, controversy remains as to whether patients with SLE have an increased risk of non-haematological malignancies. To address this question large cohorts with long-term longitudinal follow-up are required.

2.5 CYTOKINES OF THE TUMOUR NECROSIS FACTOR LIGAND SUPERFAMILY

Receptors and monoclonal antibodies neutralizing Tumour Necrosis Factor (TNF)α have revolutionized the treatment of several inflammatory rheumatic diseases, particularly RA during the past decade.[98,99] TNFα is just one of about 20 members of the TNF superfamily. The cytokines in this family share the TNF homology domain and commonly adopts a typical trimeric structure. [100] Two other members of this family have been of great interest in relation to inflammation, immune responses and malignancy: B cell activating factor belonging to the TNF family (BAFF) and A proliferation-inducing ligand (APRIL). The latter being of potential interest in SLE, which is characterized by high B cell activity and is a novel potential target of therapies.

2.5.1 BAFF

BAFF has a few synonyms, B-lymphocyte stimulator (BLyS) is the most common. Several experimental studies have shown that BAFF, as well as a BAFF specific receptor (BAFF-R) has a vital role in the maturation of peripheral B-cells. Dysregulation of BAFF influences the important stage in the spleen where elimination of self-reactive B-cells is done. [101-103] BAFF is expressed in peripheral blood leukocytes, in the spleen and the lymph nodes by stromal cells. Various proinflammatory stimuli can induce BAFF production in leukocytes, like for instance monocytes and macrophages stimulated with type I and II interferons. [104,105] Also, non-hematopoietic cells like astrocytes and fibroblast-like synoviocytes produce BAFF excited by interferon γ and TNF.[106]. It appears that there are two ways BAFF expression is regulated: one constitutional with B-cell homeostasis regulation as the main focus and the other inducible in response to inflammation.[107]

Furthermore, in human autoimmune diseases like RA, SLE, pSS and myositis elevated circulating BAFF levels have been found as well as local elevation in affected organs (for instance in salivary glands of patients with pSS). [108,109,110]. In pSS the circulating BAFF levels have been found to correlate with the level of autoantibodies and in SLE a relationship between BAFF levels and SLE disease

activity, measured by SELENA-SLEDAI score, was observed.[111,112] These observed dysregulations have led to hopes of BAFF as a suitable therapeutic target in rheumatic diseases

Finally, BAFF has also been associated with B-cells neoplasms. In vitro tests show that BAFF protects cells from B-cell chronic lymphocytic leukaemia from apoptosis and prolongs cell survival also for NHL B-cells.[113] A later study of NHL from the same group showed that BAFF is expressed in NHL tumours and that expression increased when tumours transformed to more severe NHL subtypes like DLBCL. Also BAFF serum levels correlated to transformation, disease activity and final outcome.[114] These findings along with others were substance for elaborating of pharmacological therapies. Clinical trials in patients with relapsed and refractory B-cell NHL with soluble TACI-Ig receptors neutralizing BAFF/APRIL are ongoing.[115] Whether BAFF is also associated with lymphoma in SLE and whether this could be involved in the molecular mechanisms of lymphoma development in SLE has not been investigated.

2.5.2 APRIL

Since APRIL is about 50% identical with BAFF [100] and these two cytokines share two receptors (see the receptor section below) it is not strange that they are often presented together when describing different effects on cells in the reticuloendothelial system. When first described in 1998 APRIL was found to be mainly expressed in connection with tumours and it potentiated both *in vivo* and *in vitro* growth of malignant cells.[116] In contrast to BAFF, APRIL has no clear role in the B-cell maturation,[117] but is regarded as a co-stimulator of B-cell activation.[118] Overexpression of APRIL and BAFF in animal models gives rise to B-cell neoplasms.[119] In NHL APRIL expression was first and foremost upregulated in the aggressive DLBCL subtype and mostly by *in situ* neutrophils and not much by the tumour cells. The tumour cells also expressed BAFF/APRIL receptors, that is, they have the possibility to recognize and respond to APRIL. Furthermore, an observation of a correlation between the amount of APRIL coming from the host inflammatory cells in the tumour and the outcome was also made.[118] Consequently, APRIL could influence tumour aggressiveness and negatively influence the possibility to respond to conventional lymphoma therapy. APRIL might be a key player in high-grade B cell lymphoma origin beyond BAFF.

2.5.3 BAFF/APRIL receptors

BAFF has three receptors: BAFF receptor (BAFF-R), transmembrane activator and CAML interactor (TACI) and B-cell maturation antigen (BCMA). BAFF-R is exclusive for BAFF, whereas the other two are shared by BAFF and APRIL. As mentioned above, the task of BAFF-R is primarily to put through the survival signals of BAFF during the maturation phase and to keep up and support the mature B-cells. BAFF-R gene mutated mice have a B cell phenotype similar to BAFF depleted mice.[120]

The function of TACI is in B cell homeostasis partly by being a negative B cell proliferation regulator. TACI KO B cells act hyperproliferatively.[121] The role of

BCMA is not obvious. Suggestions of maintaining homeostasis of B cells have been put forward.[122] Tumour cells from NHL of B-cell origin express BAFF-R and TACI but not BCMA.

The effects of BAFF/APRIL when dysregulated like in autoimmune disease and B cell neoplasms and the knowledge about their receptors have given rise to efforts to produce specific BAFF/APRIL-blocking treatment. In mouse models for autoimmune diseases like RA and SLE a protective effect of TACI Ig (neutralizing antibodies) has been shown in collagen-induced arthritis and proteinuria respectively.[123,124]

2.6 THE SWEDISH HEALTH CARE REGISTRIES

2.6.1 The Swedish Hospital Discharge Register (HDR)

In 1964 the first registrations were made in the HDR (or "patientregistret"). Six of the 26 County Councils began reporting hospitalizations to the Swedish National Board of Health and Welfare (SNBHW). In addition to information on treating hospital, department, and clinic, the personal code number and the discharge diagnosis were registered. Gradually the other County Councils joined with nearly 80% coverage by the end of 1983.Since 1987 the reporting has been nation-wide.

One primary diagnosis and up to five secondary diagnoses are registered for each hospital discharge and they are coded according to the seventh revision of the International Classification of Diseases (ICD) version 7 (1964-68), ICD-8 (1969-1986), ICD-9 (1987-1995) and ICD-10 (1996-). The information on diagnosis has been validated at the 4-digit level for ICD-9 in 1990 with an accuracy of 86% of the primary diagnosis. Between the years 1964 -2005 more than 49 million registrations had been made in the HDR. 99% of these have a diagnosis registered.[125] The validity for each diagnosis has been varying and depends on for instance medical department, if the diagnosis is primary or secondary. In a recent study the diagnosis of heart failure had a validity of 95 % irrespective of clinic type, but only if it was the primary discharge diagnosis.[126] From the 1970s the specificity of the RA diagnosis was studied with a result of approximately 80 % of the discharge diagnosis being correct.[127] Regarding the SLE diagnoses of the HDR no validation has been published.

The patients included in the studies of this thesis have been accessible thanks to a linkage between the HDR and the National Swedish Cancer Register (SCR). This procedure has been feasible due to the National Registration Number (NRN), a ten-digit code given to all residents in Sweden, that besides being a unique personal identifier also enables register linkages for instance for research purposes. Linkage is performed by the Centre for Epidemiology at the SNBHW on request when accompanied by relevant research program documents and ethics approvals.

2.6.2 The National Swedish Cancer Register

The Swedish Cancer register started in 1958. All physicians active in the country are obliged to report new cases. This reporting is complemented by data from death

certificates which make the completeness of the register almost 100%. With very few exceptions the cancer diagnoses are morphologically based. Data in cancer reports contain information of NRN, sex, place of residence, date of diagnosis, hospital and department, pathology/cytology department, specimen number, site of tumour with coding according to current classification and from the 1990s also histological type and stage. For quality control purposes and to get follow up information of each individual SCR is linked to the Cause of Death and population registers.

During 2007 the number of new cancer reports was 50 100 of which less than 1 % were found incidentally at autopsy. The number of deaths from cancer was 22 815. [45]

3 AIMS OF THE THESIS

From the very early days of planning this thesis - at the end of the recent millennium - the main purpose was to further investigate the issue of cancer occurrence in SLE. Utilizing an assembled Swedish SLE cohort, providing a large number of patients and enough statistical power, the specific aim was to further study the cancer occurrence in SLE, both the overall cancer risk, and specific sites and cancer types.

A second aim was to identify risk factors for some defined haematological malignancies, NHL and leukaemia, in SLE,. The question of whether the use of cytotoxic drugs in the management of the SLE could be a risk factor for haematological malignancies in SLE was one important question to address.

4 PATIENTS AND METHODS

The identification of the patients for the studies of this thesis is here presented briefly with some complementary information. For further details see the methods section of each paper.

All patients in studies I-III were identified through a register linkage between the Swedish hospital discharge register (HDR) and the Swedish cancer register. Study IV is principally based on RA and SLE patients included in this manner with comparator patients (lymphoma patients without rheumatic disease) randomly chosen from one pathology department in Sweden.

4.1 PAPER I

For patients with a diagnostic code of SLE in the HDR their first registered discharge from hospital with a diagnostic code of SLE between 1964 and 1994 was chosen for inclusion in the study base (the national Swedish SLE cohort) –by this, they had the *exposure* (SLE). These cases were followed to the end of the observation period for the possible *outcome*, the registration of (the first) cancer, by linkage to the SCR, using the NRN. Each patient included in the cohort contributed with a number of years (being) at risk of developing cancer depending on date of inclusion and date of exclusion (date of cancer, death or end of 1994)

This national SLE cohort consisted initially of 9,076 patients. 3,361 patients were excluded due to: first discharge before the age of 20 (n=361), death before or at first discharge (n=308), data inconsistencies (n=354), a prior cancer (n=601), cancer within one year after the first discharge (n=122) and a diagnostic code for other chronic inflammatory diseases (n=1624). Thus, the cohort consisted of 5,715 patients after exclusions.

The standardized incidence ratio (SIR) was calculated as a measure of relative risk. That is, the ratio of the observed to the expected number of diagnosed (incident) cancers in the Swedish population. Overall as well as site specific SIRs were calculated and stratifications for years of follow-up were performed (1-5, 5-10, 10-15 and >15 years).

4.2 PAPERS II AND III

These are nested case-control studies using the SLE cohort from the first paper as the study base. Medical records were retrieved and scrutinized for defined variables including disease manifestations, laboratory data and treatment. From the patient records we can estimate the time of onset of SLE diagnosis with higher accuracy as the date of onset is seldom the same as the first SLE discharge diagnosis date in the HDR. Prior cancer or cancer within one year after inclusion has not been among the exclusion criteria from the SLE cohort in searching for cases, in order not to miss any incident case of lymphoma or myeloid leukaemia in SLE. So in these studies the SLE cohort consisted of 6438 patients. In all cases of SLE and lymphoma/myeloid leukaemia that have remained after validation of the ACR criteria of SLE and the

WHO classification of tumours the time interval between onset of SLE and cancer diagnosis have exceeded one year, most often a considerable longer time.

In paper II the register linkage yielded 42 cases with SLE and non Hodgkin´s lymphoma. We first scrutinized the medical records of the 42 registered NHL cases. Cases that fulfilled at least four ACR criteria of SLE remained in the study. From their medical records data on the clinical and serological manifestations of their SLE as well as their medication were registered. The tissues on which their lymphoma diagnoses were made were retrieved, reviewed and (re-)classified according to the WHO classification of lymphomas. Additional immunohistochemical stainings were performed (EBV investigation by EBER in situ hybridization and markers for distribution of GC/non GC of the DLBCLs).

For each remaining case of SLE after medical record review with incident lymphoma (n=17) five cancer/lymphoma-free controls from the national SLE cohort were randomly chosen. The controls, which were also matched for gender, were scrutinized in the same way as the cases. Less than four fulfilled ACR criteria resulted in exclusion. Data on SLE manifestations and medications in those fulfilling the ACR classification criteria for SLE were collected.

In paper III a similar methodology was applied. All registered cases of myeloid leukaemia (acute/chronic) and SLE (n=13) were investigated by scrutinizing medical records (for clinical/laboratory manifestations and drug exposure, as well as fulfilment of the ACR criteria). In those cases where SLE was confirmed the haematological diagnosis was checked histologically. From our national SLE cohort five gender-matched controls for each case were randomly selected. Controls had to have an observation-period free of cancer as long or longer than the case.

The relative risks (RR), estimated by odds ratios (OR), for the different SLE manifestations, as well as the medication, and malignancy development were calculated with the Statistical Package for the Social Sciences (SPSS).
In the study of SLE and myeloid leukaemia, paper III, a Med-line search on previously published cases of SLE and acute/chronic, myeloid/non-lymphocytic leukaemia was performed and summarized.

4.3 PAPER IV

In this study, expression of APRIL in NHL tissue was performed in DLBCL in RA, SLE and in cases without any chronic inflammatory disease from the general lymphoma population.

RA: From a previous study by Baecklund et al of RA and NHL, where incident lymphoma cases between 1964 and 1994 had been identified with the same methodology (linked register study and verifying both diagnoses clinically and histologically [43]) as in paper II, 343 reclassified RA-lymphoma cases were identified of whom 165 were DLBCLs. 111 of these had enough lymphoma tissue left to be used for the tissue microarray (TMA).

SLE: Nine of our 10 SLE-DLBCL cases (from paper II) were suitable for TMA. The tenth was analyzed separately. Of the initial 42 cases in the SLE-NHL study there were two DLBCL patients that did not strictly fulfil ≥4 SLE ACR criteria, but as a clinical SLE diagnosis was obvious, they were also included in this study. Of the original cohort of SLE patients with malignancy eight cases were reported to the cancer register as Hodgkin lymphoma. They were as well reviewed and one of these cases was reclassified as DLBCL and was also included in the study. Two more cases were identified and included in the tissue study; one from a collaboration with DrTom Pettersson, Helsinki University, Finland, and one recent SLE-DLBCL case identified from Mälar hospital, Eskilstuna. These two cases were analyzed separately. In total the study comprised 15 SLE-DLBCL cases, 12 with lymphoma tissue in TMA blocks and three with traditional whole sections from paraffin-embedded lymphoma tissues

General population: 74 lymphoma specimens of DLBCL cases at the department of pathology, Akademiska hospital, Uppsala were also included for TMA. These cases had been used in a study by Berglund et al subtyping of the DLBCL and had from medical records been checked to remove any cases with concomitant inflammatory diseases, particularly rheumatologic.[128]

Clinical information of the lymphoma and the rheumatological disease was registered for all cases along with the DLBCL-subtype (GC/non-GC) and the EBV status of the lymphoma (pos/neg). Follow-up for survival for all cases without known death dates was made.

Tissue Micro Array (TMA) blocks were produced. Formaline fixed, paraffin embedded lymphoma tissues that were retrieved for previous studies, two pieces for each specimen, 0,5 μm in diameter small pieces were punched out and were put on a paraffin block. The TMA blocks contained beside the DLBC lymphoma tissue specimens also other RA- and SLE lymphoma subtypes but they were not structurally examined only subject to a qualitative evaluation.

Immunohistochemical stainings of the TMA blocks (and the separate specimens) with anti-APRIL antibodies (Alexis) were performed. Two independent investigators (BL,CS) estimated the staining results of the DLBCL cases (from all three categories) using a conventional light microscope. The final estimate used was the percentage of stained cells out of the total counted stained/not stained cells from both investigators.

The results of the stainings of the DLBCL of the general population (the controls) were divided in quartiles and the results of the stainings of the RA and SLE cases were compared to these.

5 RESULTS

5.1 PAPER I

After linking the HDR to the SCR, and a number of exclusions, we identified 5,715 patients for our national Swedish SLE cohort with a total follow-up time of 50,246 patient years.

During the observation period (1964-1995) there were 443 incident cancers registered. The overall cancer risk was increased by 25 % (SIR 1.25 CI 95: 1.14-1.37). The risk persisted even after >15 years of follow-up (SIR 1.48 CI 95: 1.15-1.86).

The cancer sites that numerically (together with reliable statistical significant confidence intervals) contributed most to the increased observed cancers were the haematological and respiratory ones. NHL was the most common haematological malignancy, with a nearly 3-fold increased risk (SIR 2.86) which persisted up to ten years after start of follow-up. The HL risk was even higher (SIR 4.34 CI 95: 1.59-9.45) but there was only six cases making the risk estimate uncertain. During the five first years of follow-up there was also an excess risk of acute myeloid leukaemia (SIR 6.06 CI 95: 1.97-14.1), but again only a few (n=5) cases. The lung cancer risk was statistically significantly increased during the whole observation period and became most evident after 15 years of observation (SIR 2.79 CI 95: 1.39-4.98). The non melanoma skin cancer risk was also increased but first after that long observation period (>15years), whereas for melanoma skin cancer, on the contrary, the risk was decreased. No significantly increased risks of bladder or cervix uteri cancer were observed but there was an increased risk for cancer of other female organ (vagina/vulva) (SIR 2.70 CI 95 1.09-5.57).

5.2 PAPER II

Register linkage of HDR and SCR yielded 42 patients with SLE and NHL, which after exclusions were reduced to 16 cases. Two were excluded because of incorrect diagnosis code and 23 had to be excluded for not fulfilling at least four ACR criteria for SLE. One SLE patient was excluded since the lymphoma diagnosis could not be confirmed histologically. The 16 remaining cases were compared to the 26 (out of 80) controls that were left after exclusions.

The cases, all women, had a mean age at SLE onset of 48 years and a mean age at NHL diagnosis of 61 years. A very marked predominance of the aggressive NHL subtype DLBCL was found, 10 out of 16 cases. EBV was detected only in two of 15 investigated cases.

The SLE-NHL cases more often than controls had haematological manifestations; most obvious was haemolytic anaemia, RR 3.2 (CI 95: 2.0-5.0). Fourteen (88%) of the cases had at least one sign of haematological aberration, which almost always preceded the lymphoma diagnosis by at least four years. Sicca symptoms and/or salivary gland swellings, as well as presence of SS-A/SS-B, were associated with

increased lymphoma risk – RR 2.7 and 2.0 respectively – as were recurrent pneumonias and/or pulmonary infiltrates, RR 2.5. SLE nephritis or CNS manifestations of the SLE disease were on the contrary more common among the controls, however not significantly – RR 0.6 (CI 95: 0.2-2.2). Usage of cytotoxic drugs did not differ between cases and controls (RR 1.1 CI 95: 0.5-2.5).

Survival after NHL diagnosis had a bimodal pattern. Seven (44%) did not survive the first year, but the five-year survival (for all 16) on the other hand was 50%. Two out of 10 DLBCL cases had the for prognostic favourable GC subtype. Interestingly enough, although seven out of these 10 DLBCLs had widespread disease (Ann Arbor III-IV) at time of lymphoma diagnosis, there was a 60 % 10-year survival. Among them the two GC subtype cases.

5.3 PAPER III

For the third study the register linkage identified 13 cases of myeloid leukaemia (11 acute, 2 chronic). Only 12 had available medical records, and the evaluation of these disclosed two cases of drug-induced SLE and two cases not fulfilling the ACR criteria for SLE. The remaining eight cases of SLE and myeloid leukaemia, were subject to retrospective bone marrow analysis,and all passed the haematological re-evaluation. They were compared with 18 (out of 65) SLE controls that remained after exclusion criteria were checked (medical records missing, diagnosis code errors, drug-induced SLE and not fulfilling SLE ACR criteria).

Surprisingly, a male predominance (5/8) was seen among the cases, with mean age at SLE onset of 60 years and a mean interval to the leukaemia diagnosis of five years.

Leukopenia was the only clinical and laboratory manifestation that was significantly more common among the cases – OR 14 (CI 95: 1.4-41), but haematological aberrations occurred most often in at least two, sometimes three of the haematological cell lines. Bone marrow analysis prior to leukaemia diagnosis was made in four cases. Two of these showed dysplastic changes indicative of MDS. Although a possible association between cytotoxic drugs and leukaemia could not totally be ruled out in two cases, cytotoxic drugs were more frequently used among the controls –OR 0.4 (CI 95: 0.1-2.1). The occurrence of serious organ manifestations of SLE, was more frequent among the controls, however not significantly different – OR (SLE nephritis) 0.2 (CI 95: 0.1-2.2).

Median survival time after diagnosis was 6.5 months. A literature search revealed similarly poor outcome in 11 of the 15 cases. The other four, all published during the last decade, had longer survival. Also many of these literature cases had been subject to bone marrow analysis before leukaemia was established. In five of six investigated myelodysplastic features were found.

5.4 PAPER IV

In 95 RA cases, 14 SLE cases and 63 comparator cases the laboratory procedures were successful allowing an estimation of APRIL stained cells. The three different groups differed in some aspects: Mean age at lymphoma diagnosis was lower in the SLE patients than the RA patients and the controls (59 years vs 71 and 70 years). SLE patients were all female whereas the male/female ratio in the other groups was ≈ 1:1. The SLE patients had a lower mean age at start of the rheumatic disease (46 years) than the RA patients (51 years). Only a low proportion of the SLE and RA patients had been treated with potent immunosuppressive/cytotoxic drugs before lymphoma diagnosis. In SLE, the use of oral glucocorticoids ever during the course of SLE was 93% and at lymphoma diagnosis 86%, corresponding figures for the RA patients, 45 % and 22%.

More advanced lymphoma stages at diagnosis were seen in the patients with SLE and RA than in comparator cases and also the prognostically unfavourable non-GC DLBCL subtype was more frequent among the SLE- (78%) and RA patients (71%) than among the comparator cases (57%). Presence of EBV was similar in the SLE- and RA lymphoma tissues (about 10%), but was unfortunately not investigated in the comparator cases.

The SLE patients had a significantly higher percentage cells expressing APRIL compared to the two other groups combined (χ^2 p=0.002) or analyzed separately. SLE patients had a mean percentage of APRIL stained cells in the DLBCL tissues of 20%, while it was 11% in the RA patients and 10 % in the comparator cases. Based on the quartile distribution (quartile 4 – most stained) of the staining pattern in comparator cases we compared the SLE and RA lymphomas. The quartile distribution was significantly different from the comparator cases in SLE (p= 0.007), but not in RA. In patients with SLE 71% of the DLBCLs were strongly positive for APRIL (quartile 4). A positive correlation between severe RA (measured as cumulative disease activity) and APRIL expression was also seen.

EBV positive lymphomas had higher APRIL expression (p=0.009), but neither the disseminated stadium of the lymphoma disease at diagnosis, nor the non GC-subtype was associated with higher APRIL expression.

The best outcome was seen among the SLE patients with a mean survival time of 8.7 years. The RA patients survived on average only 2 years, the comparator cases 5 years. The amount of APRIL expression had no impact on prognosis. Lymphomas with APRIL high (quartile 3,4) and APRIL low (quartile 1,2) expression did not differ in survival.

6 DISCUSSION

6.1 SLE AND CANCER

To determine the cancer risk in SLE at various sites, for every cancer type one must have a, for all subsets of the SLE disease, representative patient population to follow for a large enough amount of time. To exclude random associations between a rare disease like SLE and many types of rare malignancies also a large population is necessary to achieve statistically significant results. Iceland, for example, a prominent country in SLE research has 320,000 inhabitants. The Icelandic SLE database linked to the Icelandic cancer registry showed an overall cancer risk of 1.38 in this unselected cohort of SLE patients followed for up to 44 years, but the size of the population, probably impeded the risk estimate to reach statistical significance.[129]

Our study design, retrospective and nationwide, enabled both a long observation time and gave the opportunity for many phenotypes of the SLE disease to be captured from many different medicine disciplines. Using a similar methodology, the results of our study have many similarities with the findings from Mellemkjaer´s Danish national SLE cohort.[81] The magnitude of the overall increased cancer risk in SLE was about the same in our Swedish cohort as well as the pointing out of NHL and lung cancer as some of the cancer sites at increased risk. There is, however, no absolute conformity between the risks at different sites in these two Scandinavian register studies. The very basis of the risk calculations, the number of observed patient years at risk, is different inasmuch as our study had more than 50,000 patient years, five times greater. The possible length of follow-up also differed as we could follow some patients for up to thirty years, while Mellemkjaer et al were limited to twelve years of follow-up. The importance of this circumstance could possibly explain the observed difference in the risk of non melanoma skin cancer. In the Danish study there was no increased risk at all; in our study there was a 50 % increased risk (with borderline significance) which rose to a statistically significant three times elevated risk after 15 years of follow up.

Since our publication in 2002 some further reports of SLE and malignancy in different cohorts and settings have emerged. A large international cohort study from SLE referral centres in the US, Canada, UK, Korea, Sweden and Iceland, including in total 9547 patients, published in 2005 found an overall increased risk of cancer of 1.15 (CI 95 1.05-1.27). Again the NHL risk was the most conspicuous finding.[54] The California patient discharge data, like the national Swedish and Danish SLE cohort, creates a cohort from SLE diagnoses of hospitalizations in a geographically defined area (State of California) and by linking to the correspondent cancer register demonstrated an increased cancer risk in SLE. Their relative risk estimate was 1.14 (CI 95 1.07-1.20) and based upon more than150,000 patient-years at risk, although the maximum follow-up time was 11 years.[130]

In summary, as of the date of this thesis, the take-home message across all these investigations, with their strengths and weaknesses, suggests a 15-30 % increased

risk of cancer in patients with SLE. The recurrent finding of an increased NHL risk in SLE suggests that this association may be real. However, the risk of cancer at different sites differs widely which will be dealt with in the following sections.

6.2 SLE AND HAEMATOLOGICAL CANCER

6.2.1 SLE and non-Hodgkin´s lymphoma

As presented in the introduction section the question of NHL risk in SLE has time and again indicated a more pronounced association than any other cancer in SLE. Although most often reaching statistical significance, the relative risk figures of NHL in SLE in table 4 vary considerably due to the statistical uncertainty that comes with few cases in a study. Our study pointed to a three times elevated risk (SIR 2.86). The international cohort of Bernatsky et al showed a SIR of 3.64 (CI 95 2.63-4.93) [54] and a SLE cohort study from California calculated the SIR to 2.74 (CI 95 2.22-3.34).[130]

NHL is a heterogeneous disease entity with some 40 subtypes, some of which are more often diagnosed, for instance DLBCL and follicular lymphoma. With the number of incident NHL cases in the SLE cohort cancer studies reaching a two-digit level comes an ambition also to look at the subtype, comparing the distribution with lymphoma in the general population.

Observations of NHL subtyping in other rheumatic diseases have shown for RA, an increased risk of the aggressive subtype DLBCL. Furthermore DLBCL subtyping into GC-like/non-GC-like showed a predominance of the prognostically unfavourable non-GC type (70%).[44,131] Primary Sjögren´s syndrome (pSS) has been associated with MALT lymphomas - approximately 85% of the lymphomas in pSS are MALT lymphomas.[46] However, in a recent study from a mono-centre primary Sjögren´s syndrome cohort - with patients fulfilling the American-European Consensus Group criteria [132] –a predominance of DLBCL was found.[133]

With regards to SLE, the NHL subtype question has been dealt with in the same three cohorts mentioned above. Our study did show an extreme predominance of DLBCL (10 out of 16, 62%). Although our ten DLBCL are too few to draw any conclusions, further DLBCL subtyping resulted in 8 out of 10 (80%) being non-GC type, a distribution far from those of the previously published of the general lymphoma population.[62,128] In the international cohort from SLE referral centres 11 out of the 21 cases (52%) that could be found in tumour registers were DLBCLs. More than 50% of the total number of cases (n=42) had died a median of 1.2 years after NHL diagnosis.[134] The linked registers study in California did, beside indicating an increased NHL risk in SLE, also show increased risks for both DLBCL and follicular lymphoma at about the same magnitude; 3.26 (CI 95 2.33-4.39) and 2.89 (CI 95 1.88-4.22) respectively. Information of prognosis and survival is lacking.[130]

The usage of cytotoxic drugs in our case-control study did not differ between the lymphoma patients and the controls, making treatment induced lymphoma a less likely explanation for development of NHL. Serious SLE manifestations, like

nephritis, were more uncommon among the cases than the controls. Similar observations were made by King and Costenbader in their analysis of characteristics of patients with SLE and NHL.[135] Our group comparisons displayed more often clinical SLE-manifestations like haematological manifestations (autoimmune haemolytic anaemia, hypergammaglobulinemia etc), recurrent pneumonias or pulmonary infiltrates among the lymphoma cases compared to the controls with SLE. The (SLE-lymphoma) cases also beyond having a definite SLE according to classification criteria often showed signs of a simultaneous or overlapping Sjögren´s syndrome (Sicca symptoms/glandular swellings, SS-A- positivity significantly more common among the cases).

In summary, the magnitude of the excess risk of NHL in SLE seems to be about three times the normal risk. A figure that possibly will be adjusted slightly downwards if the selection issue in these studies has excluded the possible milder forms of the SLE disease that never see a rheumatology specialist, a hospital bed or perhaps not even a doctor. A vast majority of this excess NHL risk comes from DLBCLs. Their predominance is, at least, just as marked as in RA. There are indications of a similarity between the two rheumatic diseases also regarding the GC/non GC distribution. Activated B-cell like DLBCLs of SLE patients with lymphoma give a hint of that the aetiology in many of the SLE-NHL cases could rise from an uncontrolled expansion of an activated B-cell clone. On the other hand, there is little evidence of treatment induced lymphomas.

6.2.2 SLE and myeloid leukaemia

Being a less common malignancy than lymphoma (approximately five times more unusual than lymphoma) it is understandable that a possible association between myeloid leukaemia and SLE is difficult to study. Here we tread on virgin soil.

In previously published studies that have reported an association between a rheumatic disease and leukaemia, have had in common patients that have been exposed to one of the few known risk factors of leukaemia, ionizing radiation or alkylating drugs.[30,31,136,137] The leukaemia cases in our case-control study were not more likely to be exposed to cytotoxic drugs, on the contrary, and only a minority of the leukaemias that arose might have been therapy-related. As in the case of SLE and NHL, the subset of SLE patients with incident leukaemia did not more often have serious manifestations like nephritis, but haematological abnormalities were more common. Here leucopenia was the only clinical manifestation that reached statistical significance (OR 14 CI 95 1.4-41). Moreover signs of a preceding MDS were seen both in our cases and those from our literature review.

Two previous cohort studies of SLE and cancer showed an increased leukaemia risk though results were not statistically significant.[80,81] Our linked register cohort study did demonstrate a significant leukaemia risk of 1.98 (CI 95 1.18-3.13). This risk was most prominent in the five years after inclusion, in contrast to the SLE-NHL cases where median time span between SLE onset and lymphoma diagnosis was 13 years. The SLE-myeloid leukaemia cases also were older at SLE onset

(median 60 years) and the male gender was in majority. This SLE subset of "survivors" has escaped an earlier SLE debut, serious SLE organ manifestations but is taken ill with SLE in an age where when cancer is becoming increasingly more common and where also MDS can occur. A speculation regarding the pathogenesis is that an exogenous agent causes a chromosome alteration, which in turn causes the SLE disease and a clonal proliferation. Alternatively, the SLE disease itself is inflicted and the defects of immunosurveillance and apoptosis of this disease make the normal clean-up of defect stem cells ineffective or insufficient, facilitating a clonal expansion of myeloid cells.

The results of the Californian SLE cohort showed a similar SLE-AML risk estimate 2.13 (CI 95 1.49-2.77).[130] A recently published study from the US, a case-control study of patients aged 67 or older based on information in the Medicare database showed that several autoimmune conditions, among them SLE (OR AML 1.92), were associated with AML and MDS. They suggest – beside medications – a shared genetic predisposition and an infiltration of the bone marrow by the autoimmune disease as possible explanations of these excess risks.[138]

Thus: There is a doubled increased risk of myeloid leukaemia in SLE without significant association with cytotoxic drug usage. The SLE subset at risk differs a bit from the "traditional" SLE patient conception by an older age at disease onset, a more equal gender ratio and predominating haematological aberrations.

6.2.3 SLE and Hodgkin´s disease

What about the risk of Hodgkin´s disease (HD) in SLE? Can we take for certain that by being a lymphoma the risk of HD, like NHL, is increased? Only 165 cases of HD were diagnosed in Sweden 2007- about one-tenth of the NHL cases. A fact that urges a very high risk and/or large cohort studies to assess an association with SLE. In their multi-site cohort, Bernatsky and colleagues found five cases of HD yielding a SIR of 2.36 (CI 95 0.75-5.51). By pooling this result with the result from the Swedish and Danish national SLE cohorts from the studies by Björnådal and Mellemkjaer a pooled SIR for HD in SLE became 3.16 (CI 95 1.63-5.51).[139] Parikh-Patel got in their Californian SLE cohort a very similar SIR of 3.02 (CI 95 1.60-5.13).[130]
Our national Swedish SLE cohort displayed a statistical significant SIR of 4.34. We did, as with the NHL and leukaemia cases, a follow-up of registered cases of HD. Lymphoma tissues from our nine cases were retrieved and reclassified. Unfortunately, we couldn't get hold of more than six of them. In the reclassification three of these six instead were classified as NHLs.

There are indeed observations of an increased risk of HD in SLE though the continuous development in lymphoma diagnosing and classification and the differential diagnostic difficulties between certain HD and NHL subtypes puts some question marks in this issue. Furthermore due to the rarity of this haematological disease it does not stand out as a large and immediate threat for the SLE patients.

6.3 SLE AND OTHER CANCER SITES

6.3.1 SLE and Respiratory cancer

Several cohort studies including ours have shown a statistical significant increased risk of lung cancer in SLE. [54,81,130] Our registry linkage first caught 76 cases of respiratory cancer, SIR 2.70 (CI 95 2.13-3.38). After exclusions of cancer cases occurring within less than one year after the first discharge from hospital with a SLE diagnosis, this number was modified to 50 cases (SIR 1.79, CI 95 1.33-2.36). A closer follow-up of these patients for SLE diagnosis confirmation and risk factor analysis had been interesting. Unfortunately, it is probable that the medical records would not have been complete enough to enable evaluation of such an important risk factor as cigarette smoking. Smoking has been shown to be a risk factor of SLE development.[12] A recent study, not surprisingly, showed that the lung cancer risk was increased in smoking SLE patients compared to non-smokers.[140] There are two possibilities to explain the overall increased lung cancer risk in SLE only by smoking. Either that the risk between smoker-non-smokers should be more pronounced in SLE patients – possibly due to potentiating cytotoxic drug use – than in the general population. Alternatively smoking should be more frequent among SLE patients. Another plausible explanation is cancer being the result of low-grade inflammation of many years duration in the respiratory mucosa.

6.3.2 SLE and Squamous cell carcinoma

Consequently, we could after 15 years of follow-up demonstrate an increased risk of non-melanoma skin cancer (or squamous cell carcinoma (SCC)) of 3.05 (CI 95 1.39-5.78), emphasizing the importance of long time follow-up in epidemiological investigations of issues like cancer development. In the Icelandic SLE cohort, with still longer observation time, SCC was the only cancer site associated with a statistically significant increased risk.[129]. Other SLE cohort studies – with shorter observation time – have failed to confirm this association.

The observed increasing risk to develop NHL and skin cancer (particularly melanoma) worldwide is, along with the strong association between these two malignancies, interesting and has put focus on ultraviolet light exposure as a possible common environmental risk factor.[141,142] Highly increased risks of skin cancer as well as NHL are also noted among transplanted patients, who without exceptions are treated with cytotoxic and/or immune modulating drugs in order to reduce the risk of transplant rejection. In a cohort of renal transplanted patients in Oxford followed for up to 21 years, the cumulative incidence of skin cancer was as high as 61%. [143] Regarding the increased risk of malignant lymphoma seen in transplanted patients (PTLD), a strong association with EBV was found.[50]

Our, and others, observations in SLE of an increased risk of NHL as well as SCC, occurring 5-10 years and more than 15 years after SLE disease onset respectively, do not contradict an association between the malignancies. However, UV light exposure as an (common) explanation of the increased risks of NHL and SCC in SLE is less likely since photosensitivity is a common clinical manifestation in this disease and most SLE patients avoid and protect themselves from sun exposure.

Immunosuppressive drug use – or EBV - does neither appear as prominent possible risk factors according to our experiences from the case-control studies of haematological malignancies in SLE. On the other hand, a common denominator to SLE and post transplantation is the state of immunosuppression or disturbed immunosurveillance per se.

6.3.3 SLE and other cancer sites

Female genital cancer and breast cancer:
Despite frequent reports of increased cervical dysplasia in women with SLE [87-89] investigations on larger SLE cohorts, including ours, have not convincingly showed an increased risk of cervix uteri cancer.[54,81,130]
Contrary to that, the risk of cancer of the vagina and vulva in SLE has not been drawn attention to. Our study shows in similarity to other large enough SLE cohort studies, an increased risk, SIR 2.70 (CI 95 1.09-5.57). The recent Californian SLE cohort study, with the, so far, largest amount of patient years got a risk estimate of SIR 3.27 (CI 95 2.41-4.31).[130] A connection with the similarly increased risk of SCC is probable. For the uterus and the ovary, the results of the four largest SLE cohort studies suggest a decreased risk. Our observed decreased risk of breast cancer SIR 0.72 (CI 95 0.54-0.95) is well in line with Bernatsky´s and Parikh-Patel´s of 0.76.[54,130] This diminished risk of the absolutely most common cancer of women contributes considerably to lessen the total cancer burden in SLE.

Bladder cancer:
No SLE cohort study – ours not an exception - has been able to show a statistically significant risk of bladder cancer. With observed relative risks just above 1, pooled together an increased risk is not totally excluded but is, at the most, marginal.[54,81,130]

Liver cancer:
The national Swedish SLE cohort did show an increased risk of liver cancer of SIR 1.61 but only with borderline significance. The three SLE cohort studies frequently mentioned in the last sections could all demonstrate statistically significant increased liver cancer risks. Mellemkjaer in Denmark had five cases and a SIR of 8.0 (CI 95 2.6-18.6). All patients were elderly and cirrhosis most often preceded both the cancer and the SLE diagnosis.[81] The other two got a similar SIR 2.6-2.7 but no further information about the patients.[54,130] A possible explanation to the different risk estimates might be differences in the populations regarding for instance use of alcohol, exposure to hepatotoxic drugs, hepatitis etc.

6.4 APRIL IN SLE AND IN SLE PATIENTS WITH DLBCL

SLE is characterized by a chronic activation of the immune system, including B cell activation with among other things auto-antibody production. The driving force of B cell activation is far from being fully understood. The cytokines BAFF and APRIL are of profound interest as they are both essential for B cell survival and development and overexpression is seen in connection with autoimmune disease and tumour development.[116] Elevated serum levels of APRIL (and BAFF) have been

observed in patients with SLE (and RA) [144-146] and local production of these cytokines in synovial fluid of inflamed joints has been demonstrated. [147] Thus these cytokines, which are normally occurring signal substances, have been associated with states of rheumatic disease when the B cell is active and the homeostasis of these signal substances are dysregulated. If they are causative factors or just contributing participators in the inflammatory process remains to elucidate.

We noticed in our lymphoma TMA blocks a difference between low-grade lymphomas, like for instance the follicular and chronic lymphocytic leukaemia, and high-grade, like the DLBCL, regarding APRIL expression inasmuch as APRIL being (almost) absent in the low-grades and highly expressed in the high-grades. This observation is inline with a previous study of APRIL and NHL, where they found that about APRIL was up-regulated in half of their DLBCLs. [118] Beside this they also found that infiltrating neutrophils – and not the tumour cells themselves - were the main source of secreted APRIL, which bound to the tumour cells via proteoglycan binding. The DLBC lymphoma cells could also express the receptors BCMA and TACI, which APRIL can bind to and induce signals. Thus the inflammatory cells and APRIL seem to have important functions in high-grade lymphoma formation.

The results of the SLE-NHL studies showed in this thesis point to the fact that there is an about three- to fourfold increased risk of lymphoma in SLE and that the DLBCL subtype dominates. So the excess lymphoma risk in SLE is to the greatest part due to DLBCL. The result of our study as well as King´s did not speak in favour of treatment induced lymphomas but certain clinical and laboratory SLE manifestations were associated with increased lymphoma risk.[135] Most prominent were certain haematological manifestations as well as sicca symptoms/glandular swellings and pulmonary infiltrates/recurrent pneumonias. All these symptoms are consistent with an active SLE with ongoing B-cell activity with possible APRIL overexpression and dysregulation. All of our SLE-DLBCLs had one or two (more common) of these three types of manifestations. APRIL was up-regulated in all but one of the SLE-DLBCLs.

APRIL expression was however not unique for the SLE DLBCLs but existed in DLBCLs of RA patients as well as in the DLBCL of the general population to a varying extent. In our study comparing DLBCL expression in SLE-, RA- and patients in the general population without concomitant inflammatory disease the highest expressions was found among the SLE patients and a subset of RA patients characterized by high cumulated RA clinical activity, a state that very well can be associated with high B cell activity. The effect of treating therapy-resisting RA with a B-cell depleting monoclonal antibody during later years demonstrates the importance of the B cell in this inflammatory process.[148,149]

Thus, APRIL was seen in connection with development of DLBCL also in SLE. The observed high APRIL expression among our SLE DLBCLs and the subset of RA patients with longstanding, severe inflammatory disease might suggest not only that APRIL per se could be a risk factor for lymphoma development but the possibility that the extra high APRIL expression could reflect the B cell dysregulation of the

autoimmune disease and consequently that a not adequately treated rheumatic disease could be a risk factor for these patients to develop DLBCL.

6.5 METHODOLOGICAL CONSIDERATIONS

Rare diseases like SLE, with a prevalence of less than one per thousand, are limited in epidemiological studies due to sample size. To study yet another rare event in these patients, such as incident cancer makes even greater demands of a large study population. In a heterogeneous disease like SLE, for the generalizability of the results, also another issue has to be handled with - the selection.

Regarding the sample size, given the population of Sweden and estimated SLE prevalence one might expect a sample of patients well into the thousands, particularly if you calculate that the greater part of them some time during a long observation period will be hospitalized for some reason. This number of patients has a reasonable chance to minimize the role of chance.

Many SLE cohorts used in epidemiologic investigations, for instance regarding cancer development, are composed of patients from tertiary rheumatology referral centres. In SLE the most prominent and serious organ involvement is variable and, along with it, the type of specialist treating it. It could be a rheumatologist, a nephrologist, or a dermatologist. To get a representative sample of all kinds of SLE is an important aspect of the selection issue. The study population should contain SLE patients with the milder forms as well as the more serious forms of the disease, as well as include patients that are not only treated and monitored by a rheumatologist. Our method, using the patients with a primary or secondary SLE discharge diagnosis in the Hospital Discharge Register during an era when hospitalizations were more common to investigate and treat diseases such as rheumatic diseases, made it possible to;
A) Gather patients treated and hospitalized at different hospital clinics. Interestingly, in our nested case-control study of SLE-NHL it turned out that eight out of the sixteen patients with NHL in SLE had never seen a rheumatologist;
B) Gather a majority of the SLE patients in Sweden. Studies from two other Nordic countries from the same time period showed a high percentage of hospitalization in their SLE populations. [150,151] However, we cannot rule out some due to the hospitalization per se. In paper I we excluded patients with cancer at the first SLE diagnosis discharge or during the first year of follow-up in order to decrease the risk that the admission to hospital was due to an underlying malignancy. Our nested case-control studies (paper II,III) showed that this method of avoiding confounding by indication was not really necessary as it stood clear from reading the medical journals that the SLE disease was in almost all cases existing long before this first discharge.

Our rather long follow-up time – up to thirty years – is also a big advantage. The importance of a long follow up time when studying cancer was previously discussed in section 6.1 with examples of different results in the national SLE cohorts of Denmark, Sweden and Iceland regarding squamous cell skin cancer.

Register studies always have to rely upon what information is put in the register. The Swedish Cancer Register is a well-established register, considered trustworthy, and probably very few incident cancers that escape reporting. The accuracy of the cancer diagnosis reported depends of the skill of the clinician and the pathologist. Moreover, from the early 1960s when the first cases in our study were reported, up until the 2000s when our haematological malignancies were subject to reclassification there have been numerous pertinent medical developments (for instance the lymphoma classification has changed four times). During our reclassification process there were, however, several specimens that not only got a new a designation due to change of classification system but also were changed to another cancer type.

The diagnoses in the Hospital Discharge Register, as mentioned in the Background section, have been validated with an accuracy of at least 80% for several diagnoses.[152] To our knowledge a validation of the SLE diagnosis has not been performed before. As it turned out during our nested case-control studies there was a falling off of "SLE cases" in the cancer cases as well as non-cancer SLE patient controls, but fortunately the exclusions were more frequent in the control group. In the case of the SLE diagnosis there were some administrative imperfections of the International Classification of Disease code numbers, like misnumbering of tuberculosis and the lack of a separate diagnosis code for drug-induced SLE.

The retrospective design also means that the information there is, for instance in the medical journals, what you get. Beside the fact that medical journals were not always possible to obtain – and which was the case in < 10% - there might be important information missing, for instance data on symptoms, diseases and states associated with SLE and of laboratory investigations and findings. This lack of information has in a number of cases (and controls) unfortunately led to exclusion as we decided to adhere to the ACR classification criteria and used fulfilling ≥ 4 of those criteria for inclusion.

Beside the patients where an SLE diagnosis was probable but the available information not allowing inclusion due to the SLE ACR criteria, there were also many patients during the medical journal scrutinizing process where the SLE diagnosis obviously – according to my opinion and available information – were erroneous. This circumstance could reflect the rarity and heterogeneity of the SLE disease and the fact that the diagnosis sometimes is hard to settle.

In summary, the specificity of the SLE diagnosis in the Hospital discharge register due to all circumstances described above did only reach an accuracy of about 50%. These random errors do not negatively affect our cancer risk estimates, on the contrary, as there was a greater number of excluded controls (the denominator) than cases (the numerator) in our nested case-control studies of the cohort.

In the nested case-control studies (paper II,III), the controls are matched for sex and had to have an observation-period free from cancer as long as or longer than the matched NHL-SLE case. To match for other characteristics like age at inclusion,

duration of SLE disease at inclusion and same inclusion period (decade) had been desirable but was unfortunately not possible.

In paper IV, in order to investigate the importance of an inflammatory rheumatic disease and its BAFF/APRIL dysregulation, we set the APRIL expression of DLBC lymphomas of RA- and SLE patients against the expression in a material of DLBCL patients without any inflammatory, autoimmune or rheumatic disease. The last-mentioned group is used as "controls" but since they are not be regarded as "healthy" and they have not been selected from age, gender etc a perhaps more proper designation should be comparator cases.

6.6 SOME CLINICAL REFLECTIONS OF THE FINDINGS

SLE patients in the year of 2009 live considerably longer than their sisters (and brothers) fifty years ago. Beside flares with general as well as organic symptoms in their autoimmune disease, which most often can be handled with pharmacological treatment, they also to a large extent contract bad health as the general population regarding cardiovascular and malignant diseases. Additional factors of atherosclerosis development in rheumatic disease make the risk of cardiovascular disease *and death* in SLE tripled and consequently the most important cause of death. Naturally, experts in vascular disease and SLE strongly recommend that the treatment and monitoring of SLE patients should strive for minimizing risk factors like blood pressure, blood lipids and smoking for instance.

From what has appeared in these studies of cancer in SLE there are some issues that might be of value for those who regularly see, treat and monitor SLE-patients.

No serious and responsible rheumatologist treating SLE patients shut their eyes to serious SLE manifestations like nephritis. Although the immunomodulating treatment options nowadays are more numerous, with non-cytotoxic alternatives, the alkylating drug cyclophosphamide is still often chosen for induction treatment due to proven efficacy and for saving the kidney. For SLE patients with other not so dramatic symptoms of ongoing systemic inflammatory disease ready treatment recommendations most often are lacking. In our nested case-control studies of SLE and NHL, and SLE and myeloid leukaemia we could not find an association between these haematological malignancies and cytotoxic drug usage. The lymphoma and leukaemia cases neither had the serious organ involvement more often than the SLE controls without malignancy, on the contrary. On the other hand they more often had several signs of ongoing systemic inflammatory disease with pulmonary infiltrates, lymph glandular enlargements, elevated immunoglobulins and aberrations of haematological cell lines. If this symptom constellations should be considered just as serious as for instance SLE nephritis and be treated more aggressively pharmacologically for the potential malignancy risk this thesis cannot really tell. However, these patients really do deserve the attention and the considerations of the treating rheumatologist/physician in the monitoring process. Clinicians caring for SLE patients should keep the lymphoma risk in mind and regularly check for lymphadenopathy, especially in those who do not show up a state of complete remission. Likewise SLE patients with prolonged cytopenias and

in particular leukopenia should , irrespective of treatment, be subject to a bone marrow investigation due to a little, but all the same increased, risk that an MDS or myeloid leukaemia might have developed.

7 CONCLUSIONS

7.1 THE OVERALL CANCER RISK, THE RISK AT DIFFERENT SITES IN SLE

Patients with SLE have a 15-30 % overall increased risk of cancer.

The risk of a non Hodgkin´s lymphoma is about threefold and constitutes the major risk of malignancy in SLE patients.

The aggressive NHL subtype DLBCL was the most common subtype, constituting a greater part of the excess lymphoma risk in SLE patients.

A doubled increased risk of myeloid leukaemia and a certain excess risk, of the more unusual, Hodgkin´s lymphoma , along with the NHL risk make haematological malignancies to the principal enemy of the SLE patients in the cancer issue.

Several unanimous cohort studies, ours included, also indicate a doubled increased risk of respiratory cancer and a two-threefold increased risk of cancer of the vagina and vulva. According to our and other SLE cohort studies with long follow up time there is also an increased risk of squamous cell carcinoma (non-melanoma skin cancer), most obvious after > 15 years of the SLE disease. There is conflicting risk estimates from other SLE cohort studies regarding the size of increased risk of liver cancer in SLE. The findings from the national Swedish SLE cohort do not totally contradict a possible excess risk but for our population the risk estimate did not reach statistical significance.

The most common female cancer, breast cancer, has a decreased risk in SLE patients.

7.2 RISK FACTORS FOR HAEMATOLOGICAL CANCER IN SLE

Certain clinical SLE manifestations like haematological aberrations, Sjögren-like disease and pulmonary involvement were associated with increased lymphoma risk.

On the contrary, treatment with cytotoxic drugs was not associated with increased lymphoma risk

APRIL expression was constantly up-regulated in the SLE DLBCLs. This finding must so far be interpreted with caution, but may indicate that APRIL has a role in DLBCL development – as in the general population – but also that this finding could reflect a dysregulation of the autoimmune disease per se.

Leucopenia was the only clinical manifestation associated with an increased risk of myeloid leukaemia. Leukaemia diagnosis was frequently preceded by an MDS.

Low-dose chemotherapy was not a major cause of myeloid malignancy in our population-based cohort of SLE patients.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

För att försvara sig mot sjukdomar, orsakade av bakterier, virus, parasiter eller förändrade cancerförstadieceller, har kroppens ett immunförsvar bestående av blodceller och vissa proteiner. Vid sjukdomen Systemisk Lupus Erytematosus (SLE) reagerar cellerna i kroppens immunförsvar även på normala kroppsegna celler och delar av celler med inflammation och sjukdom som följd. Symtom från leder och hud är vanligast men allvarligare livshotande engagemang från organ som njurar, hjärna och blodkärl kan också förekomma. Därför kräver ibland sjukdomen omfattande medicinering med bl a höga kortisondoser och immundämpande läkemedel

Om olika cancerformers uppkomst vid SLE också är en yttring av själva sjukdomen eller beror på slumpmässiga orsaker är huvudfrågan som denna avhandling försöker besvara. I arbetet med att hitta orsaker till den riskökning för cancer som ses vid SLE analyseras vilka patienter som löper större risk att drabbas.

Patientmaterialet för alla fyra delstudier utgår ifrån den grupp av patienter med SLE som vi identifierat utifrån det Svenska slutenvårdsregistret genom att ta med alla patienter som vårdats och utskrivits från sjukhus med SLE-diagnos mellan 1965 och 1995.

I det första delarbetet undersöktes hur många av dessa SLE-patienter som under samma tid också har registrerats för en cancerdiagnos i Svenska cancerregistret och dessa jämfördes med ”normalbefolkningen”, d v s den risk som man utifrån mångårig statistik räknar med att vi alla har att drabbas av cancer. Vi fann att SLE patienterna har en 30 % ökad risk att drabbas av cancer och att en stor andel av denna riskökning utgjordes av cancer i blod och blodbildande organ. Risk för hud- och lungcancer var också något ökad medan andra cancerformer, som exempelvis bröstcancer, förekom mindre ofta vid SLE.

I delarbete 2 och 3 har de patienter ur SLE-kohorten som drabbats av lymfcancer (lymfom) och blodcancer (leukemi) undersökts närmare och jämförts med andra icke cancerdrabbade SLE-patienter. Sjukjournaler och cancervävnader har granskats. De som fick lymfom resp. leukemi hade INTE en ”svårare” SLE, behandlades INTE oftare med immundämpande men hade oftare vissa drag av SLE, framför allt påverkan på olika typer av blodvärden.

I delarbete 4 studeras ett speciellt hormon, ett protein som populärt benämns APRIL. APRIL behövs för utvecklingen av vita blodkroppar, men har tidigare också hittats i ökade mängder i tumörer och i blodet hos reumatiker. Vi fann att APRIL förekom lite oftare och i större utsträckning hos patienter med SLE och en elakartad form av lymfom jämfört med patienter med bara detta lymfom. Detta tolkas som att APRIL kan vara viktig för uppkomsten av denna cancertyp och att de lite högre värdena för SLE patienterna speglar en mycket aktiv reumatisk sjukdom. Möjligen innebär alltså en långvarig och aktiv SLE sjukdom en risk för denna cancertyp.

9 ACKNOWLEDGEMENTS

There are of course a lot of people to whom I owe many thanks. Without you the tale of the wolf and the crayfish (cancer) wouldn't exist! Thank you…..

….to begin with, Professor **Ingrid Lundberg**, my supervisor, whose energy and enthusiasm never runs dry. She is probably also the most important person regarding my choice of discipline in medicine. It has been a great pleasure to work with you and your optimism and attitude

….Professor **Anders Ekbom**, my co-supervisor, you are if possible, an even more optimistic and cheerful tutor. I am sorry, I always let you down regarding news and gossip from the department of surgery of Eskilstuna. Anders, your enormous knowledge about almost anything in the field of epidemiology and medicine has been the backbone of this project.

….Professor **Christer Sundström.** Your support and evaluations of the hematopathological and immunohistochemical parts gave these studies a raise.

…., and your invaluable work, **Carin Backlin**. You came in times of doubt and despair and gave me help with lots of practical and absolutely necessary but time-consuming things like bringing in the lymphoma specimens, doing all the stainings and many more things.

…."deputy" supervisor **Eva Baecklund.** For always being friendly, your knowledge and your passion for the rheuma-lymphoma issue.

….co-author **Lena Björnådal**. For your brief introduction in practical clinical epidemiology. And yes Lena, Sörmland is indeed one of the most beautiful counties in Sweden!

…..**Eva Hellström-Lindberg** and **Tom Pettersson**, co-authors and prominent in their fields of knowledge. For valuable points of view in the writing process. Tom, for giving my work a little international touch.

….**Hans G Eriksson**, my statistician, for your help with p-values, your figures, your encouraging interest in my work and for your ability to appreciate a game of chess.

…. my bosses **Birgir Arge** and **Åke Thörner** and my colleagues at the department of Rheumatology in Eskilstuna. For giving me the opportunity to do research.

….**Tomas Svensson**, **Tomas Ljungberg**, **Anna Lundström** and **Carina Pettersson** at the centre of Research and Development, Sörmland county council. For practical and financial support.

….**Julia Fridman Simard** for linguistic advice with the reviewer hat on.

….last but not least my wife **Gun**, my sons **Ludvig** and **Olov.** For being the most important persons in my life, giving me the possibility to recover my strength from a funny conversation at the kitchen table at home, from the travels to Ljungdalen, the games at Stockholm Stadion, from the walking-tours on Sörmlandsleden -or other nice things we do together.

10 REFERENCES

1. Estes D, Christian CL. The natural history of systemic lupus Erythematosus by prospective analysis. Medicine 1971;50:85-95

2. Systemic Lupus Erythematosus/ edited by Robert Lahita – 2nd edition. Churchill Livingstone 1992

3. Nived O, Sturfelt G, Wollheim F. Systemic Lupus Erythematosus in an adult population in Southern Sweden: incidence, prevalence and validity of ARA revised classification criteria. Br J Rheum 1985;24:147-

4. Michet CJ, McKenna CH, Elveback LR et al. Epidemiology of Systemic Lupus Erythematosus and other connective tissue disorder in Rochester, Minnesota, 1950-79. Mayo Clin Proc 1985;60:105

5. Weinstein A, Bordwell B, Stone B, Tibbetts C, Rothfield NF. Antibodies to native DNA and serum complement (C3) levels. Application to diagnosis and classification of systemic lupus Erythematosus. Am J Med 1983;74:206-16

6. Notman DD, Kurata N, Tan EM. Profiles of antinuclear antibodies in systemic rheumatic diseases. Ann Intern Med 1975;83:464-9

7. Sam Lim S, Drenkard C. Epidemiology of systemic Lupus Erythematosus: Capturing the Butterfly. Current Rheumatology Reports 2008;10:265-72

8. Hochberg MC. Updating the American college of Rheumatology revised criteria for the classification of systemic lupus Erythematosus. Arthritis Rheum 1997;40:1725

9. Collins DA, Bourke BE. Systemic lupus Erythematosus: an occasional misdiagnosis. Ann Rheum Dis 1996;55:421-2

10. Ramos-Casals M, Brito-Zerón P, Font J.The overlap of Sjögren´s syndrome with other systemic autoimmune diseases. Semin Arthritis Rheum 2007;36:246-55

11. Hochberg MC. The application of genetic epidemiology to Systemic Lupus Erythematosus. J Rheumatol 1987;14:867-9

12. Costenbader KH, Kim DJ, Peerzada J, Lockman S, Nobles-Knight D, Petri M et al. Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. Arthritis Rheum 2004;50:849-57

13. Gunnarsson I, Kanerud L, Pettersson E, Lundberg I, Lindblad S, Ringertz B. Predisposing factors in Sulphasalazine-induced Systemic Lupus Erythematosus. British Journal of Rheumatology 1997;36:1089-94

14. Dubois EL, Wierzchowieck M, Cox MB, Weiner JM. Duration and Death in Systemic Lupus Erytematosus. An Analysis of 249 Cases JAMA 1974;227:1399-1402

15. Abu-Shakra M, Urowitz MB, Gladman DD, Gough J. Mortality Studies in Systemic Lupus Erytematosus. Results from a Single Center. I Causes of Death. J Rheumatol 1995;22:1259-1264)

16. Urowitz MB, Gladman DD, Abu-Shakra M, Farewell VT. Mortality studies in Systemic Lupus Erythematosu. Results from a single center. III Improved survival over 24 years. J Rheumatol 1997;24:1061-5

17. Ståhl-Hallengren C, Jönsen A, Nived O, Sturfelt G. Incidence studies of Systemic Lupus Erythematosus in southern Sweden: increasing age, decreasing frequency of renal manifestations and good prognosis. J Rheumatol 2000;27:685-91

18. Björnådal L, Yin L, Granath F, Klareskog L, Ekbom A. Cardiovascular disease a hazard despite an improved prognosis in patients with systemic lupus erythematosus: Results from a Swedish population-based study 1964-1995. J Rheumatol 2004;31:713-9

19. Somers EC, Thomas SL, Smeeth L, Schoonen WM, Hall AJ. Incidence of Systemic Lupus Erythematosus. Arthritis Care &Research 2007;57:612-8

20. Uramoto KM, Michet Jr CJ, Thumboo J, Sunku J, O´Fallon WM, Gabriel SE. Trends in the incidence and mortality of Systemic Lupus Erythematosus. Arthritis Rheum 1999;42:46-50

21. D´Cruz DP, Khamashta MA, Hughes GRV. Systemic Lupus Erythematosus. Lancet 2007;369:587-96

22. Hochberg MC. The incidence of Systemic Lupus Erythematosus in Baltimore, Maryland, 1970-1977. Arthritis Rheum 1985;28:80-

23. Barnett EV, Dornfeld L, Lee DB, Liebling MR. Longterm survival of lupus nephritis patients treated with azathioprine and prednisone. J Rheumatol 1978;5:275-87

24. Donadio JV Jr, Holley KE, Ferguson RH, Ilstrup DM. treatment of diffuse lupus nephritis with prednisone and combined prednisone and cyclophosphamide. N Engl J Med 1978;299:1151-5

25. Abu-Shakra M, Shoenfeld Y. Azathioprine therapy for patients with systemic lupus Erythematosus. Lupus 2001;10:152-3

26. Ginzler EM, Dooley MA, Aranow C, Kim MY, Buyon J, Merrill JT et al. Mycophenolate Mofetil or Intravenous Cyclophosphamide for Lupus Nephritis. N Engl J Med 2005;353:2219-28

27. Austin HA 3rd, Klippel JH, Balow JE, le Riche NG, Steiberg AD, Plotz PH et al. Therapy of Lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med 1986;314:614-9

28. Valeri A, Radhakrishnan J, Estes D, DÁgati V, Kopelman R, Pernis A et al. Intravenous pulse cyclophosphamide treatment of severe lupus nephritis: a prospective five-year study. Clin Nephrol 1994;42:71

29. Houssiau F. Thirty years of cyclophosphamide: assessing the evidence. Lupus 2007;16:212-6

30. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener´s Granulomatosis. Int J Cancer 2002;100:82-85

31. Knight A, Askling J, Granath F, Sparen P, Ekbom A. Urinary bladder cancer in Wegener´s Granulomatosis: risks and relation to cyclophosphamide. Ann Rheum Dis 2004;63:1307-11.

32. Carneiro JR, Sato EI. Double blind, randomized, placebo controlled clinical trial of methotrexate in systemic lupus erytematosus. J Rheumatol 1999;26:1275-9.

33. Radhakrishnan J, Kunis CL, DÁgati V, Appel GB. Cyclosporine treatment of lupus membranous nephropathy. Clinical Nephrology 1994;42:147-54

34. Chan TM, Tse KC, Tang CS, Mok MY, Li FK. Long-Term Study of Mycophenolate Mofetil as Continous Induction and Maintenance Treatment for Diffuse Proliferative Lupus Nephritis. Am Soc Nephrol 2005;16:1076-84

35. Klareskog L, Saxne T, Enman Y. Reumatologi. Studentlitteratur 2005

36. Arnett F, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of Rheumatoid Arthritis. Arthritis Rheum 1988;31:315-24

37. Isomäki HA, Hakulinen T, Joutsenlahti U. Excess risk of lymphomas, leukemia and myeloma in patients with rheumatoid arthritis. J Chron Dis 1978;31:691-6

38. Mellemkjaer L, Linet MS, Gridley G, Frisch M, Moller H, Olsen JH. Rheumatoid arthritis and cancer risk. Eur J Cancer 1996;32A:1753-7

39. Kauppi M, Pukkala E, Isomäki H. Elevated incidence of hematologic malignancies in patients with Sjögren´s syndrome compared with patients with rheumatoid arthritis (Finland). Cancer Causes Control 1997;8:201-4.

40. Thomas E, Brewster DH, Black RJ, Macfarlane GJ. Risk of malignancy among patients with rheumatic conditions. Int J Cancer 2000;88:497-502

41. Gridley G, McLaughlin JK, Ekbom A, Klareskog L, Adami HO, Hacker DG et al. Incidence of cancer among patients with rheumatoid arthritis. J NatlCancer Inst 1993;85:307-11

42. Ekström K, Hjalgrim H, Brandt L, Baecklund E, Klareskog L, Ekbom A et al. Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. Arthritis Rheum 2003;48:963-70

43. Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F et al. Chronic inflammation, not its treatment, increases lymphoma risk in rheumatoid arthritis. Arthritis Rheum 2006;54:692-701

44. Baecklund E, Sundström C, Ekbom A, Catrina AI, Biberfeld P, Feltelius N et al. Lymphoma subtypes in patients with rheumatoid arthritis: increased proportion of diffuse large B cell lymphoma. Arthritis Rheum 2006;54:1543-50

45. Cancer Incidence in Sweden 2007. Stockholm: Epidemiologiskt Centrum, Socialstyrelsen, 2008. www.socialstyrelsen.se/epc

46. World Health Organization Classification of Tumours: Pathology and Genetics of tumours of Haematopoietic and Lymphoid Tissues. IARC Press, 2001.

47. Sveriges onkologiska centra. Svenska lymfomregistret. Rapport för år 2000-2005. www.ocsyd.lu.se

48. Smedby KE, Hjalgrim H, Melbye M et al. Ultraviolet radiation exposure and risk of malignant lymphomas. J Natl Cancer Inst. 2005;97:199-209.'

49. Dal Maso L, Franceschi S. Epidemiology of non-Hodgkin´s lymphomas and other haemolymphopoietic neoplasms in people with AIDS. Lancet Oncol 2003;4:110-9

50. Taylor AL, Marcus R, Bradley JA. Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. Critical Reviews in Oncology/Hematology 2005;56:155-67

51. Wootherspoon AC, Ortiz-Hidalgo C, Falcon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991;338:1175-6

52. Wang SS, Slager SL, Brennan P, Holly EA, De Sanjose S, Bernstein L et al. Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (Interlymph) Blood 2007;109:3479-88

53. Kassan SS, Thomas TL, Moutsopoulos HM, Hoover R, Kimberley RP, Budman DR et al. Increased risk of Lymphoma in Sicca Syndrome. Ann Intern Med 1978;89:888-92.

54. Bernatsky S, Boivin JF, Joseph L, Rajan R, Zoma A, Manzi S et al. An International Cohort Study of Cancer in Systemic Lupus Erythematosus. Arthritis Rheum 2005; 52:1481-90

55. Askling J, Linet M, Gridley G, Halstensen TS, Ekstrom K, Ekbom A. Cancer incidence in population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. Gastroenterology 2002; 123:1428-35

56. Askling J, Brandt L, Lapidus A, Karlén P, Björkholm M, Löfberg R et al. Risk of haematopoietic cancer in patients with inflammatory bowel disease. Gut 2005;54:617-22.

57. Askling J, Grunewald J, Eklund A, Hillerdal G, Ekbom A. Increased risk for cancer following sarcoidosis. Am J Respir Crit Care Med 1999;160:1668-72

58. Carbone PP, Kaplan HS, Musskoff H, Smithers DW, Tubiana M. Report of the Committee on Hodgkin´s Disease Staging Classification. Cancer Res 1971;31:1860-1.

59. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin´s lymphoma. N Engl J Med 1993;329:987-94

60 Alizadeh AA, Eisen MB, Davis RE, Chi Ma, Lossos IS, Rosenwald A et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11

61 Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large B-cell lymphoma. N Engl J Med 2002; 346:1937-47.

62. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275-282

63. Onkologiskt centrum. Quality register (Sweden). Adult acute leukaemia. Report nr 4 2004.

64. Svensk förening för Hematologi, Sveriges Onkologiska centra. Blodcancerregistret Akut leukemic hos vuxna. Rapport nr 5, Maj 2008.

65. Malcovati L, Nimer SD. Myelodysplastic syndromes: Diagnosis and Staging. Cancer control 2008;15 No 4 Supplement

66. Bennett JM, Catovsky D, Daniel MT et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189-99

67. Greenberg P, Cox C, LeBeau MM et al. International scoring system for evaluating Prognosis in Myelodysplastic syndromes. Blood 1997;89:2079-88.

68. Malcovati L, Germing U, Kuendgen A et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25(23):3503-10

69. Lee SL. Clinical experiences with the LE cell test. J Mount Sinai Hosp NY 1955;22:74-8

70. Cammarata RJ, Rodnan GP, Jensen WN. Systemic Rheumatic Disease and Malignant Lymphoma. Archives of Internal Medicine 1963 pp 330-7

71. Nilsen LB, Missai ME, Condemi JJ. Appearance of Hodgkin´s disease in a patient with Systemic Lupus Erythematosus. Cancer 1967;20:1930-3

72. Penn I, Halgrimson CG, Starzl TE. De novo malignant tumors in organ transplant recipients. Transplant Proc 1971;3:773-8

73. Canoso JJ, Cohen AS. Malignancy in a Series of 70 Patients with Systemic Lupus Erythematosus. Arthritis Rheum 1974;17:383-90

74. Lewis RB, Castor W, Knisley RE, Bole GG. Frequency of Neoplasia in Systemic lupus Erythematosus and Rheumatoid Arthritis. Arthritis Rheum 1976;19:1256-60

75. Mellors RC, Landy DA, Bardell D. Autoimmune Disease in NZB/BI Mice II: Autoimmunity and Malignant Lymphoma. Blood 1966;27:435-48

76. Casey TP. Azathiprine (Imuran) administration and the development of malignant lymphomas in NZB Mice. Clin exp Immunol. 1968;3:305-12.

77. Grünwald HW, Rosner F. Acute Leukemia and Immunosuppressive Drug Use. A review of Patients undergoing Immunosuppressive Therapy for Non-neoplastic Diseases. Arch Intern Med 1979;139:461-6

78. Pettersson T, Pukkala E, Teppo L, Friman C. Increased risk of cancer in patients with systemic lupus Erythematosus. Ann Rheum Dis 1992;51:437-9

79. Sweeney DM, Manzi S, Janosky J, Selvaggi KJ, Ferri W, Medsger Jr TA et al. Risk of Malignancy in Women with Systemic Lupus Erythematosus. J Rheumatol 1995;22:1478-82

80. Abu-Shakra M, Gladman DD, Urowitz MB. Malignancy in Systemic Lupus Erythematosus. Arthritis Rheum 1996;39:1050-4

81. Mellemkjaer L, Andersen V, Linet MS, Gridley G, Hoover R, Olsen JH. Non-Hodgkin´s Lymphoma and other cancers among a cohort of patients with Systemic Lupus Erythematosus. Arthritis Rheum 1997;40:761-8

82. Ramsey-Goldman R, Mattai SA, Schilling E, Chiu YL, Alo CJ, Howe HL et al. Increased Risk of Malignancy in Patients with Systemic Lupus Erythematosus. Journal of Investigative Medicine 1998;46:217-22

83. Sultan SM, Ioannou Y, Isenberg DA. Is there an association of malignancy with systemic lupus Erythematosus? An analysis of 276 patients under long-term review. Rheumatology 2000;39:1147-52

84. Cibere J, Sibley J, Haga M. Systemic Lupus Erythematosus and the risk of malignancy. Lupus 2001;10:394-400

85. Nived O, Bengtsson A, Jönsen A, Sturfelt G, Olsson H. Malignancies during follow-up in an epidemiologically defined systemic lupus erythematosus inception cohort in southern Sweden. Lupus 2001;10:500-4

86. Nyberg G, Eriksson O, Westberg NG. Increased incidence of cervical atypia in women with Systemic Lupus Erythematosus treated with chemotherapy Arthritis Rheum 1981;24:648-50

87. Blumenfeld Z, Lorber M, Yoffe N, Y Scharf. Systemic Lupus Erythematosus: Predisposition for Uterine Cervical Dysplasia. Lupus 1994;3:59-61

88. Dhar JP, Kmak D, Bhan R, Pishorodi L, Ager J, Sokol RJ. Abnormal Cervicovaginal Cytology in Women with Lupus: A Retrospective Cohort Study. Gynecologic Oncology 2001;82:4-6

89. Ognenovski VM, Marder W, Somers EC, Johnston CM, Farrehi JG, Selvaggi SM et al. Increased Incidence of Cervical Intraepithelial Neoplasia in Women with Systemic Lupus Erythematosus Treated with Intravenous Cyclophosphamide. J Rheumatol 2004;31:1763-7

90. Boumpas DT, Austin HA, Vaughn EM, Klippel JH, Steinberg AD, Yarboro CH et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. Lancet 1992;340:741-5

91. Plotz PH, Klippel JH, Decker JL, Grauman D, Wolff D, Brown BC et al. Bladder complications in patients receiving cyclophosphamide for systemic lupus erythematosus or rheumatoid arthritis. Ann Intern Med 1979;91:221-3

92. Cox PJ. Cyclophosphamide cystitis – Identification of Acrolein as the causative agent. Biochemical Pharmacology 1979;28:2045-9

93. Brock N, Stekar J, Pohl J. Antidot gegen urotoxische Wirkungen der Oxazaphosphorin-derivate Cyclophosphamid, Ifosfamid und Trofosfamid. Naturwissenschaften 1979;66:60-1

94. Radis CD, Kahl LE, Baker GL, Wasko MC, Cash JM, Gallatin A et al. Effects of Cyclophosphamide on the development of malignancy and on long-term survival of patients with Rheumatoid Arthritis. Arthritis Rheum 1995;38:1120-7

95. Elliott RW, Essenhigh DM, Morley AR. Cyclophosphamide treatment of systemic lupus erythematosus: risk of bladder cancer exceeds benefit. BMJ 1982;284:1160-1

96. Thrasher JB, Miller GJ, Wettlaufer JN. Bladder leiomyosarcoma following cyclophosphamide therapy for Lupus nephritis. The Journal of Urology 1990;143:119-21

97. Ortiz A, Gonzalez-Parra E, Alvarez-Costa G, Egido J. Bladder cancer after Cyclophosphamide Therapy for Lupus nephritis. Nephron 1992;60:378-9

98. Moreland LW, Baumgartner SW, Schiff MH et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. NEJOM 1997;337:141-7

99. Maini RN, Breedveld FC, Kalden JR et al Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor α monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis- Arthritis Rheum 1998;41:1552-63

100. Bodmer JL, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. Trends Biochem Sci 2002;27:19-26

101. Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J. BAFF mediates survival of peripheral immature B-lymphocytes. J Exp Med 2000;192:1453-65

102. Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S et al. Identification of a novel receptor for B-lymphocyte stimulator that is mutated in a mouse strain with severe B-cell deficiency. Curr Biol 2001;11:1547-52

103. Sutherland APR, Mackay F, Mackay CR. Immunomodulation for autoimmune diseases and lymphomas. Pharmacology & Therapeutics 2006;112:774-86

104. Schneider P, Mackay F, Steiner V, Hofmann K, Bodmer JL, Holler N et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates cell growth. J Exp Med 1999;189:1747-56

105. Nardelli B, Belveder O, Roschke V, Moore PA, Olsen HS, Migone TS et al. Synthesis and release of B-lymphocyte stimulator from myeloid cells. Blood 2000;97:198-204

106. Ohata J, Zvaifler NJ, Nishio M, Boyle DL, Kalled SL, carson DA et al. Fibroblast-like synoviocytes of mesenchymal origin express functional B-cell activating factor of the TNF family in response to proinflammatory cytokines,J immunol 2005;174:864-70

107. Mackay F, Mackay CR. The role of BAFF in B-cell maturation,. T-cell activation and autoimmunity. Trends Immunol 2002;23:113-5

108. Zhang J, Roschke V, Baker KP, Wang Z, Alarcon GS, fessler BJ et al. A role for B cell stimulator in Systemic Lupus Erythematosus. J Immunol 2001;166:6-10

109. Groom J, Kalled SL, Cutler AH, Olson C, Woodcock SA, Schneider P et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren´s syndrome. J Clin Invest 2002;109:59-68

110. Krystufková O. Vallerskog T, Helmers SB, Mann H, Putová I Belácek J et al. Increased serum levels of B cell activating factor (BAFF) in subsets of patients with idiopathic inflammatory myopathies. Ann Rheum Dis 2009;68:836-43

111. Mariette X, Roux S, Zhang J, Bengoufa D, Lavie F, Zhou T et al. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren´s syndrome. Ann Rheum Dis 2003;62:168-71

112. Petri M, Stohl W, Chatham W, McCune J, Chevrier M, Ryel J et al. Association of Plasma B Lymphocyte Stimulator Levels and Disease Activity in Systemic Lupus Erythematosus. Arthritis Rheum 2008;58:2453-9

113. Novak AJ, Bram RJ, Kay NE, Jelinek DF. Aberrant expression of B-lymphocyte stimulator by B chronic lymphocytic leukemia cells: a mechanism for survival. Blood 2002;100:2973-9

114. Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM et al. Expression of BLyS and its receptors in B-cell non-Hodgkin

lymphoma: correlation with disease activity and patient outcome. Blood 2004;104:2247-53

115 Ansell SM,Witzig TE, Inwards DJ, Porrata LF, Ythier A, Ferrande L et al. Phase I clinical study of Ataticept in patients with relapsed and refractory B-cell Non-Hodgkin´s lymphoma Clinical Cancer Research 2008;14:1105-10

116. Hahne M, Kataoka T, Schröter M, Hofmann K, Irmler M Bodmer JL et al. APRIL, a new ligand of the Tumor Necrosis Factor Family, Stimulates Tumor cell Growth. J Exp Med 1998;188:1185-90

117. Schneider P, Takatsuka H, Wilson A, Mackay F, Tardivel A, Lens S et al. Maturation of marginal zone and follicular B cells requires B cell activating factor of the tumor necrosis factor family and is independent of B cell maturation antigen. J Exp Med 201;194:1691-7

118. Schvaller J, Schneider P, Mhawech-Fauceglia P, M;cKee T, Myit S, Matthes T et al. Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlated with human B-cell lymphoma aggressiveness. Blood 2007;109:331-8

119. Planelles L, Carvalho-Pinto CE, Hardenberg G, et al. APRIL promotes B-1 cell-associated neoplasm. Cancer Cell 2004;6:399-408

120. Sasaki Y, Casola S, Kutok J, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and independent roles for BAFF in B cell physiology. J Immunol 2004;173:2245-52

121. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T et al. Activation and accumulation of B-cells in TACI-deficient mice. Nat Immunol 2001;638-43

122. Thompson JS, Schneider P, Kalles S, Wang L, Lefevre EA, Cachero TG et al. BAFF binds to the tumor necrosis factor receptor-like molecule B cell matruration antigen and is important for maintaining the peripheral B-cell population. J Exp Med 2000;192:129-35

123. Wang H, Marsters SA, Baker T, Chan B, Lee WP, Fu L et al. TACI-ligand interactions are required for T cell activation and collagen-induced arthritis in mice. Nat Immunol 2001;2:632-7

124. Gross JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R et al. TACI-Ig Neutralizes Molecules Critical for B Cell Development and Autoimmune Disease: Impaired B Cell Maturation in Mice Lacking BLyS. Immunity 2001;15:289-302

125. Hospital Discharge Register 1964-2005; Quality and contents: National Board of Health and welfare, Stockholm 2006

126. Ingelsson E, Arnlöv J, Sundström J, Lind L. The validity of a diagnosis of heart failure in a hospital discharge register. Eur J Heart Fail 2005;7:787-91

127. Allebeck P, Ljungström K, Allander E. Rheumatoid arthritis in a medical information system. How valid is the diagnosis. Scand J Soc Med 1983;11:27-32

128. Berglund M, Thunberg U, Amini RM, Book M, Roos G, Erlanson M et al. Evaluation of immunophenotype in diffuse large B-cell lymphoma and impact on prognosis. Mod Pathol 2005;18:1113-20

129. Ragnarsson O, Gröndal G, Steinsson K. Risk of malignancy in an unselected cohort of Icelandic patients with systemic lupus Erythematosus. Lupus 2003;12:687-91

130. Parikh-Patel A, White RH, Allen M, Cress R. Cancer risk in a cohort of patients with systemic lupus Erythematosus (SLE) in California. Cancer Causes Control 2008;19:887-94

131. Baecklund E, Backlin C, Iliadou A, Granath F, Ekbom A, Amini RM et al. Characteristics of diffuse large B cell lymphomas in rheumatoid arthritis. Arthritis Rheum 2006;54:3774-81

132. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE et al Classification criteria for Sjögren´s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-8

133. Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson L. Lymphoma and other malignancies in primary Sjögren´s syndrome. A cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis 2005;64:1507-9

134. Bernatsky S, Ramsey-Goldman R, Rajan R, Boivin JF, Joseph L, Lachance S et al. Non-Hodgkin´s lymphoma in systemic lupus Erythematosus. Ann Rheum Dis 2005;64:1507-9

135. King JK, Costenbader KH. Characteristics of patients with systemic lupus Erythematosus (SLE) and non-Hodgkin´s lymphoma (NHL). Clin Rheumatology 2007;26:1491-4

136. Weiss HA, Darby SC, Fearn T, Doll R. Leukemia mortality after X-ray treatment for ankylosing spondylitis. Radiat Res. 1995;142:1-11

137. Wick RR, Nekolla EA, Gaubitz M, Schulte TL. Increased risk of myeloid leukaemia in patients with ankylosing spondylitis following treatment with Radium-^{224} Rheumatology 2008;47:855-9

138. Anderson LA, Pfeiffer RM, Landgren O, Gadalla S, Berndt SI, Engels EA. Risks of myeloid malignancies in patients with autoimmune conditions. British Journal of Cancer 2009;100:822-8

139 Bernatsky S, Ramsey-Goldman R, Isenberg D, Rahman A, Dooley MA, Sibley J et al. Hodgkin´s lymphoma in systemic lupus Erythematosus. Rheumatology 2007;46:830-2

140. Bernatsky S, Joseph L, Boivin JF, Gordon C, Urowitz M, Gladman D et al. The relationship between cancer and medication exposures in systemic lupus erythaematosus: a case cohort study. Ann Rheum Dis 2008;67:74-9

141. Adami J, Frisch M, Yuen J, Glimelius B, Melbye M. Evidence of an association between non-Hodgkin´s lymphoma and skin cancer. BMJ 1995;310:1491-5

142. Hu S, Federman DG, Kirsner RS. Skin cancer and non-Hodgkin´s lymphoma: examining the link. Dermatol Surg 2005;31:76-82

143. Bordea C, Wojnarowska F, Millard PR, Doll H, Welsh K, Morris PJ. Skin cancers in renal-transplant recipients occur more frequently than previously recognized in a temperate climate. Transplantation 2004;77:574-9

144. Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic , diseases. Arthritis Rheum 2001;44:1313-9

145. Zhang J, , Roschke V, Baker KP, Wang Z, Alarcon CS, Fessler BJ, et al. Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus J Immmunol 2001;166:6-10

146. Koyama T, Tsukamoto H, Miyagi Y, Himeji D, Otsuka J, Miyagawa H, et al. Raised serum APRIL levels in patients with systemic lupus erythematosus. Ann Rheum Dis 2005;64:1065-7

147. Tan SM, Xu D, Roschke V, Perry JW, Arkfeld DG, Ehresmann GR et al. Local production of B lymphocyte stimulator protein and APRIL in arthritic joints of patients with inflammatory arthritis Arthritis Rheum 2003;48(4):982-92.

148. Edwards JC, Leandro MJ, Cambridge G. B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders. Biochem Soc Trans 2002;30:824-8

149. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 2006;54:2793-2806

150. Gudmundsson S, Steinsson K. Systemic lupus Erythematosus in Iceland 1975 through 1984. A nationwide epidemiological study in an unselected population. J Rheumatol 1990;17:1162-7

151. Nossent HC. Systemic lupus erythematosus in the Arctic region of Norway. J Rheumatol 2001;28:539-46

152. Nilsson AC, Spetz CL, Carsjö K, Nightingale R, Smedby A. Slutenvårdsregistrets tillförlitlighet. Diagnosuppgifterna bättre än sitt rykte (The reliability of the Hospital Discharge Register. The data on the discharge diagnosis more accurate than their rumours) Läkartidningen 1994; 91:598-605. In Swedish