Associations Between Rheumatoid Arthritis and Malignant Lymphomas

EVA BAECKLUND
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Abstract

Patients with rheumatoid arthritis (RA) are at increased risk of developing malignant lymphoma, although details about this association remain unclear. The aims of this thesis were to investigate risk factors for lymphoma in patients with RA and to characterize these lymphomas regarding subtype, presence of Epstein-Barr virus (EBV), clinical manifestations and prognosis.

The Swedish hospital discharge register and the cancer register were used to identify RA patients with lymphoma. Two case-control studies were performed, one smaller including RA patients with lymphoma hospitalised in Uppsala health care region 1964-1993 (n=41) and one larger study of hospitalised RA patients with lymphoma in Sweden 1964-1995 (n=378). RA patients from the same cohorts, but without lymphoma, were matched as controls. Medical records for cases and controls were scrutinized for exposure information. The lymphoma tissues were reclassified according to the WHO classification, and presence of EBV was analysed by EBER in situ hybridisation.

The most important risk factor for lymphoma development was high RA disease activity. No association was determined between treatment with traditional disease modifying drugs, non-steroidal anti-inflammatory drugs, aspirin, peroral and intra-articular corticosteroids and lymphoma risk. Diffuse large B-cell lymphoma (DLBCL) was more frequent in RA patients than in lymphoma patients in the general population and displayed stronger association with RA disease activity than other lymphoma subtypes. RA patients with DLBCL had increased extranodal involvement and more advanced lymphoma stage at presentation than DLBCL patients in general, and the prognosis was poor.

A further subdivision of DLBCL into germinal centre (GC) and non-GC subtypes by the expression patterns of CD10, bcl-6 and IRF-4 showed a predominance of the non-GC subtype. This suggested peripheral activated B-cells as the cells of origin in these lymphomas.

The presence of EBV was low in lymphomas in RA patients (12%).

Keywords: rheumatoid arthritis, malignant lymphoma, diffuse large B-cell lymphoma, disease activity, disease modifying anti-rheumatic drug, Epstein-Barr virus, germinal centre-like/non-germinal centre-like subtype

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List of papers

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


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<td>ACR</td>
<td>American College of Rheumatology</td>
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<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CLL</td>
<td>chronic lymphocytic leukaemia</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>DAS</td>
<td>disease activity score</td>
</tr>
<tr>
<td>DMARD</td>
<td>disease modifying antirheumatic drug</td>
</tr>
<tr>
<td>DLBCL</td>
<td>diffuse large B-cell lymphoma</td>
</tr>
<tr>
<td>GC</td>
<td>germinal centre</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IPI</td>
<td>International Prognostic Index</td>
</tr>
<tr>
<td>MALT</td>
<td>extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>PTLD</td>
<td>post-transplant lymphoproliferative disorder</td>
</tr>
<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
</tr>
<tr>
<td>RAAD</td>
<td>rheumatoid arthritis articular damage (score)</td>
</tr>
<tr>
<td>TNF-alfa</td>
<td>tumour necrosis factor-alfa</td>
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Introduction

This work investigated different risk factors for lymphoma development in patients with rheumatoid arthritis (RA) and some of the characteristics of these lymphomas.

RA and lymphoma are two common diseases or disease entities that each affects many people worldwide. Patients with RA are at increased risk of developing lymphoma, but details about this association, including the cause and the features of these lymphomas, remain unclear.

The four papers in this thesis include two case-control studies of risk factors for lymphoma, one smaller “pilot” study (Paper I) and one larger (Paper III). Papers II and IV focus on the characteristics of the lymphomas.

Just as rheumatologists, pathologists and oncologists all are involved in the care and treatment of RA patients with lymphoma, this thesis is the result of cooperation between different fields of research; epidemiology, rheumatology, pathology and oncology. For readers, more specialized in a particular field and maybe not so familiar with both RA and malignant lymphomas, an introduction to the two diseases will be presented in the following sections.

Rheumatoid arthritis

Classification

RA is a chronic, systemic, inflammatory disease, characterized by symmetrical arthritis in peripheral joints (1). There is no single clinical manifestation or test to establish a diagnosis of RA, instead the classification is based on sets of criteria representing symptoms and findings that are common in RA patients. The first criteria for the classification of RA proposed in 1956 (2) have been followed by several revisions, the most recent from 1987 (3). These criteria, the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA (Table 1), have since been used to define RA in most scientific investigations, including those presented in this thesis.
Table 1. The 1987 American College of Rheumatology criteria for the classification of RA.

<table>
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<th>Criteria</th>
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<tr>
<td>1. Morning stiffness</td>
</tr>
<tr>
<td>2. Arthritis in three or more joint areas</td>
</tr>
<tr>
<td>3. Arthritis of hand joints</td>
</tr>
<tr>
<td>4. Symmetric arthritis</td>
</tr>
<tr>
<td>5. Rheumatoid nodules</td>
</tr>
<tr>
<td>6. Rheumatoid factor</td>
</tr>
<tr>
<td>7. Radiographic changes</td>
</tr>
</tbody>
</table>

Patients fulfilling 4 or more criteria for at least 6 weeks are classified as having RA.

Epidemiology

RA is a common disease with worldwide distribution. The estimated prevalence in Europe and the USA is between 0.5-1.0% (4-6) with an annual incidence of 25-50/100 000 (4, 7, 8). The disease can occur at any age, but incidence increases with age; the peak incidence is between the fifth and sixth decades and prevalence is 2.5 times higher in women than in men.

Some studies (5, 9) have indicated a decrease in the incidence of RA over time and a shift towards a higher mean age at onset (5). It has been suggested (9, 10) that time trends in incidence could be due to different susceptibility to RA in certain birth cohorts.

Disease course

For many years, RA was considered a benign disorder, in which most patients did well; however, this view has changed. A number of studies have shown that in most patients RA is a progressive disease causing joint damage, disability, and marked psychological and socio-economic decline (11). In patients with severe forms of the disease, life-expectancy is reduced by 10-15 years, predominately from cardiovascular mortality, compared with the general population (9, 11-13). The severity of RA encompasses a wide spectrum. In some patients the disease is limited to a small number of joints and does not lead to significant impairment, whereas others may have severe involvement of many joints, as well as systemic and extra-articular manifestations of the disease e.g. vasculitis, rheumatoid nodules, Felty’s syndrome, and secondary Sjögren’s syndrome. The varying clinical pictures of this disease have led to proposals that the current classification of RA actually includes more than one disease entity.
The proportion of RA patients with mild versus severe RA is unclear, but in a study of patients with early RA who were followed for 6 years, three patterns of clinical course were described (14). About 20% of the patients experienced a mild disease with long remissions of disease activity, 70% had intermittent or continuing disease activity with incomplete remissions and moderate progression over time, and in 10% of patients a progressive pattern with severe joint involvement was seen. During 10 years of follow-up in another study of patients with early RA diagnosed between 1985 and 1989 (15), 21% of the patients had continuously active disease, and after 10 years, 6% of patients had severe problems with daily life activities (Steinbrocker class III-IV [16]). During the course of RA, the individual patient may experience fluctuating disease activity; disease flares may be followed by periods with inactivity of the disease (17, 18).

Factors that predict a more severe, persistent disease course and also premature mortality include the presence of anti-citrullinated protein antibodies (19, 20), rheumatoid factor (20), extra-articular manifestations (21) and the human leukocyte antigen (HLA)-DR4 haplotype (22).

Measures of disease activity and outcome

There is no single standard measure to assess and monitor the clinical status and outcome in patients with RA. Instead, there are a number of available measures, used depending on purpose and which aspects of the RA disease that are being addressed (23).

For assessment of disease activity, the most common measures are tender and/or swollen joint counts, erythrocyte sedimentation rate (ESR) and C-reactive protein values, and global assessments of disease activity by patient and/or physician. Different joint counts include different joints, but reduced joint count of e.g. 16 joints/joint groups has been shown to be as sensitive in assessing disease activity as counts including more joints (23). The combination of tender and swollen joints is more accurate in assessing inflammation than a swollen or a tender joint count (23). ESR has been the most widely used laboratory test to assess inflammation, but is not always elevated in RA. For example, in a recent cross-sectional evaluation of consecutive patients, 75% with longstanding RA and 50% with early RA had an ESR value of less than 28 (24). A number of indices combining different disease activity measures have been introduced and now e.g. the disease activity score (DAS) (25) is widely used in clinical trials and investigations of RA, and to some extent in clinical practice. DAS consists of swollen joint count, tender joint count, acute-phase reactant, and patient’s assessment of global status.

Joint damage is assessed by radiographic scores (26), as well as clinical scores of joint deformity, e.g. the rheumatoid arthritis articular damage
The number of deformed joints is highly correlated to radiological damage (28).

Introduced in 1949, the Steinbrocker functional class became a widely used and simple method for classifying functional capacity (16). One limitation of these criteria is that in practice few patients fall in classes I or IV. The criteria were revised in 1991 (29), although latterly, patient self-report questionnaires, such as the health assessment questionnaire (HAQ), have become the most commonly used measure of functional status.

Table 2. **Steinbrocker functional class**

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Ability to carry on full functional activity without handicaps.</td>
</tr>
<tr>
<td>II</td>
<td>Functional activity adequate to carry out normal activity with discomfort or limited mobility of one or more joints.</td>
</tr>
<tr>
<td>III</td>
<td>Functional activity adequate to perform little or none of the duties of activity of occupation or self-care.</td>
</tr>
<tr>
<td>IV</td>
<td>Largely or wholly incapacitated with the patient bed-ridden or wheelchair-bound.</td>
</tr>
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</table>

In everyday clinical practice, and in contrast to clinical trials, only a limited number of measures are generally used and documented.

**Care and treatment of patients with RA**

Since the 1950s, the care and treatment of patients with RA have changed dramatically (30, 31). As this change has affected the patients included in the works of this thesis, a short historical summary is justified.

Developed in 1899, aspirin was the most important medical treatment for RA in the first half of the 20th century; this was later followed by the non-steroidal anti-inflammatory drugs (NSAIDs). The first NSAID, phenylbutazone, was introduced in 1949.

Important progress in the medical treatment of RA was the discovery of the efficacy of the gold salts in 1927 and the introduction of cortisone in 1949. After gold salts, other disease-modifying anti-rheumatic drugs (DMARDs) followed. Sulfasalazine was synthesized in 1938, but although suggested to be effective in RA, the drug was not widely used in this disease until the 1980s. The first positive effects of antimalarials in RA/chronic polyarthritis was described in 1951, azathioprine was first used in RA in the
early 1960s, and Prorecid in 1969. In 1970, cyclophosphamide was introduced for the treatment of severe RA. D-penicillamine was formally shown to be effective in RA in 1973 and the first study of cyclosporine as a treatment for RA was reported in 1979. Methotrexate, shown to be effective in RA in 1951, was not formally approved for the treatment of RA in USA until 1987 and was not in general use in Sweden until the early 1990s.

Although drugs that could potentially change the course of RA were available, the preferred therapeutic approach was for a long period the so-called therapeutic pyramid, as suggested in a textbook of rheumatology as late as 1993 (32). The foundation of the pyramid was rest, physical therapy, heat and education combined with high-dose salicylates and/or NSAIDs. If necessary, DMARD therapy could be added, with less toxic drugs (antimalarials) used first, and if response was unsatisfactory, more effective, but less well-tolerated drugs were used later. During the 1980s and 1990s, the therapeutic strategy gradually changed towards use of effective DMARDs as early as possible after onset of the disease.

In 1915 in Sweden, the state (pensionsstyrelsen) started the first institutions specifically aimed at patients with “threatening disability”, later followed by hospitals specialized in rheumatic diseases. RA patients were commonly referred to these institutions for long and recurrent periods. The documentation of treatments (drugs, baths, plaster, rest, electrotherapies, vitamins, surgery etc), joint status and ESR values was often detailed (33).

An important hallmark in the history of RA was also the discovery of the rheumatoid factor, analysed by different methods since the 1940s.

The advances in RA therapy appear to have improved short-time outcome of RA, but despite treatment, a large proportion of patients in follow-up studies still continue to have active disease (34).

Etiology and pathogenesis of RA

The primary cause of RA is still unknown, but there is evidence that both genetic factors and the environment play important roles in the etiology of this disease (35, 36). It has been suggested that there could be, not one but several agents or events that may initiate an aberrant immune response in genetically predisposed individuals leading to the synovial inflammation typical of RA.

Genetic risk factors, which may account for 50-60% of the population liability to RA (37), also correlate with disease severity and phenotype. RA is strongly associated with HLA-type, in particular HLA-DRB1 variants, but many different genes appear to contribute to disease susceptibility and RA may include several genetically different subsets (35).

Some non-genetic factors of importance in RA etiology have been identified (35, 36). Hormonal factors appear to be of importance as illustrated by
the influence of pregnancy on RA disease activity (4, 35). Increasing age and its effects on the immune system is an important variable in RA (38), and smoking has emerged as a major environmental risk factor (39, 40). Various bacteria and viruses have been proposed as potential triggers of the disease, but there is no consistent evidence for an infectious cause (4, 35).

Although no exogenous antigens or autoantigens have been recognized in the synovial tissue that trigger the disease, many factors involved in the disease pathogenesis have been identified (36, 41), and various therapeutic strategies have been developed to target one or more of these factors. Important factors in RA pathogenesis include disease-causing immune cells (T- and B-lymphocytes, macrophages, neutrophils), a broad array of cytokines (e.g. tumour necrosis factor (TNF)-alfa and Il-1), signal transduction factors (e.g. NF-kappaB, STAT1), defects in apoptosis of cells involved in RA inflammation, and a specific role of rheumatoid fibroblasts.

The role of T-cells in the pathogenesis of RA is well established based on for example the prominent infiltration of T-cells in the rheumatoid synovium and the strong association with HLA-DR4, of which the main function is to present antigenic peptides to T-cells (42).

In the past years, it has been shown that B-cells also have many potential key roles in the pathogenesis of RA (43). They are precursors of plasma cells, the cells which produce rheumatoid factors and other auto-antibodies, secrete co-stimulatory molecules and pro-inflammatory cytokines, including TNF-alfa, and act as efficient antigen-presenting cells. Evidence suggests that T-cell activation in rheumatoid synovium is B-cell dependent (44).

There is also evidence of both T- and B-cell activation by (unknown) antigens and oligoclonal cell expansions, indicating antigen-specific responses, have been found in synovium, and in peripheral blood in RA patients (45-47).

A number of other factors involved in the complex RA pathogenesis have also been identified (41). The various factors may play their roles during different phases of the disease and are probably not equally important in all patients or subgroups of RA patients. Consequently, one important task is to identify subgroups of RA patients that will benefit from a specific therapy. To target therapy of the right factors at the right time in the disease in the right patient, could improve outcome and prognosis of RA.

Malignant lymphomas

Lymphomas comprise a heterogeneous group of malignancies derived from cells in the immune system. This is a brief survey of some aspects of lymphomas of relevance to the work of this thesis.
Classification

The classification of lymphomas has changed over the years. The first classifications relied entirely on the histological appearance of the tumors stained with eosin and haematoxylin, but along with advances in technology, genetics and immunology, new classifications schemes have been introduced (48). Among the most well-recognized classifications are the Willis classification introduced in 1948, the Rappaport classification in 1966, the Kiel and Lukes-Collins classifications in 1974, the Working Formulation in 1982 and the Revised European-American lymphoma classification system (REAL) from 1994 (48). The different sets of lymphoma criteria have made comparison of lymphoma studies conducted at different times and in different countries difficult.

In the 1960s, the period when the first lymphoma patients included in the works of this thesis were diagnosed, Swedish pathologists recognized four different lymphoma subtypes: lymphosarcoma, reticulum cell sarcoma, Brill-Symmers disease and malignant lymphogranulomatosis/Hodgkin’s disease. This may be compared with the current lymphoma classification, the WHO classification of tumours of haematopoietic and lymphoid tissues (49), introduced in 2001 and now in worldwide use. In this classification, about 40 distinct disease entities are described based on a constellation of features, including histological appearance, immune phenotype, genetic abnormalities, and clinical features. For each neoplasm, a cell of origin is postulated. The three main categories of lymphoid neoplasms in this classification are B-cell neoplasms, T- and NK-cell neoplasms, and Hodgkin lymphoma (HL). As some of the entities in the WHO classification are still heterogeneous, application of newer analyses such as microarray technology will likely continue to introduce modifications and improvements in lymphoma classification.

Epidemiology

In Sweden in 2003, about 2100 new lymphomas were diagnosed, making lymphoma the eighth most frequently diagnosed malignancy among males and the tenth most common in females in the Swedish population (50). In the same year, the total incidences in Sweden for non-Hodgkin lymphomas (NHL i.e. the B- and T/NK-cell lymphomas grouped together) were 17.6 per 100,000 population in males and 13.7 per 100,000 in females. The incidence of HL, consistently lower than that of NHL, was 1.9/100,000 in men and 1.8/100,000 in women (50). The incidence of NHL increases with age and is about 50% higher in men than women.

NHL and HL are more common in developed countries, and the highest incidence rates are reported from the USA, Europe, Australia and New Zealand. In most parts of the world, the B-cell phenotype is dominant (80%),
whereas T/NK-cell neoplasms and HL each constitute approximately 10% of all lymphomas (49). The occurrence of NHL subtypes varies with geographic area (51). The most common NHL subtypes in developed countries are diffuse large B-cell lymphoma (DLBCL) (30-40%) and follicular lymphoma (20-30%) (52). The most recent data on NHL subtypes in Scandinavia derives from the Scandinavian Lymphoma Etiology (SCALE) study comprising more than 80% of incident lymphomas (chronic lymphocytic leukaemia [CLL] included) occurring in people aged 18-74 years in Sweden and Denmark between late 1999 and 2002 (53). Of all NHLs (n=3055), 26% were DLBCLs and 19% were follicular lymphomas; the corresponding figures excluding CLL cases are 34% for DLBCL and 25% for follicular lymphoma.

Since the middle of the 20th century, time trends reveal a considerable increase in NHL incidence in most countries, including Sweden (2-4% annually, with a levelling out of the increase from the 1990s (54, 55). The increase is seen across all age groups and in both sexes. Among lymphoma subtypes, DLBCL has seen the largest increase. The reason(s) for this increase is unknown. In contrast to NHL, the overall incidence of HL is slightly declining (49, 50).

Clinical aspects and prognosis

Lymphoma was an almost uniformly fatal disease early in the 20th century (56). In patients with localized disease, the use of radiotherapy produced long-term survival only on rare occasions. However, the introduction of combination chemotherapy in the early 1970s led to the recognition that some patients could be cured, even when widespread disease was present. Now, a broad distinction between aggressive or high-grade lymphomas and indolent or low-grade lymphomas is commonly used for deciding treatment approach. In localized low-grade NHL and in patients with high-grade NHL the goal of therapy is cure. Patients with advanced low-grade NHL are considered incurable and the goal of therapy in these patients is to attain good response rates and delay relapse. In up to 60% of patients with low-grade NHL, the disease eventually undergoes transformation to a higher-grade histology. Prognosis in these patients is worse than for patients presenting de novo with high-grade lymphoma. Examples of high-grade lymphomas are DLBCL, Burkitt lymphoma, follicular lymphoma grade 3 and several T-cell lymphomas such as anaplastic large cell lymphoma, peripheral T-cell lymphoma and angioimmunoblastic lymphoma. Low-grade lymphomas include: follicular lymphomas grades 1 and 2, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), small lymphocytic lymphoma and its leukaemic counterpart CLL, and lymphoplasmacytic lymphoma.
To decide the degree to which the lymphoma has spread within the body, the Ann Arbor staging system, modified in Cotswald 1988 (57), is still used for both NHL and HL, and is presented in the table below. Stages I and II are usually defined as localized disease and stages III and IV as widespread disease.

Table 3. Ann Arbor staging classification of lymphomas, modified in Cotswold 1988.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Involvement</th>
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<tbody>
<tr>
<td>I</td>
<td>Single lymph node group</td>
</tr>
<tr>
<td>II</td>
<td>Two or more lymph node groups on same side of diaphragm</td>
</tr>
<tr>
<td>III</td>
<td>Lymph node groups on both sides of diaphragm</td>
</tr>
<tr>
<td>IV</td>
<td>Disseminated extranodal involvement</td>
</tr>
</tbody>
</table>

Stages are further divided into A: No B-symptoms, B: B-symptoms: unexplained weight loss >10% in 6 months, unexplained fever >38ºC, night sweats, E: Involvement of a single extranodal site, or contiguous or proximal to known nodal site, X: Bulky disease: tumour >10cm

A number of general factors associated with poor lymphoma prognosis have been identified. In clinical practice, the system most commonly used to predict survival in lymphoma patients is the International Prognostic Index (IPI) score (58), which assesses prognosis on the basis of five factors: the pre-treatment serum level of lactate dehydrogenase (LDH), patient’s age at presentation, Ann Arbor stage, number of extranodal sites and performance status. An adverse prognosis is indicated by age over 60 years, Ann Arbor stages III and IV, elevated LDH levels, the involvement of more than two extranodal sites and a performance status ≥2 Eastern Cooperative Oncology group or equivalent.

Lymphomas can present in many different ways depending on the site of involvement, natural history of the lymphoma subtype, and presence or absence of B-symptoms (59). Virtually any organ in the body can be affected and accordingly, patients can present with ‘lumps’ almost anywhere. Approximately a quarter of NHL patient presents with disease at an extranodal site (i.e. sites other than lymph nodes, spleen, Waldeyer’s ring and thymus): the main site being the gastrointestinal tract. Indolent lymphomas frequently present with peripheral lymphadenopathy, which generally waxes and wanes over time and may be asymptomatic unless it causes compression. With aggressive lymphomas, rapid tumour growth takes place and patients might quickly become very ill.
In general, the 5-year overall survival following NHL is about 40% (60), with the most favourable prognosis in low-grade lymphomas such as follicular lymphoma and MALT lymphoma (5-year overall survival >70%) and the shortest 5-year overall survival in subtypes such as mantle cell lymphoma and peripheral T-cell lymphoma (<30%). Prognosis is somewhat better in women than in men. For HL, the 5-year overall survival has improved and is now approximately 80% (49).

Currently, many attempts are being made to identify subgroups of patients who may benefit from specific treatment-strategies; these attempts include combinations of new genetic and immuno-markers with the IPI score (61).

Diffuse large B-cell lymphoma

DLBCL, the lymphoma subtype of specific interest in Papers II, III and IV, is so named as the tumour cells are large blasts with a nuclear size of more than twice that of a normal lymphocyte that diffusely invade the normal architecture of the tumour site. DLBCLs typically express pan-B-cell markers such as CD19, CD20, CD22 and CD79a and are divided into different morphologic variants, the most common being centroblastic, immunoblastic, T-cell rich, and anaplastic (49). In most studies, morphologic subtype is not linked to prognosis. DLBCL usually arises de novo, but may also occur by transformation of a previous low-grade lymphoma.

DLBCL incidence increases with age and the median age at diagnosis is around 65 years, and is slightly more common in men than in women (49). The most common clinical presentation of DLBCL is a rapidly enlarging mass at a nodal or extranodal site. At presentation, approximately 40% of patients have extranodal disease (62) and 25-30% have disseminated disease at Ann Arbor stage IV (60). Patients with DLBCL have highly variable clinical courses and less than 50% of patients are cured or receive a durable remission with chemotherapy (63, 64). Since the 1970s, the standard treatment for DLBCL has been different variants of the CHOP-regimen i.e. cyclophosphamide, Adriamycin, vincristine, and prednisone. Radiotherapy has also been part of the treatment, but it is only recently that new treatment modalities such as high-dose chemotherapy with autologous stem cell transplantation and anti-CD20-therapy have been introduced. In different studies, the 5-year overall survival in DLBCL has ranged between 35 and 50% (63).

In 2000, gene expression profiling of DLBCL demonstrated that this lymphoma is composed of at least two different subtypes (65), characterized by different cells of origin and prognosis (65, 66). One type has an expression profile characteristic of germinal centre (GC) B-cells (GC B-like DLBCL), whereas the other type, associated with the worst prognosis, has a profile
similar to that of in vitro activated peripheral blood B-cells (activated B-cell (ABC)-like DLBCL). As the gene expression profiling technique requires fresh or frozen lymphoma tissue and is still not generally available, alternative ways of identifying the different DLBCL subtypes have been investigated. Immunohistochemistry via the expression patterns of CD10, bcl-6 (GC-markers) and IRF-4 (marker of later stages of B-cell development) is reported to determine the GC and ABC subtypes of DLBCL and even improve survival prediction compared to gene expression profiling (67, 68). Expression profiling has also indicated a poorly characterized type 3-DLBCL entity, and this subset has been grouped together with the ABC-DLBCLs in studies using immunostainings, as patients in these entities have similar outcomes. Together these two types are called non-GC DLBCL. In lymphomas in the general population, about 50% of DLBCL appears to be of the GC subtype and 50% of the non-GC type (65-68). The GC-like DLBCL patients have superior outcome with an estimated 5-year survival rate of 60-75%, compared to around 35% for the non-GC subtype (65-68).

Risk factors for lymphoma

There are relatively few clearly identified risk factors for lymphomas, and in most cases, the cause is unknown (69, 70). The following section summarizes some of the postulated risk factors for the development of lymphoma.

Immunosuppression

The strongest known risk factors for NHL are different states of primary and secondary immunodeficiency (71). The greatest NHL risk is seen in association with acquired immunodeficiency syndrome (AIDS), which carries a 100-300-fold increase in risk compared to the general population (72). These lymphomas are typically B-cell lymphomas with high-grade histology including DLBCL and Burkitt lymphomas, which frequently occur in extranodal sites. Lymphoma localized in the brain is common. Epstein-Barr virus (EBV) and human herpes virus 8 are found in about 50% of AIDS-related lymphomas (73).

Organ and bone marrow transplantation have consistently been associated with markedly increased risks of lymphoproliferative malignancies (74). Post-transplant lymphoproliferative disorder (PTLD) is described as a heterogeneous group of abnormal lymphoid proliferations, generally of B-cells that occur in the setting of ineffective T-cell function because of pharmacological immunosuppression after organ transplantation. Characteristic of PTLD is extranodal involvement, aggressive clinical course and poor outcome. The association with EBV infection is strong, as manifested by the presence of
EBV within the malignant tissue in about 80% of cases. EBV-positive cases tend to occur early after transplantation with peak incidence three to six months post-transplant.

It has been suggested that EBV-negative PTLD, arising at a median of 50 months post-transplant should be considered as a distinct subgroup of PTLD characterized by more malignant-appearing histology, and worse outcome than EBV-positive cases (75). In the largest survey to date of lymphomas after solid organ transplantation, outcome was, however, poor regardless of time of appearance after transplantation (76). The type of transplanted organ strongly influences the risk of lymphoma. In the large transplantation-lymphoma study mentioned above (76), relative risk for lymphoma in heart-lung transplants during the first five years increased 240-fold, in contrast to the 12-fold increased risk seen in renal transplant recipients. This has been explained by the more intense immunosuppression in cardiopulmonary organ recipients than in renal transplants.

In patients with primary, congenital immunodeficiency, e.g. Wiskott-Aldrich syndrome, X-linked lymphoproliferative syndrome, and combined immunodeficiency, up to 25% of patients develop lymphomas (77). Although high-grade B-cell lymphomas predominate, a wide variety of lymphoma subtypes has been reported. EBV seems to be less frequent (overall about 30%) than in AIDS- and post-transplant lymphomas and the frequency, as well as the lymphoma subtype appears to vary with type of underlying immune disorder.

**Infectious agents**

**Epstein-Barr virus**

EBV, a herpes virus with growth transforming and oncogenic capacity, has been linked to lymphoma development, in particular in immunocompromised hosts (78). More than 90% of the human population worldwide is infected by EBV, and after the primary infection, the virus persists in B-cells as a lifelong, and usually harmless latent infection. In healthy individuals, the development of malignant clones of infected B-cells is prevented by the immune system by in particular EBV-specific cytotoxic T-cells. In situations of immunosuppression, the normal control of EBV-infected cells may be impaired resulting in an uncontrolled growth of the EBV-infected cells. EBV is also found in lymphomas in immunocompetent hosts e.g. in African Burkitt lymphomas, some unusual types of T-cell lymphomas and in HL.

Apart from associations with lymphoma subtype, the presence of EBV in lymphomas may also vary with other factors such as gender, age and geographic location. In HL, EBV is more frequent in the mixed-cellularity subtype (32-96%) than in the other subtypes, e.g. nodular sclerosis HL (10-50%).
DLBCL in AIDS patients harbour EBV infection in about 80% of cases and lymphomas with early onset after transplantation are almost all EBV-positive.

Other infectious agents
Infectious agents other than human immunodeficiency virus (HIV) and EBV are also connected with lymphomas, although only a small number of infected people ever develop e.g. the virus-induced malignancy (79, 80). Human herpes virus 8 is associated with Kaposi’s sarcoma and primary effusion lymphoma, T-cell lymphotropic virus-1 is causally linked to adult T-cell leukaemia/lymphoma. An association between hepatitis C virus and NHL has also been determined. Chronic immune stimulation by Helicobacter pylori is an etiologic factor for gastric MALT lymphoma, infection with Borrelia burgdorferi is associated with the development of primary cutaneous B-cell lymphoma, and Chlamydia psittaci is associated with MALT lymphoma in ocular adnexa.

Family history
A family history of lymphoma or leukaemia has been associated with a 2-4-fold increased risk of developing lymphoma of different subtypes (81, 82). This suggests that the same gene may be linked to the development of more than one type of lymphoid malignancy, although, no specific genes explaining this association have been identified. Lymphomas may also cluster within families, not because of inherited susceptibility, but because of shared environmental factors or perhaps an increased susceptibility to such factors. In one study, alcohol use was associated with an increased risk of NHL, but only among men with a family history of haematopoietic malignancy (83).

Medical conditions and treatment
Increased risk of developing lymphoma has been reported among persons with a variety of autoimmune and inflammatory conditions, and in fact, in most of the diseases seen by rheumatologists there are indications of increased lymphoma risks. Increased risks have been reported in RA (84), cryoglobulinaemia (85), celiac disease (86), chronic gastritis, dermatitis herpetiformis (86), diabetes, inflammatory bowel disease (87), primary Sjögren’s syndrome (88), poly- and dermatomyositis (89), sarcoidosis (90), systemic lupus erythematosus (91), systemic sclerosis (92), thyroiditis (93), and Wegener’s granulomatosis (94). There are no studies of lymphoma risk in psoriatic arthritis, but in patients with psoriasis, several studies have reported increased risks (95). Cancer incidence in ankylosing spondylitis has been reported in only one large population-based study, and in this study, lymphoma risk was not significantly increased (96).
The magnitude of the reported risks has varied, and in some diseases, results have been inconsistent. In general, large population-based studies have shown lower risks than smaller studies of selected groups of patients. Lymphoma risk in most studies has been assessed for lymphoma overall, or “neoplasms of the immune system” overall, and not by the separate lymphoma subtypes. Many studies are also based on few cases contributing to low statistical power and uncertainty over the results. The underlying cause or causes of increased lymphoma risk in most of the disorders have not been studied in detail, and therefore, remain unclear.

One disorder that has been studied in more detail in connection with lymphoma risk is primary Sjögren’s syndrome; this was one of the first recognized associations between an autoimmune disease and lymphoma, and the risk initially reported was very high. In the first study estimating the magnitude of the risk, a 44-fold increased risk for NHL compared with the general population was determined in patients with sicca syndrome who were referred to a university clinic (88). Three of the seven cases had sicca syndrome secondary to RA. In subsequent studies of lymphoma risk restricted to patients with primary Sjögren’s syndrome, increases in relative risk have varied between 9 and 13, and although these risk estimates are lower than initially reported, lymphoma remains an important complication in patients with primary Sjögren’s syndrome (97). Lower risks have been reported in patients with Sjögren’s syndrome secondary to RA (98) and other rheumatic diseases. MALT lymphoma located in the salivary glands appears over represented in primary Sjögren’s syndrome, but a number of other NHL subtypes have also been reported (99), but are in general of low-grade type and prognosis is relatively good.

RA is the other rheumatic disease, which has been subject to many case-reports and epidemiological studies of lymphoma risk. Associations between RA and lymphoma will be discussed in more detail below.

A number of drugs have been investigated in relation to lymphoma risk. Chemotherapy for malignant diseases appears to be followed by an increased risk of NHL (100, 101), but for most other common medications, the existing literature concerning lymphoma risk is conflicting. Medication used in RA will be discussed in more detail later, but use of antibiotics, histamine2-receptor antagonists, psychotropic drugs, anti-convulsants, oestrogen replacement therapy, antidepressants, anti-anxiety drugs, amphetamines, and digitalis have been associated with increased, as well as no or even decreased risks of lymphoma (102-105). A common limitation in these studies is that the underlying disorder that the drug was intended to treat is unknown.

**Lifestyle factors**

A number of occupational exposures and lifestyle factors have been investigated in the etiology of lymphomas, and in particular factors that have
changed in parallel with the rise in NHL incidence (69). In conclusion, the evidence is inconclusive in most cases.

**Diet, vitamins, alcohol and cigarette smoking**

Intake of dairy products and red meat has been associated with small increased risks of NHL, whereas fruit and vegetables consumption has been reported to reduce NHL risks (106). Results from different studies are, however, inconsistent. Long-term, regular use of vitamin supplements appears to have no association with the risk of NHL (107). The role of alcohol in the development of NHL is unclear; most studies have shown no or decreased lymphoma risks associated with alcohol consumption (108, 109). Cigarette smoking appears to have no or weak association with NHL (109). A recent pooled analysis of nine case-control studies of NHL determined an increased risk for follicular NHL subtype with smoking, but not with other lymphoma subtypes (110).

**Blood transfusion**

Epidemiological evidence for an association between blood transfusion and NHL is weak (111). Early studies, which were less detailed and enrolled fewer cases than studies that are more recent, showed moderately elevated risks, whereas subsequent studies have not been able to confirm this association.

**Anthropometric measures, physical activity and stress**

Although several studies have shown increased lymphoma risks linked to obesity, most studies have not. In a recent study, a weak but statistically significant association was seen between high body mass index and increased risk of DLBCL (112), but not with lymphoma in general.

A number of immune changes are demonstrated in association with physical activity, but no studies have reported increased lymphoma risks linked to physical activity or exercise (113). In one study of former female athletes risks for some types of cancer including haematopoietic malignancies were decreased (114).

Stress-induced immune-modulation (115) and chronic widespread body pain (116) have been reported to influence the incidence or progression of some malignant diseases. However, it is unknown whether stress and pain may influence the risk or course of lymphoma.
Occupational exposures
Few consistent associations with occupations have been reported and the risks that have emerged have been small. Exposure to pesticides, herbicides, and insecticides has been associated with increased risks for NHL, but not consistent (117). In particular, a link between pesticide use and NHL has been proposed many times but remains unproven, possibly because the agents involved have been grouped into classes rather than considered as separate substances in most studies.

Ionising radiation and ultraviolet light exposure
There is little to suggest increased risk of lymphoma in association with ionising radiation, either in individuals with therapeutic, diagnostic (118, 119) or occupational exposure (69). An increased risk of multiple myeloma has, however, been reported among patients who were frequently exposed to X-rays (118). Ultraviolet radiation has been hypothesized as a risk factor for NHL, but contrary to previous beliefs, two recent case-control studies have found strong epidemiological evidence that a history of high ultraviolet radiation exposure is associated with reduced risk of NHL (120, 53).

Aspects on lymphoma pathogenesis
As with all malignant diseases, lymphomas are clonal expansions from normal cells that have transformed and acquired the ability to outgrow its neighbours (121). Lymphomagenesis is a multi-step process in which a succession of genetic changes leads to the progressive conversion of a normal lymphoid cell into a cancer cell. This accumulation of genetic lesions may extend over several years and even decades. The various genetic lesions that occur may disrupt normal lymphoid homeostasis in different ways: by enhancing cell growth and proliferation, by preventing the normal induction of cell death and by blocking the terminal cell differentiation (122).

The B-cell neoplasms can derive from any stage of the B-cell differentiation and, depending on the stage of differentiation of the original cell, tumour subtypes with unique characteristics are formed. Accordingly, a transformation in an early pre-B-cell gives rise to acute lymphatic leukaemia, and a plasma cell transformation to a plasma cell myeloma. The B-cell genome is particularly vulnerable during the rearrangement of the immunoglobulin genes of the immature B-cell in the bone marrow and during the response of a mature B-cell to antigen, which takes place in the germinal centre of the lymphoid tissue (123). These processes involve double-stranded DNA breaks and
occasionally these breaks are resolved aberrantly, leading to chromosomal translocations.

Based on the epidemiologic studies of risk factors for lymphoma development some specific biologic mechanisms causing the genetic lesions leading to NHL have been suggested. Chronic antigenic stimulation could increase B-cell proliferation, which in turn would increase the probability of a random genetic mistake. Oncogenic viruses may infect a normal cell and turn it into a malignant cell capable of self-replication by integration of viral DNA in the host genome. Environmental carcinogens can cause mutations leading to disturbed cell growth and regulation. Even if biological mechanisms involving antigenic stimulation, oncogenic viruses and environmental factors are suggested, it is clear, that few such factors are identified, and that the cause of NHL remains largely unknown.

Rheumatoid arthritis and lymphomas

Epidemiological associations

RA has been associated with increased lymphoma risk in a number of studies, the first larger one published in the 1970s (84). The magnitude of increased risk has varied, but differences in study design, selection of patients and time of follow-up may be part of the explanation. The highest risk, a 23-fold increased risk for lymphoma compared to the general population, was reported in patients selected from a university rheumatology clinic (124), whereas larger cohort studies consistently have found lower risks and report two- to three-fold increased lymphoma risks in RA patients compared to the general population (Table 4).

The study by Gridley et al., reporting a relative lymphoma risk of 1.98 (131) in patients with RA, encompass the same RA population that was studied in more detail in Paper I in this thesis, and the study by Ekström et al., reporting a relative lymphoma risk of 2.0 (135) includes the study-base of the case-control study in Paper III.
Table 4. Studies of lymphoma risk in patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Author, year ref</th>
<th>RA patients</th>
<th>Time period studied</th>
<th>Malignancy, number of patients</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allebeck, 1982 ¹²⁵</td>
<td>1165</td>
<td>1971 follow-up 1978</td>
<td>cancer in lymphatic and haematopoietic tissue, 5</td>
<td>1.9*</td>
</tr>
<tr>
<td>Prior, 1984 ¹²⁴</td>
<td>489</td>
<td>1964-1978 follow-up 1981</td>
<td>lymphoma, 6</td>
<td>23.0 (8.4-50.1)</td>
</tr>
<tr>
<td>Symmons, 1985 ¹²⁶</td>
<td>See Prior</td>
<td>See Prior follow-up 1983</td>
<td>NHL, 7, HL, 2</td>
<td>24.1, 12.5</td>
</tr>
<tr>
<td>Katusic, 1985 ¹²⁷</td>
<td>521</td>
<td>1950-1974 follow-up 1984</td>
<td>lymphoma, 3</td>
<td>1.2 (0.2-3.4)</td>
</tr>
<tr>
<td>Hakulinen, 1985 ¹²⁸</td>
<td>See Isomäki</td>
<td>See Isomäki follow-up 1978</td>
<td>lymphoma, 57</td>
<td>2.7</td>
</tr>
<tr>
<td>Laakso, 1986 ¹²⁹</td>
<td>1000</td>
<td>1959-1968 follow-up 10 years</td>
<td>cancer in lymphatic and haematopoietic tissue, 10</td>
<td>5*</td>
</tr>
<tr>
<td>Tennis, 1993 ¹³⁰</td>
<td>646</td>
<td>1978-1980 follow-up 1987</td>
<td>lymphoma, 4 and myeloma, 2</td>
<td>4.1 (1.7-9.9)</td>
</tr>
<tr>
<td>Gridley, 1993 ¹³¹</td>
<td>11 683</td>
<td>1965-1983 follow-up 1984</td>
<td>NHL, 36, HL, 12</td>
<td>2.0 (1.5-2.6), 1.9 (1.3-2.6), 2.3 (1.2-4.1)</td>
</tr>
<tr>
<td>Mellernkjaer, 1996 ¹³²</td>
<td>20 699</td>
<td>1977-1987 follow-up 1991</td>
<td>NHL, 85, HL, 14</td>
<td>2.4 (1.9-2.9), 3.4 (1.8-5.6)</td>
</tr>
<tr>
<td>Cibere, 1997 ¹³³</td>
<td>862</td>
<td>1966-1974 follow-up 1995</td>
<td>NHL, 3, HL, 0</td>
<td>0.6 (0.1-1.6)</td>
</tr>
<tr>
<td>Kauppi, 1997 ¹³⁴</td>
<td>9 469</td>
<td>1970-1991 follow-up 1993</td>
<td>NHL, 34, HL, 4</td>
<td>2.2 (1.5-3.1), 2.2 (0.6-5.7)</td>
</tr>
<tr>
<td>Thomas, 2000 ¹³⁵</td>
<td>7 080 males</td>
<td>1981-96 follow-up 1996</td>
<td>NHL, 30, HL, 8</td>
<td>2.4 (1.6-3.4), 5.5 (2.4-10.8)</td>
</tr>
<tr>
<td></td>
<td>19 543 females</td>
<td>1996 follow-up 1996</td>
<td>NHL, 71, HL, 9</td>
<td>2.0 (1.6-2.6), 3.0 (1.4-5.8)</td>
</tr>
<tr>
<td>Ekström, 2003 ¹³⁶</td>
<td>76 527</td>
<td>1964-1999 follow-up 1999</td>
<td>lymphoma, 535</td>
<td>2.0 (1.8-2.2)</td>
</tr>
</tbody>
</table>

* endpoint mortality, NHL=non-Hodgkin lymphoma, HL=Hodgkin lymphoma
Risk factors for lymphoma development in RA

The underlying reason or reasons for the increased lymphoma risk in RA are still incompletely understood, although they have been subject to debate during the last decades.

Multiple potential mechanisms of lymphoma development in this population have been proposed; the main ones are displayed below. The factor that has caused most concern is the treatment used in RA, in particular the role of immunosuppressive therapy and its influence on the risk of lymphoma.

Suggested risk factors for lymphoma development in RA:
1. The treatment used in RA
2. RA disease-related factors
3. Shared genetic predisposition to both RA and lymphoma
4. Environmental factors common to both RA and lymphoma

RA treatments and lymphoma risk

DMARD

During the last decades, numerous case-reports have described RA patients developing lymphoma during treatment with a certain DMARD (136-164). In analytical studies designed to assess the lymphoma risk linked to treatment, results, however, have been conflicting (165). Some studies have looked at the risk of cancer in groups of patients treated with a variety of DMARDs (Table 5), and results indicate increased risks in RA patients treated with DMARDs compared to non-exposed RA patients (166, 167) or the general population (168, 169). However, no specific DMARD has clearly been linked to an increased lymphoma risk (170).
Table 5. Studies of lymphoma risk in DMARD treated patients with rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Author, year ref</th>
<th>No. treated RA patients, type of treatment</th>
<th>Follow-up</th>
<th>No. lymphomas</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirsner, 1982</td>
<td>126, cytotoxic drugs*</td>
<td>4.9 years</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Kinlen, 1985</td>
<td>643, immunosuppressive drugs**</td>
<td>1-12 years</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Mattesson, 1991</td>
<td>530, DMARDs***</td>
<td>mean 30 months</td>
<td>4 lymphoproliferative malignancies</td>
<td>8 (3.3-21)</td>
</tr>
<tr>
<td>Jones, 1996</td>
<td>259, immunosuppressive drugs**</td>
<td>10 years</td>
<td>7 neoplasms of the immune system</td>
<td>7 (0.9-56)</td>
</tr>
</tbody>
</table>

*83% cyclophosphamide, **mainly azathioprine, cyclophosphamide, chlorambucil, *** antimalarials, azathioprine, D-penicillamine, gold, methotrexate

Azathioprine

The risk of lymphoma associated with azathioprine has been investigated, with conflicting results. In one study of 202 RA patients treated with azathioprine, followed for 11 years on average and compared with matched RA controls, two lymphomas occurred among the controls and four in the azathioprine-exposed group. Compared with the general population, lymphoma risk was increased 4.8-fold in RA controls and 10-fold in the azathioprine-treated RA patients (171). In another study of all RA patients treated with azathioprine from one clinic, three out of 41 patients developed NHL, indicating a high lymphoma risk linked to azathioprine therapy (172). Conversely, in two surveys, together including 484 azathioprine-treated patients with RA, no lymphomas occurred during 10 years of follow-up, suggesting that azathioprine treatment was not linked to increased lymphoma risk (173, 174). Studies of azathioprine in a variety of other inflammatory conditions have also shown conflicting results regarding lymphoma risk (170).

Cyclophosphamide

Occasional cases of lymphomas, solid tumours and bladder carcinomas have been reported in association with cyclophosphamide in RA, but few cohort studies have evaluated the risk of lymphoma associated with this drug. In one study of 81 RA patients treated with cyclophosphamide, the risk of maligna-
cies of the “haematological and lymphoreticular systems” was increased (RR 15; 95% CI 4-38; 3 lymphomas) (175), but in another study no increased lymphoma risk was determined (176).

**Cyclosporine**

Treatment with cyclosporine has not been linked to increased lymphoma risk in RA (177, 178). One study has even suggested a lymphoma protective effect of cyclosporine as RA patients treated with cyclosporine for over than one year had a lower risk of lymphoma than those receiving the drug for less than one year (179).

**Intramuscular gold and Prorecid**

Compared to the general population, increased lymphoma risks in RA patients exposed to parenteral gold or Prorecid, has been determined, although only significant in the gold-treated group (5 lymphomas: RR 9; 95% CI 3-22) (180). There was no correlation with dosage or duration of either therapy. Another study assessing cause of mortality in almost 600 RA patients treated with intramuscular gold, found no excess deaths due to lymphoma (181).

**Methotrexate**

An accumulated number of case reports have specifically drawn the attention to methotrexate-induced lymphomas. However, so far four large cohort studies have evaluated the risk of lymphoma in methotrexate treated RA patients, and none of them found significantly increased lymphoma risks. Accordingly, in one study of 426 RA patients treated with methotrexate and followed-up for a mean of 4.6 years, the relative risk of developing lymphoma was not increased compared with other RA patients or with individuals in the region (182). Two lymphomas occurred in the methotrexate-treated patients and one among the non-methotrexate treated RA controls.

In a retrospective study, involving more than 16 000 RA patients (183), all patients diagnosed with a malignant disease were identified and divided into groups based on the use of methotrexate versus other DMARDs. Nine of the 27 identified lymphoma patients had received methotrexate and 18 had never been treated with this drug. There was also no relationship between the cumulative or peak doses, or duration of methotrexate treatment.

In another study, an attempt was made to collect all cases of lymphoma appearing in French RA patients treated with methotrexate over a period of 3 years (184). A total of 25 cases of lymphoma were identified, which is the hitherto the largest number of lymphomas reported in methotrexate-exposed
RA patients in the same study. Compared to the estimated annual lymphoma incidence rates in the population, there was no increased overall lymphoma risk among the methotrexate-treated RA patients.

Similarly, in a prospective study of 18,572 RA patients enrolled in a national data bank for rheumatic diseases (185), the lymphoma incidence, based on 10 cases, was not significantly increased in methotrexate-treated patients compared to the general population, or to RA patients who were not exposed to methotrexate.

In addition, no cancer was reported in four smaller studies assessing the efficacy and safety of methotrexate in RA over periods ranging from 18.5 months to 13.3 years (186-189).

All these data suggest that methotrexate does not augment the incidence of lymphoma in RA, but concerns about the role of methotrexate in lymphomagenesis still remain; this is based on the large number of case reports of lymphomas in methotrexate treated RA patients, some of whom have partially, or completely, regressed after methotrexate was withdrawn (141, 144, 148). Stemming from data in these case-reports and case-series a specific lymphoma entity, “Methotrexate-associated lymphoproliferative disorders” has been included in the WHO classification of lymphomas (49). These lymphomas are described to show features typical of lymphomas in immunosuppressed patients including large B-cell NHL subtype, high frequency of EBV in the lymphoma tissue, extra-nodal involvement and spontaneous remission of the lymphoma if methotrexate is withdrawn (49, 71). The frequency of “Methotrexate-associated lymphoproliferative disorders” is not known. A serious limitation when discussing lymphomas in methotrexate treated RA patients is incomplete knowledge about “background” characteristics of lymphomas in RA patients not treated with methotrexate or other potentially immunosuppressive drugs.

Other DMARDs
Regarding lymphoma risk in relation to other DMARDs, no studies have specifically assessed lymphoma risk in RA patients treated with antimalarials, auranofin, D-penicillamine or sulfasalazine. Apart from a few case-reports (136, 137) there is no suggestion that the use of these drugs increases the risk of lymphoma (170).

Other treatments
Treatment in RA also includes the frequent use of NSAIDs, aspirin, corticosteroids (oral and intra-articular) and orthopaedic surgery. Studies on lymphoma risk in association with NSAIDs, aspirin, and corticosteroids, have shown conflicting results and there is lack of robust and specific data about RA patients. In three case-control studies of patients with
NHL and self-reported RA/"rheumatism", one showed increased risk with use of NSAID and aspirin (190), and two showed no increased risks (104, 191). However, a major argument against a critical role of NSAIDs in lymphoma development is that osteoarthritis, which accounts for a larger number of NSAID users than RA does, has generally not been associated with an increased risk of lymphomas (190, 192).

By using pharmacy records as source of exposure information, two studies on steroid use and NHL risk arrived at different results; one found an increased risk of lymphoma (193), the other determined no increased, and even decreasing risk with prolonged use (105). In a third study, long-time use of steroids was associated with increased risk of NHL (194). None of the studies reported the reason for the use of steroids.

Other non-pharmacological treatments assessed as potential risk factors for lymphomas in patients with RA include joint replacement (192, 195) and yttrium radiosynovectomy of the knee joint (196), but neither of these studies observed any increase in the lymphoma risk.

RA disease-related factors and lymphoma risk

Factors related to the RA disease per se have repeatedly been hypothesized as potential risk factors for lymphomagenesis in RA patients. Despite such suggestions, few studies have actually addressed, or been able to address, this issue. Small numbers of lymphoma cases, lack of robust and consistent data on RA disease severity measures, non-RA subjects as controls, or that studies in general have focused exclusively on treatment and not the RA disease, contribute to the limited knowledge of RA disease severity and lymphoma risk. Register-based studies, which usually encompass larger number of lymphoma cases, are hampered by the often limited clinical information included in the registers.

However, indirect support for an association between RA disease severity and lymphoma risk has derived from such studies. The increased risk of lymphoma seen in RA patients already in the 1970s, before the current widespread use of immunosuppressive therapy, indicates an increased background risk of lymphoma in RA independent of immunosuppressive therapy (84, 125, 131). In a Canadian study, the risk of lymphoma was increased only in patients hospitalised by rheumatologists, presumably patients with more severe disease than those not seen by rheumatologists, and was independent of DMARD use (130).

Other indirect support for a link between RA disease severity and lymphoma risk includes a study of patients with the Felty syndrome, a complication of severe RA, that reported a 13-fold relative risk for lymphoma in these patients compared to the general population, but found no correlation with a specific therapy (197). Similarly, in RA patients with secondary Sjögren’s
syndrome, an extra-articular manifestation that is more common in severe RA, increased lymphoma risk compared to RA patients without secondary Sjögren’s syndrome has been reported (98).

Presuming that an association between disease activity and lymphoma does exist, this may have influenced the results in studies of patients treated with different immunosuppressive drugs. As patients selected for immunosuppressive therapy are likely to have active disease, studies comparing individuals requiring treatment with untreated individuals may have falsely exaggerated the risk associated with treatment.

Shared genetic and/or environmental risk factors

Another possible explanation for the association between RA and lymphoma would be the existence of common risk factors for the two diseases. However, there has been little evidence for any such factor, and although a common distinct genetic risk factor may exist, it has still to be identified.

Few environmental risk factors for lymphoma development have been identified, and evidence for such factors in RA is even weaker. Smoking could be such a risk factor, as it is common enough to have an impact on the population level, and has recently shown to be of importance in RA pathogenesis (39, 40). There is, however, little support for smoking as a risk factor for lymphoma (109, 110).

Involvement of common infectious agents, perhaps still unidentified, cannot be excluded. EBV, linked to the development of some lymphoma entities and to lymphoma development in immunosuppressed individuals, has been suggested as a potential etiologic factor also in RA, but studies have failed to clearly demonstrate a role of EBV in RA pathogenesis (4, 35).

Characteristics of lymphomas in patients with RA

Descriptions of lymphoma characteristics in RA-related lymphomas have derived from studies, case-series and case-reports of patients treated with different DMARDs.

A wide range of lymphoma subtypes has been reported in RA patients. Although interpretation is hampered by different lymphoma classification systems, it is obvious that high-grade B-cell lymphomas predominate in case-reports (136, 140-142, 144, 147, 148) whereas no distinct pattern of lymphoma subtypes has been reported by others (126, 183). Some register-based studies have indicated higher risks for HL than NHL (Table 4). It has, however, been shown in Swedish reclassification studies that earlier register-based documentation about NHL and HL diagnoses must be interpreted with some caution, as a significant proportion of NHLs have been misclassified as HL (54).
Information about EBV in lymphoma tissue has been documented in case-reports of RA patients with lymphoma since the 1990s and onwards, and the majority of reported cases has been EBV-positive. In case series of DMARD treated RA patients, 21-41% of NHLs and a higher proportion HLs has been EBV-positive (141, 144, 148 156). In one study of MTX-treated RA patients three of 18 NHLs (17%) and five of seven HLs (71%) were EBV-positive (184). There is one study which arrived at somewhat different results. In that study, 42 patients with NHL and RA and 49 NHL controls without RA were identified from the general population (198). No significant differences were seen regarding NHL subtypes or presence of EBV between RA patients and controls. One of the lymphomas in the RA patients (2%) and one among the controls (2%) were EBV-positive. Few of the patients were DMARD-treated. A factor of uncertainty in this study is that, in most patients, the RA diagnosis was self-reported.

Case-reports also suggest that extranodal lymphoma involvement is common in DMARD-treated RA-lymphoma patients and that some of these lymphomas have a poor prognosis (141).

From the above, it may be summarized that the background of the increased lymphoma risk in patients with RA is incompletely understood. Controversy still exists whether there is an increased lymphoma risk linked to therapy. Lymphoma risk linked to RA disease severity has not been explored and studies have not been able to separate effects of disease activity from influence of therapy. Information about lymphoma characteristics is mostly based on case-reports of DMARD-treated patients and information about “background” lymphoma characteristics in unselected/non-DMARD-treated RA patients with lymphoma is lacking.
Aims of the thesis

The aims of this thesis were

- to investigate risk factors for the development of lymphomas in patients with RA with particular focus on the role of RA disease severity and different RA treatments (Papers I and III)

- to characterize lymphomas in RA patients regarding subtype, presence of EBV, clinical manifestations and prognosis (Papers II and IV)
Subjects and methods

All patients in study I-IV were identified by register linkage between the Swedish hospital discharge register and the Swedish cancer register.

The Swedish hospital discharge register

The Swedish in-patient institutions have county-wise and in a computerised fashion reported data on in-patient care to the hospital discharge register (www.sos.se/epc/par) since 1964 (199). The coverage of the register has been nation-wide since 1987. For each discharge, the main and up to five secondary discharge diagnoses, hospital, department, date of admission, date of discharge, surgical procedures, the personal identification number and county of residence of each individual are recorded. The diagnoses are coded according to the International Classification of Diseases (ICD); ICD7 (1964-1968), ICD8 (1969-1986), ICD9 (1987-1996) and ICD10 (1997- ). Date of death in the hospital discharge register is ascertained by linkage with the Swedish cause of death register (www.sos.se/epc.dors).

The quality and content of the register is regularly validated, and completeness is estimated to be close to 100%. Validation studies of discharge information have indicated a diagnostic validity between 85% and 90%, with variations depending on diagnosis and calendar time (199). In RA, a study of specificity of the diagnosis performed in the 1970s found that around 80% of discharge diagnoses coded as RA were correct (200).

The Swedish cancer register

The Swedish cancer register (www.sos.se/epc.cancer) was established in 1958 and contains data on all cancer cases in the population (50), classified according to the ICD system (ICD7). The reporting to this register is compulsory for both pathologists and clinicians and the completeness of the register is estimated to be close to 99% (50, 201). The register is regularly linked to
the cause of death register and supplies date and cause of death for all individuals with a cancer registration.

In the ICD system, CLL is included in leukaemias, and plasmocytoma/myeloma a separate entity, whereas the WHO classification of tumours of haematopoietic and lymphoid tissues sorts both CLL and plasma-cell malignancies as B-cell lymphomas.

Paper I

In this nested case-control study of risk factors for lymphoma in RA patients, the Swedish Hospital Discharge Register was used to identify all patients above 16 years of age discharged from hospital with a diagnosis of RA (ICD7:722; ICD8:712.10, 712.38-712.39) during 1964-83 in Uppsala Health Care Region. Among the 11 683 patients with RA as the main or a secondary discharge diagnosis, 42 cases of lymphoma (ICD7: 200-202) were identified through record linkages between the Hospital Discharge Register and the Cancer Register through 1984. Excluded first were patients with a prior diagnosis of cancer and those with lymphoma diagnosis within the first year after the first discharge listing RA. Controls were identified from the same RA cohort and had to be alive and without any registered cancer at the time of the lymphoma diagnosis of the case. Cases were individually matched to three controls per case by year of birth and sex. The medical records were collected and patients not fulfilling the 1987 ACR criteria for RA were excluded. After exclusions, this study consisted of 41 cases and 113 controls.

Data of drug exposure defined as four or more consecutive weeks on a specific drug, RA disease severity (overall disease activity [see below], functional class of Steinbrocker, pattern of joint involvement), and extra-articular disease manifestations were abstracted from the medical records blinded for case control status.

To account for the matched design relative risks for lymphoma was calculated as odds ratios (OR) with 95% confidence intervals (CI) using conditional logistic regression. OR for inflammatory activity and functional class was adjusted for the different treatments.

The incidence of site-specific cancers in the cohort of 11 683 RA patients has been investigated in a study by Gridley et al (131). The risk for lymphoma in these patients was increased two-fold compared with the general population (standardized incidence ratio 1.98; 95% CI 1.5-2.6).
Paper II

In this study, the lymphoma tissues of the RA-lymphoma cases in paper I were reviewed, classified according to the WHO classification of lymphomas and investigated for EBV by EBER in situ hybridisation (202). Additional information about the lymphomas was retrieved from the medical records and included the lymphoma extension in different organs, Ann Arbor stage at diagnosis and survival after lymphoma diagnosis.

After exclusion of five cases due to poor quality of lymphoma tissue and one as the lymphoma diagnosis could not be confirmed from available material, 35 of the 41 RA-lymphoma cases were reclassified and 30 were examined for EBV (five cases could not be EBV-examined due to shortage of lymphoma tissue).

Paper III

This is an extension of the first two studies. In the Swedish hospital discharge register we identified all individuals above 16 years of age discharged with a diagnosis of RA, main or secondary (ICD7: 722, ICD8: 712.38-712.39, ICD9: 714 A-C, 714 W) 1964-1994 in Sweden (n=74 651). Through linkage with the Swedish cancer register we identified as cases those individuals who were subsequently diagnosed with a malignant lymphoma (ICD7: 200-202) as their first, primary cancer through 1995 (n=424).

For each case one cancer/lymphoma-free individual from the same RA cohort was used as control. Controls had to be alive at the time of lymphoma diagnosis of the case and were individually matched by sex, year of birth, year of first RA discharge, and county of residence. For ascertainment of RA and lymphoma diagnosis, medical records were scrutinized and lymphoma tissues reviewed. Cases and controls not fulfilling RA criteria and cases not having lymphoma at pathology review were excluded. After exclusions 378 RA-lymphoma cases and 378 controls remained in the study.

Information from the medical records was abstracted blinded for case versus control status using structured forms and included potential risk factors for lymphoma such as smoking, heredity, concomitant disorders, surgery, drug therapy, RA disease activity (assessed as overall disease activity and cumulative disease activity, further described below), joint damage according to the RAAD score, functional class, extra-articular manifestations as well as presence of rheumatoid factors and antinuclear antigen. The lymphoma tissues were reclassified according to the WHO classification and EBV was searched for by EBER in situ hybridisation.

Relative risks were calculated as ORs with 95% CI using conditional logistic regression. Cumulative disease activity was estimated as area under the curve (AUC) (see further below). Analyses of treatment were adjusted for
disease activity as quartiles of AUC and (other) DMARD use. To assess effect modification, lymphoma risk linked to disease activity (quartiles of AUC) was stratified by RA disease duration, sex and DMARD use. All analyses were performed using the SAS System software.

In a study of cancer incidence in RA by Ekström et al. (135) encompassing the same source-population of RA patients as this study, the standardized incidence ratio for lymphoma was 2.0 (95% CI 1.8-2.2).

Paper IV

In paper IV, the patients identified with DLBCL in study III were studied separately for distribution of GC versus non-GC DLBCL subtypes, clinical characteristics and prognosis. Of the 165 DLBCL patients from study III, 26 cases were not included in the subclassification into GC/non-GC subtypes due to shortage of lymphoma material or histological DLBCL subtype (T-cell rich DLBCL and anaplastic DLBCL) characterized by few and scattered tumour cells making interpretation of immunostainings difficult. Patients were followed-up until death or end of the study period (December 31, 1996).

For the subclassification of DLBCLs into GC and non-GC subtypes, immunohistochemistry using the expression patterns of CD10, bcl-6 and IRF-4/MUM-1 were used in accordance with previous studies (67, 68).

Overall survival was estimated by the Kaplan-Meier method. Log-rank tests were used for comparisons of survival curves between GC and non-GC patients and differences in distributions between GC and non-GC DLBCL patients were estimated with chi-square tests. The SAS System software was used for the analyses.

Measures of RA disease activity

To adapt to the typical information given in the medical records, we have used two models to score RA disease activity. These models are referred to as overall disease activity (paper I-IV; in paper I called “inflammatory activity”) and cumulative disease activity (paper III and IV) and are both based on three common variables used to describe RA disease activity; number of swollen and tender joints (15-joint count), ESR values and physician’s global assessment of disease activity.

Overall disease activity summarized disease activity for the whole RA disease period as either low, medium or high and was based on the estimation of a score value for each reported visit, and the final score the mean of the total scores from all visits. Disease activity was considered low if the final score was 3-4, medium if the final score was >4 to 8, and high if the final score was >8 to 9 (Table 6).
Table 6. Rheumatoid arthritis overall disease activity score

<table>
<thead>
<tr>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>1-30</td>
<td>31-60</td>
</tr>
<tr>
<td>No swollen joints</td>
<td>0-3</td>
<td>4-6</td>
</tr>
<tr>
<td>Global assessment of disease activity</td>
<td>Mild</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Cumulative disease activity estimated the duration in months of inactive, low, medium and high disease activity from onset of RA until lymphoma diagnosis. The definition of the different levels of disease activity was the same in both disease activity scores; the limits of ESR scored 1 refers to low disease activity in the cumulative disease activity score etc (Table 6).

In the analyses, we integrated the monthly levels of disease activity over time and calculated the AUC. A score based on the impact of the different levels of disease activity on lymphoma risk was used, and accordingly, inactive was assigned a score of 0, and low, medium and high disease activity the scores 1, 5, and 8, respectively. The AUC of an individual with 10 months of low, 10 months of medium and 10 months of high disease activity would then equal 10x1 + 10x5 + 10x8= 140.

The 15-joint count included the following joints: shoulders, elbows, knees, wrists and ankles (1 affected: 1 point, 2 affected: 2 points), metacarpophalangeal joints (1-5 affected: 1 point, 6-10 affected: 2 points), proximal interphalangeal joints (1-5 affected: 1 point, 6-10 affected: 2 points), and metatarsophalangeal joints (1-10 affected: 1 point).
Results

Paper I

Of the 41 RA-lymphoma cases, 22 (54%) were females. The mean age at onset of RA was 52.7 years in cases and 54.5 years in controls, and lymphoma was diagnosed on an average 17.7 years (range 4-41 years) after onset of RA.

The most prominent risk factor for development of lymphoma was high inflammatory activity with an OR of 25.8 (95% CI 3.1-213.0) compared with low inflammatory activity. Other characteristics associated with RA disease severity also entailed significantly increased risks for lymphoma i.e. functional class IV of Steinbrocker (OR class IV versus class I=12.7; 95% CI 2.1-76.8), widespread joint involvement compared to involvement in only hands and feet (OR =9.3; 95% CI 2.1-41.5), and, although based on small numbers, atlantoaxial subluxation (OR= 11.2; 95% CI 1.2-100.0) and nodules (OR= 7.6; 95% CI 1.5-37.1).

Treatment with DMARDs was limited in both cases and controls and the duration of treatment was mostly short; treatment duration was shorter than one year for 22 of 24 patients treated with intramuscular gold (15 patients had a 3-months’ cure) and for 4 of the 5 D-penicillamine-treated patients. None of the cases or controls had been treated with methotrexate, cyclosporine, cyclophosphamide, azathioprine, auranofin or chlorambucil. No association between a specific drug and risk of lymphoma was detected.

Paper II

Of the 35 reclassified lymphomas, 33 (94%) were NHL and two HL. Among patients with NHL, there was a predominance of DLBCL, diagnosed in 22 of 33 NHL patients (67%). EBV was detected in 5 of 30 examined lymphomas (17%), four of these were DLBCLs and one a lymphoplasmacytic lymphoma.

Of the 22 patients with DLBCL, 20 had RA with high or medium inflammatory activity. Four of the 5 EBV-positive cases occurred in patients with high inflammatory activity. Of all lymphoma cases, 17 had never received DMARD therapy and of the DMARD treated patients, only 8 had received a DMARD for one year or more during the entire RA disease period from onset until lymphoma diagnosis. When we divided the patients into groups accord-
ing to treatment with DMARDs (never treated, treated <1 year, and treated ≥ 1 year), the different lymphoma subtypes were distributed in a similar way into the different categories. At the time of diagnosis of the lymphoma, two of the patients were receiving DMARD therapy (Prorecid).

Of the 35 lymphoma patients, 23 (66%) had generalized disease Ann Arbor stage IV at diagnosis. The median time of survival from diagnosis of lymphoma was 6 months (2 weeks-13 years) for the patients with DLBCL and 27 months (3 weeks-13 years) in the remaining cases.

Paper III

Of the 378 RA patients with lymphoma, 208 (55%) were females. The mean age at onset of RA was 50.5 years for cases and 53.3 years for controls and the mean time from RA onset until lymphoma diagnosis 20.1 years (range 1-59 years).

Risk factors for lymphoma development

High RA disease activity was the strongest risk factor for lymphoma development, measured both as overall disease activity (OR high versus low disease activity=71.3; 95% CI 24.1-211.4) and cumulative disease activity (OR10th versus 1st decile of AUC= 61.6; 95% CI 21.0-181.0) (Table 7).

Table 7. Cumulated disease activity assessed as area under the curve (AUC) and risk of lymphoma in 372 RA patients with lymphoma as unadjusted odds ratios with 95% confidence intervals.

<table>
<thead>
<tr>
<th>Decile of AUC</th>
<th>Cases n=372</th>
<th>Controls n=372</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>34</td>
<td>3.1 (0.9 – 10.8)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>38</td>
<td>0.9 (0.2 – 3.2)</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>36</td>
<td>2.3 (0.8 – 7.0)</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>39</td>
<td>2.6 (0.8 – 8.3)</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>36</td>
<td>1.4 (0.4 – 4.3)</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>38</td>
<td>4.1 (1.3 – 12.6)</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>36</td>
<td>6.4 (2.1 – 19.3)</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>37</td>
<td>9.4 (3.1 – 28.0)</td>
</tr>
<tr>
<td>10</td>
<td>226</td>
<td>37</td>
<td>61.6 (21.0 – 181.0)</td>
</tr>
</tbody>
</table>
Other measures of disease severity showed a similar pattern such as functional class of Steinbrocker (OR class IV versus class I = 67.5; 95% CI 18.9-239.8), joint damage assessed as RAAD score in hands/feet (OR severe versus partial joint damage=10.5; 95% CI 6.1-18.2) and in knees (OR severe versus partial= 28.3; 95% CI 9.0-89.6), and the mean of ESR values categorized into quartiles (OR highest quartile >45 mm versus lowest <23 mm=2.8; 95% CI 1.8-4.4).

Ever treatment with a DMARD was not associated with increased lymphoma risk (unadjusted OR=0.9; 95% CI 0.6-1.2, adjusted for quartiles of AUC OR=0.5; 95% CI 0.3-0.8). Of the individual DMARDs that the patients were exposed to (antimalarials, auranofin, azathioprine, chlorambucil, cyclophosphamide, cyclosporine, D-penicillamine, intramuscular gold, Prorecid (podophyllotoxin) and sulfasalazine) an increased risk was only seen with the use of azathioprine (unadjusted OR=2.3; 95% CI 1.2-4.6, adjusted OR=4.3, 95% CI 1.6-12.0). We found no clear risks linked to duration of DMARD treatment.

NSAIDs and aspirin did not increase lymphoma risk (adjusted OR=1.0; 95% CI 0.5-1.9 and 1.2; 95% CI 0.8-1.9, respectively), neither did use of oral corticosteroids (adjusted OR= 0.6; 95% CI 0.4-0.9) nor intra-articular steroids (adjusted OR=0.4; 95% CI 0.2-0.6). When we stratified lymphoma risk linked to disease activity (as quartiles of AUC) by disease duration, sex and DMARD treatment (figure 1), the risk remained essentially the same.

Figure 1. Cumulated disease activity as quartiles of area under the curve (AUC) and lymphoma risk as odds ratios with 95% confidence intervals in 372 RA patients treated and not treated with DMARDs.
Other potential risk factors for lymphoma such as smoking, and family history of RA or haematopoietic malignancy were reported with less consistency in the medical records, but based on smoking information in 51% of patients, RA family history in 68% and history of hematopoietic malignancy in 50%, no significantly increased lymphoma risks linked to these factors were detected.

Lymphoma reclassification and EBV analyses
The proportion DLBCL was increased (165 of 314 NHLs; 53%), but the distribution of other lymphoma subtypes unremarkable (Table 8). There were another 31 high-grade NHLs, that could not be ultimately reclassified according to the WHO classification, but which probably were DLBCLs. Adding these to the DLBCLs, 62% of NHLs would be of the DLBCL subtype.

A strong correlation between the risk of developing DLBCL and RA disease activity was seen (OR 3rd versus 1st tertile of AUC= 93.7; 95% CI 11.8-747.3), a smaller risk increase was also seen for the combined group of other lymphoma subtypes (OR 3rd tertile=5.0; 95% CI 2.1-11.5). EBER in situ hybridisation for EBV was positive in 37 of 304 (12%) investigated lymphoma cases, showed a similar distribution between lymphoma subtypes as in non-RA related lymphomas and was not associated with AUC (e.g.12% of cases in the 10th decile were EBV-positive) or the treatment with a specific drug. In patients ever treated with methotrexate two of 19 cases (10%) were EBV-positive. Around 30% of EBV-positive cases had never been exposed to DMARDs.

Reversible lymphomas
Four of the 378 lymphoma cases showed a spontaneous regression without specific lymphoma treatment. These cases were all EBV-negative and included one patient who had never been treated with DMARDs or corticosteroids (DLBCL in salivary gland), and three in which the lymphoma regressed upon withdrawal of sulfasalazine (follicular lymphoma grade 1 in lymph nodes, methotrexate (follicular lymphoma grade 2 in lymph node) and azathioprine (DLBCL in stomach). The last three patients were all treated with corticosteroids for their RA.
Table 8. Results of lymphoma reclassification in 343 patients with rheumatoid arthritis and lymphoma and in situ hybridization for Epstein-Barr virus (EBV) in 304 of these lymphomas. Ratio describes the number of EBV-positive lymphomas out of the tested number of the same subtype.

<table>
<thead>
<tr>
<th>Lymphoma subtype, WHO classification</th>
<th>All lymphomas n=343 (%)</th>
<th>Proportion EBV-positive lymphomas n=304 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-cell neoplasms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small lymphocytic lymphoma</td>
<td>17 (5%)</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
<td>7 (2%)</td>
<td>3/7 (43%)</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Plasma cell myeloma</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>MALT-lymphoma</td>
<td>3 (0.9%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Follicular lymphoma, grade 1</td>
<td>21 (6%)</td>
<td>0/19 (0%)</td>
</tr>
<tr>
<td>Follicular lymphoma, grade 2</td>
<td>7 (2%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>Follicular lymphoma, grade 3</td>
<td>4 (1.2%)</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>5 (1.5%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>165 (48%)</td>
<td>19/160 (12%)</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>3 (0.9%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Unspecified high-grade B-cell lymphoma*</td>
<td>16 (4.7%)</td>
<td>1/13 (8%)</td>
</tr>
<tr>
<td>Unspecified low-grade B-cell lymphoma*</td>
<td>10 (2.9%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Unspecified B-cell lymphoma*</td>
<td>9 (2.6%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td><strong>T-cell and NK-cell neoplasms</strong></td>
<td>16 (5%)</td>
<td>2/15 (13%)</td>
</tr>
<tr>
<td>T-lymphoblastic lymphoma</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>1 (0.3%)</td>
<td>0/0</td>
</tr>
<tr>
<td>Angioimmunoblastic T-cell lymphoma</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma, unspecified</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>8 (2.3%)</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>Unspecified high-grade T-cell lymphoma*</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Unspecified low-grade T-cell lymphoma*</td>
<td>3 (0.9%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td><strong>Hodgkin lymphomas</strong></td>
<td>21 (6%)</td>
<td>9/19 (47%)</td>
</tr>
<tr>
<td>Nodular lymphocyte predominant HL</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Nodular sclerosis classical HL (grade 1 and 2)</td>
<td>9 (5.5%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>Lymphocyte-rich classical HL</td>
<td>1 (0.3%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Mixed cellularity classical HL</td>
<td>7 (2%)</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Lymphocyte-depleted classical HL</td>
<td>2 (0.6%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Unspecified HL</td>
<td>1 (0.3%)</td>
<td>0/0</td>
</tr>
<tr>
<td><strong>Unspecified lymphomas</strong></td>
<td>37 (11%)</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>Unspecified high-grade NHL*</td>
<td>15 (4.4%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Unspecified low-grade NHL*</td>
<td>8 (2.3%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Unspecified NHL*</td>
<td>6 (1.7%)</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Unspecified malignant lymphoma*</td>
<td>8 (2.3%)</td>
<td>1/4 (25%)</td>
</tr>
</tbody>
</table>

MALT=Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, HL=Hodgkin lymphoma, NHL=non-Hodgkin lymphoma, *Lymphoma material insufficient for diagnosis according to WHO classification
Paper IV

The subclassification of the DLBCLs revealed a higher proportion of the non-GC type (97 patients; 70%) than the GC-type (42 patients; 30%). There was a strong association with RA disease activity in both non-GC and GC-DLBCL as more than 70% of cases in each subtype belonged to the RA patients with the highest cumulated disease activity (decile 10). Non-GC DLBCL more often had a disseminated lymphoma at diagnosis than GC-DLBCL (Ann Arbor stage IV 64% versus 38%) and a worse 5-year overall survival (15% versus 32%). EBV was positive in two (5%) of the GC tumours and in 10 (10%) of the non-GC tumours.

The 5-year overall survival was 30% for all RA patients with lymphoma, and 20% for all patients with DLBCL. Patients with DLBCL diagnosis after 1985 had a better 5-year overall survival (28%) than those diagnosed earlier (12%; p=0.009).

Extranodal involvement was present in 68% of patients with DLBCL, and 52% of DLBCL-cases had Ann Arbor stage IV at presentation.
Discussion

Risk factors for lymphoma development

RA disease severity, treatment and risk for lymphoma

One of the main findings of this thesis was that lymphoma risk in RA patients was linked to RA disease severity and not, contrary to previous claims, to the treatment used in RA. Both Papers I and Papers III showed a strong association between disease severity in RA and the risk of developing malignant lymphoma. This was most prominent for the exposure inflammatory activity, when assessed both as overall disease activity and as cumulative disease activity, although all other exposures associated with disease severity revealed a similar pattern. It was also evident that the risk increased in a stepped fashion with grade of RA disease severity, and was highly increased in only a subset of patients, those with very severe disease. Analyses of lymphoma risk linked to RA treatment revealed no increased lymphoma risks associated with DMARD treatment generally or with the use of specific DMARDs, nor were duration of treatment and lymphoma risk associated. There was, however, one exception as ever use of azathioprine was associated with a small, but significantly increased lymphoma risk. As azathioprine was used in only a small proportion of the RA patients, (30 [8%] of the cases in Paper III, and none in Paper I had ever used azathioprine), azathioprine may only have accounted for a marginal part of the increased lymphoma risk in these patients. Other treatments used by the RA patients i.e. NSAIDs, aspirin and corticosteroids did not increase lymphoma risk.

Essential to this work was the separation of treatment effects from disease process effects on the risk of developing lymphoma. This has been a recurrent problem in previous studies on lymphoma risk in DMARD treated RA patients, mostly because of lack of robust and consistent measures of both treatments and disease severity.

We could, however, take advantage of the public health care system in Sweden and some of its regulations. The compulsory and careful filing of all medical records in accessible archives enabled almost all original records of the patients in these studies to be traced. Most records were of high quality, and data on the patients’ RA disease was rather uniformly documented. Even if a patient was not seen regularly by a rheumatologist, many of both cases
and controls had well-documented recurrent check-ups or rehabilitation periods in one of the hospitals specialized in rheumatic diseases.

We used several different measures to assess disease severity, reflecting disease activity, joint damage and impairment. Overall disease activity, the measure of disease activity in Paper I, provides a rather rough division of patients into those with low, medium or high inflammatory RA disease. Therefore, cumulated disease activity was included as the principal measure of disease activity in Paper III, a measure that better captures the fluctuating disease activity typical of RA. Both disease activity measures are based on variables similar to those included in the common disease activity scoring system “DAS” (25), although our variables by necessity had to be adapted to the information given in routine medical records. Adjusting all analyses of DMARD treatment for cumulated disease activity, and a separate stratification of cumulated disease activity by DMARD use confirmed that disease activity was a strong, independent risk factor for lymphoma development.

One problem when evaluating the role of treatment in lymphoma development is the question of timing of the drug exposure. As the development of lymphoma stretches over long time-periods, a critical event in this process may have preceded the overt lymphoma by years. Therefore, we analysed drug exposure as ever use of a specific drug (for at least four consecutive weeks) at any point during the whole RA disease period. In most studies and case reports the drug in use when the lymphoma occurred is the drug under investigation, irrespective of earlier treatment. When we specifically examined drug exposure at lymphoma diagnosis, we found, in accordance with the other analyses of RA treatment, no relation between any drug and lymphoma risk. As this information was not reported in Paper III, it should be added that most lymphoma cases were not DMARD treated when the lymphoma occurred; 76 (20%) of the cases and 98 (26%) of the controls were treated with a DMARD at lymphoma diagnosis or at lymphoma diagnosis of the corresponding case, respectively, and no specific drug was over represented.

A related problem is how to evaluate the situation when a lymphoma occurs soon after a drug treatment has started. This is illustrated by findings in the azathioprine-treated patients. Typical of these patients was severe RA and the introduction of azathioprine therapy late in the disease (after more than 10 years of RA). In some of these patients lymphoma occurred 2-3 months after the start of azathioprine treatment. This raises the question of causality. One possibility is a true causative role for azathioprine, another that the lymphoma was already present sub-clinically before the start of the drug treatment, and the contemporaneous occurrence was just coincidental. A third possibility is that the newly started drug was not the initial cause of the lymphoma, but did in some way promote lymphoma development, for example by disturbing the natural defence against already emerging malignant cells. One of the latter two suggestions appears most likely, but cannot of course be further analysed in the frame of this work. This issue is currently also of interest for lympho-
mas in patients treated with TNF-blockade, as some of these lymphomas have occurred shortly after initiation of the drug (203).

Some discrepancies between the results in Papers I and III need to be addressed. The risk estimates linked to high overall disease activity, and advanced Steinbrocker functional class were higher in Paper III than in Paper I. Even if confidence intervals overlapped and results were therefore comparable, there is an explanation for the different point estimates. In Paper I, patients with lymphoma diagnosed within the first year after the first hospital discharge listing RA were excluded, whereas in Paper III all patients, regardless of time-span between first hospitalisation for RA and lymphoma diagnosis were retained in the main analyses. This provided a more complete study of all identified RA patients with lymphoma. It was obvious that many of the patients with lymphoma diagnosis the first year after first hospitalisation for RA had severe RA. When these patients were omitted from the analyses in Paper III, (point) risk estimates decreased and approached those found in Paper I.

The proportion of patients with high, medium and low overall disease activity and the spread between functional classes of Steinbrocker was different among the patients in Papers I and III. In general, both cases and controls had milder disease in Paper III than in Paper I, but controls displayed a greater change towards milder disease than cases did. A reasonable explanation for this change is related to the altered therapeutic approach in RA, resulting in a much higher proportion of DMARD-treated cases and controls in Paper III than in Paper I. It appears, though, as if the RA controls, more than the cases, benefited from the more active anti-rheumatic treatment. This confers with observations that many of the patients with severe RA experienced drug-related problems such as recurrent drug toxicity and quick loss of efficacy of the different DMARDs.

Combined, these results support the idea that studies and case-reports linking DMARD therapy to lymphoma risk in RA patients with severe disease, may have exaggerated or misinterpreted the risk. Instead, the risk may have been associated with high cumulated RA disease activity. The same possibility of misinterpretation is evident also in studies linking increased lymphoma risk in RA to treatment with NSAIDs, aspirin or corticosteroids. Instead of an increased lymphoma risk linked to therapy in RA, our results indicate that effective RA treatment reducing disease activity should reduce the risk of lymphoma.

Although few studies have investigated lymphoma risk linked to disease activity in RA, there is some support for our findings. Strong corroboration comes from a study, reported in abstract-form (204), which demonstrated a relationship between high erythrocyte sedimentation rates, as a manifestation of inflammatory activity, and increased risk of lymphoma in RA. In 1 767 RA patients seen in an outpatient rheumatology clinic over a 25-year period, ESR
values of 40 mm/hour or more increased the risk of NHL 9-fold and was not dependent on DMARD use.

In a recent Swedish-Danish study of NHL incidence (the SCALE study), increased NHL risks were observed in association with the use of NSAIDs, systemic corticosteroids and selected immunosuppressants (azathioprine, cyclosporine, methotrexate, cyclophosphamide and chlorambucil) in subjects with RA, but not in NHL patients without RA (205). These findings also support an association between lymphoma risk and the RA disease itself, rather than its treatment.

Further, indirect support comes from one of the incidence studies of cancer and malignant lymphomas in Swedish RA patients, which encompasses the source-population of RA patients investigated in more detail in Paper III (135). Despite the increasing use of DMARDs over time, the relative risk of lymphoma in the RA patients did not increase over successive calendar periods when compared to the general population; standardized incidence ratio remained close to 2.0 during the period 1964 to 1999.

Other potential risk factors for lymphoma

In Paper III, an attempt was made to assess lymphoma risk in association with some other potential risk factors for lymphoma i.e. smoking, family history of RA and haematopoietic malignancy, but data on these exposures was only available in around 50% of patients. Based on these data no increased risks were detected. Information about other potential risk factors for lymphoma e.g. physical activity and body mass index was more seldom documented, and was not abstracted from the medical records. Accordingly, although it cannot be excluded that exposure to some of these factors may have differed between cases and controls, these potential confounders could not be accounted for in the analyses. However, considering the weak association between environmental factors and general lymphoma risk determined in other studies, it appears unlikely that such factors would be of major importance in lymphomagenesis in RA patients. A recent study also reinforced this view (135). In approximately 70 000 first-degree relatives of patients with RA, no or only a marginally increased risk for lymphomas was observed compared to healthy individuals, which strongly supports that, if shared factors exist, their significance is limited.
Characteristics of lymphomas in patients with RA

Lymphoma subtypes and EBV

One specific lymphoma subtype, DLBCL, was increased among the RA patients. DLBCL appeared to be 2-3-fold more common in the RA patients than in lymphoma patients in general (52, 54), which indicates that most excess lymphomas in RA indeed are DLBCLs. We found no association between lymphoma subtype and treatment. Instead, there was a strong association between disease activity and the development of DLBCL, but only a weak association with other subtypes. By further subdividing the DLBCLs into newly recognized subtypes, we could approach details in lymphoma development in RA. Subdivision of the DLBCLs revealed a predominance of the non-GC subtype, which was more common in the RA patients than in DLBCL patients in general.

The frequency of EBV-positive lymphomas and the distribution among the different subtypes appeared similar to that reported for lymphomas in the general population. In Paper II, four of the five EBV-positive cases occurred in patients with high overall disease activity, indicating an association between EBV-positivity and disease activity. This could not be confirmed in Paper III as the proportion EBV-positive cases was similar among the patients in the different deciles of cumulated disease activity. No relationship between treatment with a specific drug and EBV-positivity was observed. There is, however, some uncertainty about the interpretation of the frequency of EBV-positive lymphomas. Presence of EBV in lymphomas in the general population has not been investigated in detail, and most data regarding EBV derives from studies of different HL subtypes. As the presence of EBV in lymphomas appears to vary with e.g. age and gender, apart from subtype, a proper control-population of non-RA lymphomas would have been desirable.

However, the current findings are in part different from those reported in most previous studies and case-reports of lymphomas in RA patients and of lymphomas in immunosuppressed patients (71, 141). In particular, we could not confirm an association between DLBCL and DMARD treatment generally or with the use of methotrexate. The frequency of EBV-positive lymphomas was much lower among the RA patients than in AIDS (71, 73) and post-transplant patients with lymphoma (71, 74), and lower than suggested from case series of DMARD-treated RA patients (141, 144, 148, 156).

We have considered, but not found any readily recognized methodological explanation for this discrepancy. Although validation studies of the national registers have shown a very high grade of completeness (199, 201), some lymphoma cases could still have been missed through under-notification or erroneous registration, in particular during the early period of the study. However, this misclassification has most likely been non-differential and not affected the results of subtype-classification or EBV-analyses in any particu-
lar direction. It occurred earlier, that a clinically obvious and widespread malignant disease was reported as unspecified malignancy to the cancer register without detailed investigation, and some high-grade lymphomas could therefore have been missed. On the other hand, some low-grade lymphomas could also have been missed or interpreted as part of the RA disease.

Not all identified lymphoma cases could be reclassified or analysed for EBV, still 86% of lymphomas in Paper I and 89% of lymphomas in Paper III were included in both reclassification and EBV analysis. Based on the original pathology reports and information from the medical records the non-analysed cases did not differ substantially from the analysed cases; therefore inclusion of the missing cases would likely not have changed the main results.

Another potential explanation for the results of the lymphoma reclassification is a transformation of low-grade lymphomas. This theory presupposes that a substantial number of low-grade lymphomas have been missed; these lymphomas eventually transformed and the lymphoma diagnosis not evident until a high-grade lymphoma, presumably a DLBCL, was diagnosed. Although such a scenario cannot be excluded, there are arguments against this. The haematopathologist reviewing the lymphomas was well aware of the possibility of transformation, still only one DLBCL with morphological signs of a concurrent low-grade lymphoma, indicating transformation, was identified. Of the classified low-grade lymphomas, two follicular lymphomas later transformed into DLBCL, which is not an increased frequency compared to low-grade lymphoma transformation in general. Moreover, the review of the medical records indicated that documented symptoms and signs compatible with (low-grade) lymphoma were not neglected and biopsy of enlarged lymph nodes was commonly performed.

The standard method for detecting EBV, EBER in situ hybridisation (202), was used. Validated for use in paraffin-embedded tissues this method has detected almost 100% of EBV-positive tissues in other studies (202). Adding immunostains for LMP-1 could have been an option, but would probably not have increased the number of identified EBV-positive cases. Age could have influenced the quality of the tissues and the possibility to detect EBV, although is not probable as the proportion of EBV-positive lymphomas was not affected by calendar-age of the material. A "hit-and-run" theory has recently been proposed, but not proven (206), which suggests that EBV-negative cases have been previously positive, but have managed to clear the virus from the tumour cells. Although this possibility cannot be excluded, it does not explain the discrepancy between the results presented and the findings in immunosuppressed individuals.
Reversible lymphomas in RA patients

Spontaneous lymphoma regression, another feature of lymphomas that has been related to states of immunosuppression, appears to be a rare event in RA patients.

In Paper I, no lymphoma regressed spontaneously and in Paper III spontaneous lymphoma regression was observed in four of 378 lymphomas (1%). Moreover, lymphoma regression in RA-related lymphomas was not solely linked to DMARD exposure and EBV-positivity, as one of the reversible cases had never been exposed to DMARDs and all four reversible lymphomas were EBV-negative.

There is, however, rather extensive literature describing spontaneous regression of lymphomas in both non-RA and non-immunosuppressed individuals (207-213). Although not mentioned in association with RA-lymphoma case-reports, reversible lymphomas, thus, do not seem to be exclusively linked to states of known immunosuppression.

For RA, information about reversible lymphomas comes predominantly from case-reports and case-series. In approximately a hundred case-reports on reversible lymphomas in RA patients, the reversibility has been ascribed to the withdrawal of an immunosuppressive drug, especially methotrexate (147, 150, 158, 162, 164, 165). Although the timing between DMARD withdrawal and the subsequent lymphoma regression strongly supports this assumption, the current findings and the existing literature suggest that the background to lymphoma regression may be more complex, and DMARD treatment and EBV-positivity appear not to be a prerequisite for this event.

As the reversible lymphomas that we identified occurred in the normal clinical care of patients, it is possible more lymphomas could have spontaneously reversed if anti-tumour therapy had been consequently withheld and a period of expectancy included. Conversely, this is not supported by observations from those cases who did not receive anti-lymphoma treatment for other reasons and quickly succumbed to the disease.

Clinical characteristics and prognosis of lymphomas in RA

In general, RA patients with lymphoma had advanced lymphoma disease already at presentation and a poor prognosis. As DLBCL predominates in RA patients, these general findings were strongly influenced by the characteristics of this particular lymphoma subtype. It appears as RA patients with DLBCL have a worse 5-year overall survival, more extranodal involvement and more advanced lymphoma disease, according to Ann Arbor stage, than DLBCL patients in general (60, 62, 63, 67-68).

The most common DLBCL subtype, non-GC DLBCL, was associated with significantly worse prognosis than for the GC-subtype, as for DLBCL pa-
tients in the general population (65-68). This suggests that the three-marker model used to separate GC and non-GC subtypes identifies subgroups of DLBCL with significantly different prognoses also in patients with RA. Compared to GC and non-GC subtypes in general DLBCL patients the prognoses for both GC and non GC-subtypes was worse for the RA patients (65-68). Although not consequently investigated before, information about prognosis in previous studies and case-reports of DMARD-treated RA lymphoma patients indicate a similarly poor prognosis for these patients (141).

There may be several contributing factors to a rapid progressive lymphoma disease with poor survival in RA patients, which distinguishes RA lymphoma patients from the general lymphoma patient. These factors include the accelerated senescence of the immune system in longstanding RA (38), an increased comorbidity and mortality linked to RA and a number of complications and extra-articular manifestations, in particular in patients with severe RA (11). Such factors may, for example, influence treatment possibilities, impair protection against emerging malignant cells and increase the risk for fatal infections.

It appears as if RA patients with DLBCL constitute a subgroup of lymphoma patients with complicated disease and specific problems. Special attention and cooperation between oncologists and rheumatologists may be warranted to improve the outcome for these patients.

Methodological considerations

Some general methodological problems remain to be discussed. We performed nested case-control studies to study risk factors for lymphoma development. This design allowed us to identify a large number of RA patients with lymphoma, although this is a rare event in RA and the time between RA onset and lymphoma is long. Potential problems in a case-control study are, in particular, the proper selection of cases and controls and avoiding bias in assessment of exposure. However, the risk of selection bias should be minimal. Cases and controls were identified from the same cohort of hospitalised RA patients and inclusion criteria the same. Patients and controls were matched, in Paper I by sex and year of birth, and in Paper III also by year of first RA discharge, and county of residence. We could not, with the register-based matching procedure, match for the exact duration of the RA disease. When comparing the characteristics of cases and controls, they were similar regarding e.g. rheumatoid factor-positivity, proportion of DMARD-treated patients and mode of RA onset, but there was a small, but significant difference in the duration of RA between cases and controls in study III (3 years
longer in cases). Therefore, to assess the impact of this difference, we stratified cumulative disease activity by disease duration, but found no significant differences in lymphoma risk.

To avoid bias in assessment of exposure, the abstraction of data was blinded for case versus control status as was the reclassification for all clinical data. Although the intention was to maintain blinding throughout the abstraction of data, for most of the cases, and difficult to avoid, the lymphoma disease became evident in the end. At this point, however, all data about the RA disease was already abstracted, which should minimize the risk for observation bias. The result of the reclassification was not available when clinical and exposure information was abstracted from the medical records.

The restriction to RA patients who, at some point, have been hospitalised presumably led to a selection of patients with more severe disease than is found among RA patients in general. However, this selection cannot explain the results and as hospitalisation has been common in RA patients, generalizability should not have been substantially affected. Patients with RA as a secondary/contributory diagnosis were included to increase the inclusion of patients with variable indications for hospital care and not only those treated for severe RA. Although the proportion hospitalised RA patients has decreased the last years due to a general closing of hospital-beds, studies have indicated that 50-75% of all RA patients during the study-period of these works had at least one hospital stay for RA and should have been included in the study-base (214).

**Biological considerations**

It has been postulated that RA patients with lymphoma should constitute one of the lymphoma categories associated with states of immunosuppression (49, 71, 141). Presumed features of RA-related lymphomas are high-grade lymphoma subtype, in particular DLBCL, developed as a result of immunosuppressive therapy, EBV in the lymphomas and spontaneous lymphoma regression upon withdrawal of DMARD therapy. Lymphomas in immunosuppressed patients also display a high frequency of extranodal involvement and poor prognosis.

In these studies of lymphomas in patients with RA, we did find a high frequency of DLBCL with extranodal involvement and poor prognosis, but could not confirm the other features associated with immunosuppression. Instead, our results suggest that immune stimulation is the driving force behind lymphoma development in RA.
From these findings, a hypothesis for lymphoma development in RA could be outlined. In the subgroup of RA patients with severe disease and long-standing immune activation, it can be assumed that the persistent proliferative drive increases the probability for genetic aberrations and a subsequent malignant transformation. This transformation appears to occur preferentially in a peripheral activated B-cell and may be followed by the expansion of an uncontrolled peripheral B-cell clone, and eventually the development of a non-GC DLBCL. Less often the cell of origin in this multi-step process seems to be a B-cell derived from a germinal centre, which then will lead to the development of a GC-DLBCL. Germinal centers are localized both in traditional lymphoid tissues, and in the inflamed synovium in a part of RA patients (38, 45).

Apart from the fundamental proliferative drive in this process, other factors in RA may contribute to the complex process leading to a lymphoma. The accelerated ageing of the immune system that has been described in RA is linked to disease severity (38), and thus particularly pronounced in RA patients at high-risk for lymphoma. This ageing process results in a declining immunosurveillance and thereby an impaired ability to handle e.g. premalignant and malignant cell clones. An impaired defence against emerging malignant B-cells could also derive from the active RA disease itself. Studies have shown that active RA, apart from stimulating a number of immune functions, may also suppress functions of the immune system. An impaired T-cell function has e.g. been demonstrated in active RA (215).

It seems reasonable to assume that varying degrees of immune stimulation and suppression may be present in disorders associated with lymphoma development. In RA, immune stimulation predominates and endogenous and/or exogenous immune suppression is of less importance, whereas in lymphomas in post-transplant and AIDS patients, immune suppression and EBV-positive lymphomas prevail. In post-transplant lymphomas, chronic immune stimulation by the transplanted organ may be of more importance in the late occurring EBV-negative cases than in the early EBV-positive lymphomas.

Our findings also suggest that activated peripheral B-cells are important in RA pathogenesis in the subgroup of RA patients with high risk of lymphoma. Several other factors involved in both inflammation and lymphoma have been identified and may, similarly, be of interest as potential targets for therapy in severe RA as well as in lymphoma treatment in patients with inflammatory disorders. Accordingly, elevated levels of B-cell activating factor (BAFF) have been detected in synovial fluid and serum of patients with active RA. BAFF protects malignant B cells from apoptosis and is therefore also a potent survival factor for B-cell lymphomas (216). NF-kappaB, another factor that is involved in inflammation in RA, seems to be essential as tumour promoter in inflammation-associated cancer (217, 218).
Practical implications

Finally, these results may have some implications for the clinical care of patients with RA.

Firstly, we have suggested that effective treatment to reduce RA disease activity may protect against lymphoma and, accordingly, the whole current concept for care and treatment of patients with RA is strongly supported by our results. Important parts in this concept are early arthritis clinics, which seek to facilitate an early diagnosis of RA and start of DMARD treatment, remission of disease activity as aim for the therapy, patient education programs teaching patients to recognize signs of inflammation, and non-delayed access to care-provider/rheumatologist when such signs occur. Regarding the specific drugs used in RA we have found nothing to suggest, or advice against the use of any particular drug in early RA and in RA patients with low accumulated disease burden. An individual choice to keep the level of disease activity as low as possible seems be the reasonable common aim of all drug treatment in RA. The use of peroral and intra-articular corticosteroids appears from a lymphoma protective perspective to be positive.

Secondly, as the lymphoma risk in patients with severe, longstanding RA appears to be strikingly increased, awareness of this complication of RA should be specifically important in this subgroup of patients. Further, in this high-risk group for lymphoma development, therapies that include B-cell suppression or depletion may be of specific interest.

Thirdly, as clinical trials of drugs in RA are performed in patients with active RA our data provide figures of the “background” risk of lymphoma in these patients. Possibly, patients at the very highest lymphoma risk should rather not be included in trials.

Fourthly, the poor prognosis for RA patients with DLBCL could be a reason for oncologist to specifically consider this subgroup of patients. Further studies and clinical trials for optimising treatment and improving prognosis could be needed.
Conclusions

Based on the findings in Papers I-IV, the following conclusions are made:

Risk factors for lymphoma development in patients with RA

The most important risk factor for lymphoma development in RA is high RA disease activity.

Treatment with most DMARDs (antimalarials, auranofin, cyclophosphamide, chlorambucil, cyclosporine, D-penicillamine, intramuscular gold, methotrexate, Prorecid and sulfasalazine) is not associated with increased lymphoma risk in RA patients.

Use of azathioprine may be associated with a small increase in lymphoma risk.

Use of NSAIDs, aspirin, peroral and intra-articular corticosteroids does not increase lymphoma risk in RA patients.

Characteristics of lymphomas in RA patients

A specific lymphoma subtype, DLBCL is more frequent in RA patients with lymphoma than in lymphoma patients in the general population and displays a stronger association with RA disease activity than other lymphoma subtypes.

The non-GC DLBCL subtype predominates in RA patients and is more common in RA patients than in DLBCL patients in general.
The presence of Epstein-Barr virus examined by EBER \textit{in situ} hybridisation is lower in lymphomas in RA patients than in immunosuppressed patients, and the distribution among lymphoma subtypes is similar to findings in lymphomas in the general population. EBV-positivity was not correlated to a specific RA treatment.

The frequency of spontaneously reversible lymphomas appears to be low in RA patients and may occur irrespective of previous DMARD treatment or withdrawal.

Extranodal involvement and advanced lymphoma stage at presentation is common in RA patients with DLBCL.

RA patients with DLBCL have a poor prognosis. The 5-year overall survival is significantly worse in patients with the non-GC DLBCL subtype than with GC-DLBCL subtype.
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References


25. Prevoo ML, van’t Hof MA, Kupfer HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint


215. Berg L, Lamp J, Rogberg S, van Vollenhoven R, Klareskog L. Increased peripheral T cell reactivity to microbial antigens and collagen type II in


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