Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 2125

Studies on Lymphoma in Rheumatic Diseases and the Pathophysiology of ANCA-Associated Vasculitis

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ACTA UNIVERSITATIS
UPSALIENSIS
2025

ISSN 1651-6206
ISBN 978-91-513-2392-3
urn:nbn:se:uu:diva-550889

UPPSALA
UNIVERSITET

Dissertation presented at Uppsala University to be publicly examined in H:son Holmdahlsalen, Akademiska sjukhuset Ing 100-101, Dag Hammarskjölds väg 8, Uppsala, Friday, 11 April 2025 at 09:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Professor Inger Gjertsson (Avdelningen för reumatologi och inflammationsforskning, Sahlgrenska akademin, Göteborgs universitet).

Abstract

Hellbacher, E. 2025. Studies on Lymphoma in Rheumatic Diseases and the Pathophysiology of ANCA-Associated Vasculitis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 2125. 62 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-2392-3.

Patients with rheumatic diseases are at increased risk of developing malignant lymphoma, yet the mechanisms linking immune-mediated diseases to lymphomagenesis remain unclear. A deeper understanding of these processes could provide clues to the pathogenesis of both disease categories, improve early risk assessment, and inform preventive strategies. Similarly, the pathophysiological mechanisms of ANCA-associated vasculitis (AAV) and the molecular distinctions underlying the varied clinical outcomes of its subtypes, granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), remain poorly understood. Improved insights into these mechanisms could aid in developing more targeted diagnostic tools and treatment strategies.

Paper I investigated B cell-related mechanisms in rheumatoid arthritis (RA)-associated diffuse large B-cell lymphoma (DLBCL). Key findings include elevated levels of several cytokines and chemokines relevant to B-cell biology compared to RA and population controls. In particular, CXCL13 emerged as a protein of interest for its potential role in linking RA to lymphomagenesis.

Paper II examined programmed cell death protein 1 (PD-1) and its ligands PD-L1 and PD-L2 in lymphoma tissue from patients with pre-existing rheumatic diseases, with a particular focus on RA-associated DLBCL. A key finding suggests that RA disease severity may influence PD-L1 expression in DLBCL tumor cells.

Paper III characterized lymphomas in patients with pre-existing GPA, focusing on subtypes, localization, and clinical features of both the lymphomas and the underlying rheumatic disease. No clear indications of a predominance of a specific lymphoma subtype were observed, nor was there evidence suggesting local lymphomagenesis in typical GPA target organs.

Paper IV identified key proteins, biological functions, and pathways associated with both shared and distinct disease mechanisms in AAV subtypes, categorized by ANCA serotype into proteinase 3 (PR3)-AAV and myeloperoxidase (MPO)-AAV. The findings highlighted enhanced STAT3 signaling in PR3-AAV and prominent TNF signaling in MPO-AAV, suggesting partially distinct inflammatory processes driving the pathogenesis of these subtypes.

To conclude, the studies in this thesis contribute to the efforts to elucidate the link between autoimmune diseases and lymphoma, as well as the shared and distinct disease mechanisms in AAV.

Keywords: Rheumatoid arthritis, ANCA-associated vasculitis, lymphoma, diffuse large B-cell lymphoma, CXCL13, PD1, PD-ligand, proteomics, STAT3, TNF

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ISSN 1651-6206
ISBN 978-91-513-2392-3
URN urn:nbn:se:uu:diva-550889 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-550889)

To Caroline, Ebba, Carl and Ellen

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Euler, N., **Hellbacher, E**., Hansson, M., Larsson, A., Enblad, G., Malmström, V., Baecklund, E., Grönwall, C., and the AUTO-LYMPHOMA study group. (2025) Diffuse large B cell lymphoma in rheumatoid arthritis patients is associated with an elevation of several B cell driving factors including CXCL13. *Manuscript submitted*

II. **Hellbacher, E**., Sundström, C., Molin, D., Baecklund, E., Hollander, P. (2022) Expression of PD-1, PD-L1 and PD-L2 in lymphomas in patients with pre-existing rheumatic diseases—a possible association with high rheumatoid arthritis disease activity. *Cancers (Basel),* 2022 Mar15;14(6):1509.

III. **Hellbacher, E**., Hjorton, K., Backlin, C., Enblad, G., Sundström, C., Baecklund, E., Knight,A. (2019) Malignant lymphoma in granulomatosis with polyangiitis: subtypes, clinical characteristics and prognosis. Acta Oncologica, 2019 Nov;58(11):1655-1659.

IV. **Hellbacher, E**., van Hoef, V., Johansson, A., Knight, A., Gunnarsson, I., Bruchfeld, A., Eriksson, P., Ohlsson, S., Mohammad, A., Söderbergh, A., Berglin, E., Rantapää-Dahlqvist, S., Dahlqvist, J. (2025) Plasma proteome profiling identifies distinct signaling pathways associated with PR3-ANCA and MPO-ANCA positive vasculitis. *Manuscript submitted*.

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Abbreviations

AAV	ANCA-Associated Vasculitis
ANCA	Anti-Neutrophil Cytoplasmic Antibody
APCs	Antigen-Presenting Cells
CD5	Cluster of Differentiation 5
CD8a	Cluster of Differentiation 8a
CHOP	Cyclophosphamide, Hydroxydaunorubicin, Oncovin, and Prednisone
CXCL13	C-X-C Motif Chemokine Ligand 13
CYC	Cyclophosphamide
DLBCL	Diffuse Large B-Cell Lymphoma
DMARD	Disease-modifying antirheumatic drug
EBER	Epstein-Barr Encoded RNA
EBV	Epstein-Barr Virus
FLCs	Free Light Chains
GC	Germinal Center
GPA	Granulomatosis with Polyangiitis
HLA	Human Leukocyte Antigen
IL	Interleukin
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
MPA	Microscopic Polyangiitis
MPO-AAV	Myeloperoxidase-ANCA Associated Vasculitis
MTX	Methotrexate
NETs	Neutrophil Extracellular Traps
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
OSM	Oncostatin M
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
PD-L2	Programmed Death-Ligand 2
PLS-DA	Partial Least Squares Discriminant Analysis
PPI	Protein-Protein Interaction
PR3-AAV	Proteinase 3-ANCA Associated Vasculitis
RA	Rheumatoid Arthritis
RTX	Rituximab

SjD	Sjögren's Disease
SLE	Systemic Lupus Erythematosus
SNPs	Single Nucleotide Polymorphisms
STAT3	Signal Transducer and Activator of Transcription 3
TILs	Tumor-Infiltrating Lymphocytes
TME	Tumor Microenvironment
TNF	Tumor Necrosis Factor
TNFR2	Tumor Necrosis Factor Receptor 2
TNFRSF9	Tumor Necrosis Factor Receptor Superfamily Member

Introduction

Chronic inflammatory and autoimmune diseases, including various rheumatic conditions, are well-established risk factors for the development of malignant lymphoma. This association has attracted significant research interest in both oncology and rheumatology, as a deeper understanding of the mechanisms underlying this link could provide valuable insights into the pathogenesis of both diseases. Furthermore, such knowledge could potentially uncover therapeutic targets, aid in identifying high-risk patients who may require closer monitoring, guide the development of preventive strategies, and ultimately improve patient care. Factors proposed to contribute to this association include chronic immune activation, B-cell dysregulation, the pro-inflammatory milieu characteristic of rheumatic diseases, and the immunosuppressive therapies used in their management, an aspect of particular concern from a rheumatologist's perspective. However, the precise mechanisms driving this link remain incompletely understood.

This thesis includes two studies exploring mechanisms relevant to lymphoma development in rheumatic diseases, with a particular focus on rheumatoid arthritis (RA) and the lymphoma subtype diffuse large B-cell lymphoma (DLBCL). Additionally, one study, for the first time, characterizes lymphoma in patients with granulomatosis with polyangiitis (GPA), a form of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). The thesis also includes a proteomic study of AAV, encompassing both granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), aimed at identifying biological functions and pathways relevant to both shared and distinct disease mechanisms in these AAV subtypes. Although distinct from the lymphoma-focused studies, Study IV shares the overarching objective of advancing the understanding of disease mechanisms and immune dysregulation underlying these conditions, with the ultimate goal of contributing to clinically relevant advancements. Common denominators also include the proteomic approach, aligning with the protein analysis in Study I, and the focus on AAV, which connects to the GPA-related context of Study III. Additionally, the inclusion of this study reflects my personal clinical interest in vasculitic diseases.

Rheumatoid arthritis

Clinical aspects and epidemiology

Rheumatoid arthritis is a chronic autoimmune inflammatory disease characterized by symmetric polyarthritis, with a preference for involving the small joints of the hands and feet. The prevalence of RA is approximately 0.5–1% in Western countries [1]. In Sweden, the incidence is around 41 per 100,000 person-years. RA is 2–3 times more common in women than men, and the disease typically presents in middle age, with peak onset occurring between 50–60 years of age [2]. The typical clinical presentation of RA is an insidious onset of joint inflammation, gradually affecting an increasing number of joints. This inflammation results in joint pain and stiffness, often accompanied by malaise and fatigue due to systemic inflammation. Over time, the arthritis causes irreversible joint destruction leading to impaired function, progressive disability, and reduced quality of life. RA is not limited to joints but also involves systemic features. Patients with RA are at an increased risk of extra-articular manifestations, such as lung fibrosis, along with other comorbidities, including cardiovascular disease and malignancies, particularly malignant lymphoma [3].

Classification criteria

Although classification criteria for RA were primarily developed to ensure homogeneous patient groups with high specificity for clinical studies, they are often utilized in clinical practice as an aid in diagnosing patients. Two different RA criteria are applied in the studies of this thesis: the older 1987 criteria from the American College of Rheumatology (ACR) [4] (Table 1) and the newer 2010 ACR/European League Against Rheumatism (EULAR) criteria [5] (Table 2). The 1987 ACR criteria are highly specific and useful for identifying established RA in clinical trials, but less effective in recognizing disease at an early stage. To improve this, the 2010 ACR/EULAR criteria were introduced, focusing on early features of RA, enabling earlier diagnosis. Additionally, the highly RA-specific anti-citrullinated protein antibodies (ACPA), not included in the 1987 ACR criteria, were added in the 2010 criteria.

Table 1. The 1987 revised ACR criteria for the classification of rheumatoid arthritis

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint.
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry).
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician.
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).

* For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

Table 2. The 2010 ACR/EULAR classification criteria for rheumatoid arthritis

	Score
Target population: Patients who have at least 1 joint with definite clinical synovitis, with the synovitis not better explained by another disease.	
Classification criteria for RA (score-based algorithm): add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA	
A. Joint involvement§	
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints) #	2
4-10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint) *	5
B. Serology (at least 1 test result is needed for classification)	
Negative RF and negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
D. Duration of symptoms	
<6 weeks	0
≥6 weeks	1

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment; # Small joints include metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists; * In this category, at least 1 of the involved joints must be a small joint; other involved joints (large or small) are also counted.

Overview of etiology and pathogenesis

The etiology of RA is complex and involves a combination of immune dysregulation, genetic susceptibility, and environmental triggers. Smoking is the most well-established environmental risk factor, while other inhaled exposures and microbial factors have also been implicated [6]. Among genetic factors, the strongest association is linked to specific alleles of human leukocyte antigen (HLA)-DRB1 gene, collectively known as the shared epitope [7]. The HLA-DRB1 gene encodes major histocompatibility complex (MHC) class II molecules, which are responsible for presenting peptides on antigen-presenting cells (APCs) to T cells. The shared epitope is thought to influence antigen presentation and immune activation, increasing the likelihood of autoimmune responses against modified self-proteins, such as citrullinated peptides [8]. Up to 90% of ACPA-positive RA patients carry these genetic variants, whereas their prevalence is significantly lower among ACPA-negative patients [9]. In addition to HLA genes, several non-MHC genetic variants have been implicated in RA susceptibility. Among them polymorphisms in *PTPN22*, which encodes a phosphatase involved in T and B cell regulation, are strongly

associated with an increased risk of RA[10]. Variants in cytokine-related genes, such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6, have also been linked to RA risk [11, 12].

Following the breakdown of immune self-tolerance in RA, further immune dysregulation drives an inflammatory cascade targeting the joint synovium. This process involves the activation of T and B cells, as well as innate immune cells such as macrophages and neutrophils, leading to excessive production of pro-inflammatory cytokines, including TNF-α, IL-1, and IL-6 [13]. In the joint synovium, synovial fibroblasts and macrophages release additional pro-inflammatory mediators further amplifying the recruitment of immune cells to the site of inflammation. Over time, this results in synovial hyperplasia and the destruction of cartilage and bone through mechanisms such as osteoclast activation and the release of matrix-degrading enzymes [14].

Since a major focus of this thesis is the association between rheumatic diseases and B-cell lymphomas, it is relevant to highlight the role of B cells in RA pathogenesis. Beyond autoantibody production, B cells are thought to contribute to RA disease processes through multiple mechanisms, including acting as APCs that present autoantigens to T cells and secreting pro-inflammatory cytokines that drive synovial inflammation. Furthermore, B cells contribute to the formation of ectopic germinal centers and tertiary lymphoid structures within synovial tissue, supporting local autoantibody production and sustaining chronic inflammation [15]. Notably, ectopic germinal centers have also been implicated in lymphomagenesis, as they create a microenvironment that promotes prolonged B-cell activation, clonal expansion, and somatic hypermutation, processes that, under dysregulated conditions such as chronic inflammation, may elevate the risk of malignant transformation [16]. Furthermore, the central role of B cells in RA pathogenesis is underscored by the effectiveness of B-cell-depleting therapy (anti-CD20 antibodies) in treating the disease [17].

Treatment of Reumatoid arthritis

The growing understanding of pathogenetic mechanisms in RA has led to the identification of novel therapeutic targets and the development of highly effective disease-modifying antirheumatic drugs (DMARDs) over the past few decades. Beginning with the introduction of tumor necrosis factor (TNF) inhibitors (TNFi) as the first biological DMARD (bDMARD) in the late 1990s, additional therapeutic options have since emerged, including IL-6 blockade, inhibition of T-cell activation via CTLA4-Ig (abatacept), and B-cell depletion with anti-CD20 antibodies (rituximab). More recently, janus kinase (JAK) inhibitors, which belong to a new group of anti-rheumatic drugs referred to as targeted synthetic DMARDs (tsDMARDs), have emerged as an additional effective treatment, modulating intracellular signaling pathways [18]. Although glucocorticoids and conventional synthetic DMARDs (csDMARDs), particularly

methotrexate (MTX), remain a cornerstone of RA treatment, the addition of biologics and tsDMARDs has revolutionized treatment outcomes, enabling many patients to achieve stable remission, prevent joint destruction, and maintain functional ability.

Granulomatosis with polyangiitis and Microscopic polyangiitis

Clinical aspects and epidemiology

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are systemic vasculitides that, along with eosinophilic granulomatosis with polyangiitis (EGPA), comprise the group of ANCA-associated vasculitides (AAVs). GPA and MPA share several clinical and pathological features and have traditionally been grouped together as a single disease entity in clinical studies. In contrast, EGPA is more distinct and as this thesis focuses on GPA and MPA, EGPA will not be further discussed. A key feature shared by both GPA and MPA is the loss of tolerance to enzymes within neutrophil granules, resulting in the formation of ANCAs. In GPA, the ANCA predominantly targets proteinase 3 (PR3), whereas in MPA, myeloperoxidase (MPO) is the main antigenic target, although there is some overlap between diagnoses and ANCAs [19]. The vasculitis in both GPA and MPA primarily involves small- and medium-sized blood vessels, commonly affecting the kidneys and lungs, although nearly any organ can be involved. The vasculitis is necrotizing, leading to damage of the vessel walls and causing injury to affected organs, such as rapid kidney failure and pulmonary hemorrhage. Consequently, patients with GPA and MPA often present with severe organ- or life-threatening disease, although milder presentations also occur [20].

In a recent Swedish epidemiological study, the prevalence of GPA was reported to be approximately 16 per 100,000 adults, and MPA 10 per 100,000 adults. The incidence of GPA was estimated at 10 per million per year, and MPA at 6 per million per year. The median age at diagnosis for all AAV patients was 67.5 years. When divided into subgroups, the median age was 64 years for GPA and 72 years for MPA, with no clear predominance between the sexes [21]. The global geographic distribution of AAV varies, with GPA being more common in northern Europe and North America while MPA is more prevalent in southern Europe and East Asia [22].

Despite several overlapping clinical features, GPA and MPA exhibit distinct phenotypic differences, with granulomatous inflammation characteristic of GPA being the most notable distinguishing feature. Absent in MPA, this inflammation frequently affects the ear, nose, and throat (ENT) region and lungs, exhibiting tissue- and bone-destructive capacities that often lead to erosive changes in the ENT area and cavitating lung lesions [23]. Additionally,

GPA is associated with more wide-spread extra-renal organ involvement [24] and a higher rate of disease relapses [25]. In contrast, MPA is associated with higher rates of kidney involvement and a greater prevalence of fibrotic interstitial lung disease [26, 27].

Classification criteria

The first systematic approach to classify systemic vasculitides was introduced in 1990 by the American College of Rheumatology (ACR) [28]. However, these criteria had significant limitations, including the absence of MPA and the lack of incorporation of ANCA serology. In 1994, the Chapel Hill Consensus Conference (CHCC) introduced clinico-pathologic definitions for the major types of vasculitis, categorizing them by vessel size and incorporating MPA. These definitions were revised in 2012 to include a broader range of vasculitides and to highlight the importance of ANCA [19]. Building on the ACR and CHCC systems, the European Medicines Agency (EMA) introduced a stepwise classification algorithm in 2007. This algorithm incorporated aspects of the ACR and CHCC criteria along with ANCA markers, providing a systematic and consistent method for classifying small- and medium-vessel vasculitis [29]. The EMA algorithm has been widely used in epidemiological studies and was applied also in the AAV-related work of this thesis. More recently, the 2022 ACR/EULAR classification criteria for AAV were introduced, incorporating ANCA specificity and imaging findings to improve classification accuracy.

The traditional categorization of GPA and MPA has recently been questioned due to genetic and outcome studies suggesting that classification based on ANCA specificity may be more accurate. Consequently, there is increasing support for categorizing these diseases as PR3-ANCA positive (PR3-AAV) and MPO-ANCA positive (MPO-AAV) in clinical studies, rather than relying on the traditional clinical diagnoses of GPA and MPA [30]. Reflecting this shift, the proteomic study of AAV included in this thesis classified patients according to their ANCA specificity.

Overview of etiology and pathogenesis

The etio-pathogenesis of GPA and MPA is complex, and despite progress, much remains unknown. Similar to other autoimmune diseases, evidence suggests a complex interplay between polygenic susceptibility and environmental triggers. Regarding genetic contributions, the strongest associations have been found with variants of MHC class II genes, including both shared and distinct genetic risk factors for the two AAV subtypes. Specifically, genome-wide association studies (GWAS) have revealed that GPA is most strongly associated with variants in the HLA-DP region, while MPA is predominantly linked to polymorphisms in the HLA-DQ region. Among non-MHC genes, polymorphisms in the

PTPN22 gene, important for modulating immune cell signaling pathways, particularly in T and B cells, have been associated with both AAV subtypes. In contrast, *PRTN3*, which encodes proteinase 3 (the primary target of PR3-ANCA), and *SERPINA1*, which encodes alpha-1 antitrypsin (a regulator of proteinase 3 activity), have been identified as susceptibility loci specifically associated with GPA [31, 32]. Furthermore, as noted earlier, these studies demonstrated a stronger correlation between genetic loci and the presence of PR3- and MPO-ANCA, respectively, than with traditional clinical diagnoses (GPA and MPA), thereby challenging the current categorization of AAV.

Various environmental triggers, including occupational exposures, inhaled agents such as silica, and infections, have been implicated in the etiology of AAV, although the evidence remains limited. Notably, there are indications of distinct environmental factors contributing to the two AAV subtypes, with the strongest support for an association between Staphylococcus aureus colonization in the ENT area and GPA [33].

A key hypothesis regarding the pathogenic mechanisms in AAV suggests that direct interactions between ANCAs and neutrophils play a central role in disease progression, driving vessel wall inflammation and damage. Pro-inflammatory cytokines prime neutrophils, leading to the surface expression of MPO and PR3. When ANCAs bind to these membrane-expressed proteins, they trigger excessive neutrophil activation, resulting in adhesion to the endothelium and subsequent degranulation. This triggers the release of reactive oxygen species, lytic enzymes, and the formation of neutrophil extracellular traps (NETs), processes contributing to vascular inflammation and tissue damage. An important role has also been demonstrated for the alternative complement pathway, with particularly C5a acting as a key amplifier of the neutrophil-mediated inflammatory processes in AAV[34].

In addition to neutrophils, other immune cells, including autoreactive T and B cells as well as APCs such as macrophages and dendritic cells, contribute to the pathogenesis of GPA and MPA. ANCA production begins when PR3 and MPO are presented by APCs to CD4+ T cells, which subsequently stimulate B cells to differentiate into ANCA-producing plasma cells. In addition to this role, B cells also act as APCs and are involved in immune regulation through interactions with CD4+ T cells, highlighting their broader role in AAV pathogenesis [35].

Treatment of ANCA-associated vasculitis

GPA and MPA are severe and life-threatening diseases requiring intensive immunosuppression to prevent organ damage or death. The introduction of high-dose corticosteroids (CS) and cyclophosphamide (CYC) in the 1970s revolutionized treatment outcomes, changing the prognosis from approximately 90% mortality within two years to remission in most patients. However, due to the relapsing nature of the disease and the significant toxicity

associated with prolonged CS and CYC use, the need to develop less toxic treatment options became evident.

A major advancement in this regard came with the introduction of B-cell-depleting therapy using the anti-CD20 monoclonal antibody rituximab (RTX). Accumulating evidence of its efficacy, including the landmark randomized controlled trials RAVE and RITUXVAS [36, 37], has established RTX as a highly effective and less toxic alternative to CYC. Consequently, RTX has gradually replaced CYC as the preferred first-line therapy for inducing remission in recent years. Additionally, repeated RTX treatments have become the first-line option also as maintenance therapy, showing superior efficacy compared to previously used DMARDs, most commonly azathioprine or MTX [38]. Regarding the use of CS, treatment regimens aimed at reducing cumulative doses have gradually been implemented. Furthermore, the recent approval of the C5a receptor antagonist avacopan as part of AAV treatment has demonstrated significant CS-sparing efficacy [39].

Concerning future directions for AAV treatment, more personalized strategies may emerge as our understanding of disease mechanisms, risk stratification, and relapse risk improves. Notably, there is also a need to clarify whether optimal treatment strategies could differ between PR3-AAV and MPO-AAV subtypes.

Malignant lymphoma

Classification

Malignant lymphoma is a broad term covering a heterogeneous group of tumours that arise from the clonal proliferation of lymphocytes at various stages of differentiation. The cell of origin and the stage of differentiation determine the specific lymphoma subtype. Based on morphology, immunophenotype, gene expression profiling, and clinical characteristics, more than 60 distinct lymphoma subtypes have been identified and classified by the World Health Organization (WHO) [40]. Traditionally, these subtypes are grouped into two main categories: non-Hodgkin lymphoma (NHL), which accounts for approximately 90% of all lymphomas, and Hodgkin lymphoma (HL), representing the remaining 10%.

Non-Hodgkin lymphoma is a collection of a heterogenous group of lymphoid neoplasms originating from B-cells, T-cells or NK- cells. B-cell lymphoma accounts for about 85 % of NHL and T- and NK-cell lymphoma for about 15%. There are more than 40 unique NHL subtypes of which the most common specific subtypes are diffuse large B-cell lymphoma ((DLBCL), 30-40%) and follicular lymphoma ((FL), 20-30%) [41, 42]

Hodgkin lymphoma is a B-cell-derived neoplasm characterized by sparse tumour cells, known as Hodgkin and Reed–Sternberg (HRS) cells, surrounded

by a dense infiltrate of activated non-neoplastic lymphocytes. HL is divided into two main categories: classical Hodgkin lymphoma (cHL), which accounts for over 90% of HL cases, and nodular lymphocyte-predominant Hodgkin lymphoma [43].

Epidemiology

Lymphoma can develop at any age but the risk increases with advancing age, with two thirds being over 65 at the time of lymphoma diagnosis. Men are slightly more often affected than women. As a group, lymphomas represent a relatively common type of malignancy, with approximately 2500 new cases in Sweden each year accounting for around 3-4 % of all cancers [44]. The most common category of lymphoma, B-cell NHL, occurs at an incidence of approximately 20 cases per 100 000 per year in high-income countries [45].

Clinical aspects, prognosis and treatment

As previously mentioned, lymphoma is a heterogeneous disease including many subtypes, with clinical symptoms, disease progression, and prognosis varying depending on the specific type. Broadly, lymphoma subtypes can be divided into aggressive (high-grade) and indolent (low-grade) categories. Aggressive lymphomas typically present rapidly, often with enlarged lymph nodes and systemic symptoms such as weight loss, night sweats, and general malaise. In contrast, indolent lymphomas progress more slowly and are often associated with fewer symptoms. Diffuse large B-cell lymphoma is the most common aggressive lymphoma subtype, while FL is the most prevalent indolent lymphoma.[46].

Prognosis varies significantly across lymphoma subtypes. Additionally, age, patient performance status, and disease stage are important factors in assessing lymphoma severity, prognosis, and treatment options. Staging is commonly based on the Ann Arbor classification [47], which evaluates tumor burden, ranging from localized (stage I) to disseminated (stage IV) (Table 3). In recent years, PET-CT has been integrated into the Ann Arbor system, further improving the accuracy of disease severity assessment [48]. For most B-NHL subtypes, treatment typically consists of conventional chemotherapy combined with RTX as the standard of care, leading to curative outcomes in many patients and long-lasting responses in the majority [45].

In HL, conventional chemotherapy remains a cornerstone of treatment, while the integration of novel agents has further improved patient outcomes, leading to high cure rates. Immune checkpoint inhibitors targeting PD-1 signaling are increasingly used in lymphoma treatment and have shown particular efficacy in HL, where they are approved for relapsed or refractory disease.

In NHL, their efficacy has generally been less favorable, but ongoing research aims to identify subgroups who may respond better to these agents.

Whether lymphoma patients with pre-existing rheumatic conditions represent such a subgroup remains unknown and was one of the motivations for Study II, which investigated PD-1 and PD-ligand expression in lymphoma tissue from these patients [49].

Table 3. Ann Arbor staging system, modified Cotswold version

Stage	Area of Involvement
Stage I	Single lymph node group
Stage II	≥ 2 lymph node groups on the same side of the diaphragm
Stage III	≥ 2 lymph node groups on both sides of the diaphragm
Stage IV	Disseminated involvement of one or more extranodal organs
X	Bulk > 10 cm
E	Extranodal extension or single, isolated site of extranodal disease
A/B	B symptoms: weight loss > 10%, fever, night sweats

Risk factors for lymphoma and brief overview of pathogenesis

Familial aggregation of lymphoma has consistently been demonstrated in epidemiological studies, with first-degree relatives of patients having elevated risks for both NHL and HL. This strong familial association underscores a genetic component to lymphoma risk [50]. GWAS have identified over 60 susceptibility loci, including genetic variants within the HLA region and 8q24, which are associated with multiple subtypes, such as DLBCL and HL. Additionally, several subtype-specific loci have been identified, underscoring the polygenic background of lymphoma risk [51].

Factors within the tumor microenvironment (TME) are increasingly recognized as playing a critical role in lymphoma pathogenesis and progression. Research suggests that cytokines and growth factors produced by stromal and immune cells in the TME can promote lymphoma cell proliferation, inhibit apoptosis, and create an immunosuppressive environment that enables immune evasion [52].

In addition to genetic factors, autoimmune diseases are among the most well-documented risk factors for lymphoma, with the strongest associations observed in RA, Sjögren's disease (SjD) and Systemic Lupus Erythematosus (SLE)[53]. Other well-established risk factors include certain infections such as Epstein–Barr virus (EBV), Helicobacter pylori (HP), and Hepatitis C virus (HCV), as well as immunodeficient states, such as in human immunodeficiency virus (HIV) and in organ transplant patients receiving immunosuppressive therapy.

Rheumatic diseases and lymphoma

Autoimmune and inflammatory conditions, including various rheumatic diseases, are well-recognized risk factors for malignant lymphoma, as demonstrated by extensive epidemiological studies. Elevated lymphoma risks have been reported in RA, SjD, SLE, systemic sclerosis, celiac disease, Hashimoto's thyroiditis and GPA [54-56]. Additionally, clear links have been identified between certain inflammatory diseases and specific lymphoma subtypes, suggestive of shared biological processes and disease mechanisms underlying both conditions. Notably, diseases in which B cells play a significant role in pathogenesis, including SLE, SjD, and RA, are particularly associated with an increased risk of B-cell-derived NHLs, whereas mainly T-cell-driven diseases, such as celiac disease and psoriasis, are linked to a higher risk of T-cell NHLs. Risk estimates for NHL range from about twofold in RA, 3–7-fold in SLE, and 9–16-fold in SjD compared to the general population [57].

The precise mechanisms linking autoimmune and inflammatory conditions to lymphoma remain incompletely understood. Proposed contributing factors include shared genetic predisposition, chronic immune stimulation, dysregulated cytokine signaling, impaired immune surveillance, immunosuppressive therapies, and infectious agents [58-61]. The severity of the rheumatic condition is one of the factors most strongly supported by evidence as contributing to increased lymphoma risk. Strong associations between features of higher disease severity and lymphoma risk have been demonstrated, including in RA [62] and SjD [63, 64]. Chronic B-lymphocyte activation and sustained antigenic drive, possibly mediated by autoantibodies, are thought to play a key role in this process. Antigenic stimulation is hypothesized to promote B-cell clonal expansion, increasing the likelihood of genetic alterations during critical maturation processes, such as somatic hypermutation and class-switch recombination, ultimately leading to malignant transformation [65]. Further evidence suggests that local inflammatory processes can drive lymphoma development at sites of chronic inflammation in immune-mediated diseases. This is exemplified by the up to 1000-fold increased risk of mucosa-associated lymphoid tissue (MALT) lymphoma in the parotid glands of patients with SjD, enteropathy-associated T-cell lymphoma in the gastrointestinal tract of patients with celiac disease, and thyroid lymphoma in patients with Hashimoto's thyroiditis [66]. In these conditions, long-term immune activation in affected tissues may create an environment that increases the risk of genetic changes and lymphoma development [67], consistent with previously discussed mechanisms linking rheumatic diseases to lymphomagenesis.

As for shared genetic risk factors, polymorphisms in cytokine [68] and MHC genes [69] have been proposed to contribute to lymphomagenesis in rheumatic conditions. However, studies in patients with autoimmune diseases have not shown a significantly increased lymphoma risk among their relatives,

suggesting that shared genetic susceptibility plays a limited role in this association. [54].

Regarding infectious agents, EBV is particularly notable for its oncogenic potential and has been implicated in lymphomagenesis, especially in conditions of impaired immunosurveillance, such as HIV infection [70], and in organ transplanted patients in the setting of potent immunosuppressive therapy [71], but also, to a lesser extent, during immunosuppressive treatment for autoimmune diseases [72, 73]. However, its contribution to autoimmune-related lymphomas appears limited, as most studies show that the proportion of EBV-positive lymphomas and their subtype distribution in conditions like RA, SLE, and SjD resemble those in the general population [65].

The potential contribution of immunosuppression from DMARDs to the increased lymphoma risk in rheumatic diseases has long been a concern and the subject of extensive follow-up, with the most comprehensive data available for RA. While some studies have suggested a possible link with specific csDMARDs, such as azathioprine and MTX, larger studies have generally not found direct associations between csDMARD use and lymphoma risk. Similarly, bDMARDs, including TNFi and non-TNFi bDMARDs, were initially suspected of increasing lymphoma risk in RA, particularly in early studies focusing on TNFi. However, most subsequent research involving larger cohorts and longer follow-up periods has not demonstrated a clinically significant increase in lymphoma risk among TNFi-treated patients compared to those not exposed to TNFi. [65]. In a recent Swedish study by Hellgren et al., no significant difference in lymphoma incidence between bDMARD-treated and bDMARD-naive RA patients was seen [74]. Both groups demonstrated an increased lymphoma risk compared to the general population, but notably, when restricting the follow-up to more recent years, the study observed a reduced lymphoma risk among bDMARD-treated patients compared to those not receiving these treatments. In conclusion, these findings suggest that bDMARD treatment does not increase lymphoma risk in RA and may even help reduce the excess risk by improving control of disease activity. This aligns with the concept that chronic inflammation and sustained immune activation are key contributors to lymphoma development.

Lymphoma associated with Reumatoid arthritis and Granulomatosis with Polyangiitis

Since lymphoma in RA is the main focus of Studies I and II, and Study III explores lymphoma in GPA, the following section provides an overview of the associations between these conditions and lymphoma. As previously mentioned, RA patients have an approximately twofold increased risk of developing malignant lymphoma compared to the general population, with the highest excess risk observed for DLBCL. In a study by Baecklund et al., nearly 50%

of RA-associated lymphomas were DLBCL [60]. Additionally, these patients often presented with disseminated, advanced-stage disease at the time of lymphoma diagnosis. In a follow-up study of the same patient population, the prognostically less favorable non-germinal center B-cell-like (non-GCB) DLBCL subtype was overrepresented, accounting for approximately 70% of DLBCL cases [75], compared to 30-50% reported in the general population of western countries [76]. This finding suggests a significant role for activated peripheral B cells in RA-associated lymphomagenesis. As discussed previously, studies on lymphoma risk factors in RA have demonstrated strong correlations with markers of high RA disease activity, including Felty's syndrome, elevated erythrocyte sedimentation rate (ESR), and erosive joint disease. The strongest evidence for this correlation comes from the case-control study by Baecklund et al. [60], which found that patients with high cumulative RA disease activity had up to a 70-fold increased lymphoma risk compared to those with low disease activity. Notably, in contrast to SjD, lymphomas in RA rarely develop within the target organ, the joints, suggesting that lymphoma development in RA may be driven by systemic immune activation rather than localized immune responses. In this context, one proposed link between RA and DLBCL involves pro-inflammatory cytokines, which play a central role in RA pathogenesis and have also been implicated in DLBCL, even in the absence of rheumatic disease. Notably, the upregulation of TNF-α, IL-6, and IL-10 has been observed in both conditions, correlating with disease activity in RA and with negative prognostic features in DLBCL. [77]. Furthermore, the strong association between RA and B-cell lymphomas suggests that RA disease mechanisms involving persistent B-cell activation or dysregulation may contribute to lymphomagenesis in this setting. This aligns with the previously discussed mechanisms of B-cell lymphoma in rheumatic diseases and provides the rationale for Study I, which investigates B-cell-related factors in RA-DLBCL lymphomagenesis.

Concerning malignant lymphoma in GPA, previous studies have reported an increased risk, with a Swedish study by Knight et al. finding up to a fourfold higher incidence [56]. However, detailed information on the lymphomas that develop in GPA patients, including their subtypes, localization, and clinical characteristics of affected patients, has been lacking. Addressing this knowledge gap was the rationale for Study III, which characterized lymphomas in GPA and examined features of the underlying rheumatic disease. Additionally, we aimed to elucidate whether lymphoma develops at sites of chronic inflammation in GPA, similar to other immune-mediated diseases, such as the salivary glands in SjD.

Programmed cell death protein-1 signaling pathway

T-cells play a central role in immune responses by recognizing pathogenic peptides presented on MHC molecules, initiating and orchestrating immune defences against pathogens. While T-cell activation is essential for immunity, its regulation is equally important to prevent excessive or prolonged immune responses that could lead to tissue damage or autoimmunity. To maintain balance, immune checkpoints, such as the programmed cell death protein-1 (PD-1) signaling pathway, serve as key regulatory mechanisms that modulate T-cell activity, preserving immune homeostasis and self-tolerance [78]. Binding of its ligands (PD-L1 and PD-L2) to PD-1 expressed on T cells triggers inhibitory signals that suppress T-cell responses [79]. PD ligands are expressed on immune cells, including antigen-presenting cells such as macrophages, B cells, and dendritic cells, as well as various tissue cells. Their expression is inducible and upregulated by pro-inflammatory signals.

Programmed cell death protein-1 signaling pathway and rheumatic diseases

As noted, the PD-1 signaling pathway plays a pivotal role in maintaining self-tolerance, by preventing immune reactions against self-tissue and preserving immunological balance. Dysregulation or dysfunction of PD-1 signaling can increase the risk of autoimmunity, and growing evidence suggests its involvement in the development of various autoimmune diseases [80]. Notably, several single nucleotide polymorphisms (SNPs) in the PD-1 gene, contributing to PD-1 signaling dysregulation, have been linked to the development of rheumatic diseases such as RA and SLE [81, 82]. Additionally, the occurrence of immune-related adverse events from immunotherapy targeting PD-1 signaling in cancer treatment, including rheumatological conditions such as RA, SLE, and SjD [83], further underscores the connection between the PD-1 pathway and rheumatic diseases.

Programmed cell death protein-1 signaling pathway and cancer

While PD-1 signaling is essential for maintaining immune homeostasis under normal physiological conditions, many malignancies, including lymphomas, exploit this pathway by expressing PD-L1 on tumor cells. This suppresses T-cell activation and anti-tumor immune responses, allowing tumors to evade host immune defenses [84]. The discovery of this mechanism, along with other immune checkpoints used by cancer cells to evade immune surveillance, has led to the development of immunotherapies targeting these pathways. Checkpoint inhibitors, including PD-1/PD-L1 blocking agents, have revolutionized cancer treatment, showing efficacy across a growing number of cancer types (Figure 1).

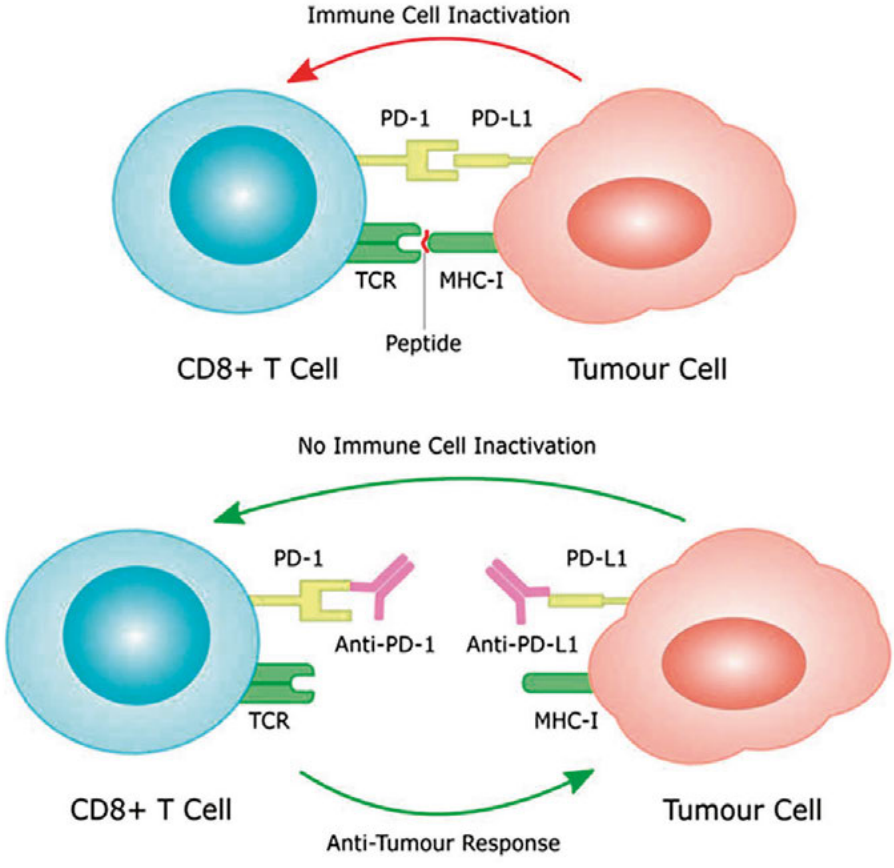

Figure 1. Overview of the PD-1/PD-L1 immune checkpoint system and its inhibition. The upper part illustrates immune cell inactivation via PD-L1 on tumor cells,binding to PD-1 on CD8+ T cells, leading to suppressed immune responses. The lower panel shows the effect of PD-1/PD-L1 inhibitors, which block this interaction, restoring T-cell activity and enabling an anti-tumor response. Image courtesy of Agnarelli et al., *Incorporating Immunotherapy in the Management of Gastric Cancer: Molecular and Clinical Implications* (PMCID: PMC9496849), used with permission.

Among lymphomas, PD-1/PD-L1 blocking agents have shown significant effectiveness in HL, as previously noted [85], whereas responses in various NHL subtypes, including DLBCL, have been more variable. However, NHL is a heterogeneous group of malignancies, and the expression of PD-1 and its ligands in tumor cells and the TME varies both across and within specific lymphoma subtypes, potentially influencing responses to anti-PD-1 therapy. Consequently, current research aims to identify NHL subgroups that are more responsive to PD-1 blockade. Whether lymphoma patients with pre-existing rheumatic conditions represent such a subgroup remains unknown and was a motivation for Study II, which investigated PD-1 and PD-ligand expression in lymphoma tissue from these patients.

Proinflammatory cytokines commonly overexpressed in rheumatic diseases, such as interferons, IL-1, IL-6, and TNF-α, can also influence the expression of PD-1 and its ligands [86-88]. Consequently, it can be hypothesized that PD-1 and its ligands may exhibit altered expression in tumor cells or within the TME in lymphoma patients with pre-existing rheumatic diseases compared to those without.

Present investigation

Aims

The general aims of this thesis were, in Studies I–III, to contribute to the understanding of the association between autoimmune diseases and lymphoma development by investigating molecular, biological and clinical characteristics that may underlie this link, and, in Study IV, to shed light on disease mechanisms and biological pathways relevant to both the shared and distinct pathophysiology of GPA and MPA.

I. To investigate RA-related B-cell activation and regulatory factors with potential implications for lymphomagenesis in RA-associated DLBCL.

II. To characterize PD-1, PD-L1, and PD-L2 expression in lymphoma tissue from patients with pre-existing RA, SLE, or SjD. Additionally, in RA-DLBCL, to investigate differences in PD-1/PD-ligand expression compared to DLBCL without rheumatic disease and relate findings to clinical characteristics of both RA and lymphoma, as well as survival outcomes.

III. To characterize lymphomas in patients with pre-existing GPA, focusing on subtypes, anatomical sites, stage, and associated characteristics of the GPA-disease.

IV. To identify key proteins and molecular signaling pathways activated in AAV and to shed light on molecular distinctions between AAV with proteinase 3 ANCA (PR3-AAV) and AAV with myeloperoxidase ANCA (MPO-AAV)

Materials and Methods

The specific methods used in the investigations of each study are described in detail in the respective manuscripts. Below is a brief summary of the study populations, study designs and key methods.

Data sources

The Swedish National Board of Health and Welfare administers several national registers that comprehensively document healthcare usage and outcomes in Sweden, with high coverage. These include registers on hospitalizations, healthcare utilization, and cancer diagnoses. Since 1947, all Swedish residents have been assigned a unique personal identity number, which is used across all national registers. This makes linkage of data from different registers to a specific individual possible, providing a significant advantage for medical research [89]. In the context of studying the association between rheumatic diseases and lymphoma, this linkage offers a unique opportunity to identify, in a population-based manner, patients initially diagnosed with rheumatic diseases who subsequently develop lymphoma, an otherwise challenging task.

The National Patient Register

The Swedish National Patient Register (NPR) is a population-based healthcare register in Sweden that includes data on patients admitted to inpatient care (the Swedish Inpatient Register) and those treated by specialists in outpatient care (the Outpatient Register). The register has recorded inpatient care data since 1964, with nationwide coverage since 1987, and began collecting nationwide data on non-primary outpatient care in 2001. For each individual, the NPR records information on all inpatient and non-primary care outpatient visits, including primary and contributory discharge diagnoses coded according to the International Classification of Diseases (ICD). Reporting to the Inpatient Register is mandatory, and hospitalization data is reported to be nearly 100% complete [90]. Coverage of the Outpatient Register is approximately 80%, with higher coverage among public care providers compared to private care [91].

The National Cancer Register

The Swedish National Cancer Register, was founded in 1958 and holds information of all incident cancers in Sweden, classified according to the ICD system. Reporting to the register is mandatory for all healthcare providers, both public and private. Data is submitted by both the diagnosing physician and the pathology laboratory, ensuring high data accuracy and a reported completeness close to 100% [92].

Study populations and study designs

Auto-Lymphoma cohort

The Auto-Lymphoma Study is a multicenter project initiated and coordinated from the Rheumatology Department at Uppsala University Hospital. It aims to investigate clinical and molecular aspects of the associations between autoimmune/inflammatory diseases and malignant lymphoma. It prospectively includes patients with initial rheumatic conditions and subsequent lymphoma development, with 14 centers regionally recruited across Sweden to enable a population-based approach. The cohort includes over 200 patients with rheumatic diseases and various lymphoma subtypes diagnosed between 2010 and 2021. The selected rheumatic diseases, RA, SLE, SjD, GPA, and ankylosing spondylitis/spondyloarthritis, were chosen based on previously reported increased lymphoma risks. Eligible patients are identified at each site by treating oncologists or hematologists, with diagnostic procedures, lymphoma staging, and treatment conducted according to national guidelines. These patients are followed for 5 years in a structured manner, with lymphoma tissue, blood, and clinical data collected as early as possible after diagnosis and additional samples and information gathered at predefined intervals during follow-up.

Study I

Study I includes 18 RA patients with DLBCL (RA-DLBCL) as cases, selected from the above-described Auto-Lymphoma cohort. These patients were diagnosed with DLBCL between 2010 and 2021 at various study sites across Sweden, with lymphoma diagnoses confirmed by a hematopathologist. All patients met the 2010 ACR/EULAR RA classification criteria, with RA diagnosed prior to lymphoma. Since the study specifically investigates B-cell-related factors in plasma and serum, and treatment for DLBCL typically involves B-cell-depleting therapy with RTX, only cases with blood samples collected prior to the initiation of lymphoma treatment were included to avoid potential impacts of treatment on the results. Similarly, the use of RTX as RA treatment was not permitted within 6 months prior to inclusion and blood sampling.

The control groups included 29 RA patients without lymphoma, selected from the Rheumatology Department at Karolinska University Hospital, with efforts made to achieve similar general and RA characteristics to the RA-DLBCL cases. Additionally, 44 population controls matched by sex and age to the RA-DLBCL cases were selected from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study [93].

Studies II-III

In Studies II and III, patients with initial rheumatic diseases and subsequent lymphoma were retrospectively identified using register-based methods, linking data from the NPR and the Cancer Register, as previously described. Rheumatic diagnoses were verified through medical record reviews to ensure that appropriate diagnostic criteria were met, and all lymphoma biopsies were reviewed by a hematopathologist to confirm the diagnoses. In both studies detailed clinical information was collected from the patients' medical records

In Study II, cases with RA or SLE and subsequent lymphoma were identified through previous population-based studies of lymphoma risk factors [94-96]. Individuals with RA (diagnosed between 1964 and 1994) or SLE (diagnosed between 1964 and 1995) were identified in the Swedish Inpatient Register. Subsequent lymphomas as the first primary cancer were identified through linkage with the Swedish Cancer Register, covering the periods 1964–2005 for RA and 1964–1995 for SLE. Additionally, patients with SjD were identified based on consecutive cases fulfilling SjD criteria, diagnosed and followed at the Rheumatology Department of Uppsala University Hospital between 1991 and 2002. Study II included two complementary parts: a cross-sectional analysis examining the intra-tumoral expression of PD-1 and its ligands (PD-L1 and PD-L2) in lymphoma tissue, and a case-control analysis comparing PD-expression in DLBCL tissue in RA and SLE patients and DLBCL controls without rheumatic diseases. The controls were identified from a previous population-based DLBCL study conducted in the Uppsala Health Care Region, Sweden, involving cases diagnosed between 1984 and 2002 [97]. Furthermore, for the RA-DLBCL patients, cumulative disease activity had been assessed in the original study using a composite score combining swollen and tender joint counts, erythrocyte sedimentation rate, and the treating physician's global assessment. These measures were repeatedly recorded from RA onset until lymphoma diagnosis. Cumulative disease activity was calculated as the area under the curve (AUC) for these repeated assessments, as described in detail in the original article [94]. These AUC values were used in Study II to examine correlations between cumulative RA disease activity and the expression levels of PD-1/PD-ligands in RA-DLBCL lymphoma tissue, serving as a means to investigate the potential impact of RA disease severity on their expression.

In Study III, a retrospective, descriptive study of clinical and lymphoma-related characteristics, 23 cases were identified in a population-based manner. These cases had an initial diagnosis of Wegener's granulomatosis (the previous name for GPA) or GPA recorded as the main or contributory diagnosis in the NPR between 1964 and 2012, followed by a lymphoproliferative malignancy registered in the Cancer Register between June 1973 and July 2011.

Study IV

Study IV is a prospective case-control study including 65 patients with newly diagnosed active AAV, characterized by systemic disease, identified across five rheumatology and nephrology centers in Sweden between 2008-2019. All patients fulfilled the EMA algorithm criteria for GPA or MPA. Blood samples were collected close to diagnosis, with patients allowed a maximum of four days of corticosteroid treatment and no other immunosuppressive therapy to minimize the impact of treatment on protein levels in this proteomic study. Patients were categorized based on ANCA serotype (PR3-AAV or MPO-AAV).

For comparison, 138 population controls matched for age and sex to AAV patients, with plasma samples collected between 1989 and 2017 by the Biobank Research Unit at Umeå University, were included. Additionally, 14 disease controls with active SLE defined by a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score ≥ 4, with blood samples collected between 1996 and 2012, and 31 disease controls with active RA, defined by a 28 joint Disease Activity Score (DAS28) ≥ 5, with blood samples collected between 2001 and 2012, were included.

Lymphoma tissue analyses

In Studies II–III, lymphoma tissue was reviewed by an experienced hematopathologist to confirm and, if necessary, reclassify the diagnosis according to the current WHO classification of tumors of hematopoietic and lymphoid tissues. DLBCLs were further classified as either GC type or non-GC subtype using the algorithm by Hans et al., based on immunohistochemical staining for CD10, Bcl-6, and IRF-4 antibodies [98]. The presence of EBV in lymphoma tissue was analyzed using EBV-encoded RNA (EBER) in situ hybridization [99].

In Study II, formalin-fixed, paraffin-embedded lymphoma biopsies were used to construct tissue microarrays (TMAs), on which immunohistochemical staining was performed to detect PD-1, PD-L1, and PD-L2 using specific monoclonal antibodies. Antigen retrieval and visualization were conducted according to standard protocols. Methods are described in detail in the article and summarized briefly here.

To distinguish PD-ligands expressed on tumor cells from those on tumor-infiltrating leukocytes, double immunostaining with the B-cell biomarker PAX-5 was performed (Figure 2). PAX-5 identifies B-cells, enabling differentiation between tumor cells (PAX-5 positive) and leukocytes (PAX-5 negative). Digital image analysis software was used as an automated method to quantify the number and proportion of PD-1, PD-L1, and PD-L2 positive tumor cells and leukocytes in the TMAs. Results for PD-L1 and PD-L2 were

expressed as the proportions of positive tumor cells of all tumor cells or positive leukocytes of all leukocytes. For PD-1, results were presented as the number of positive cells per high-power field (HPF) (0.0625 mm^2) to facilitate comparisons with manual evaluations in previous studies.

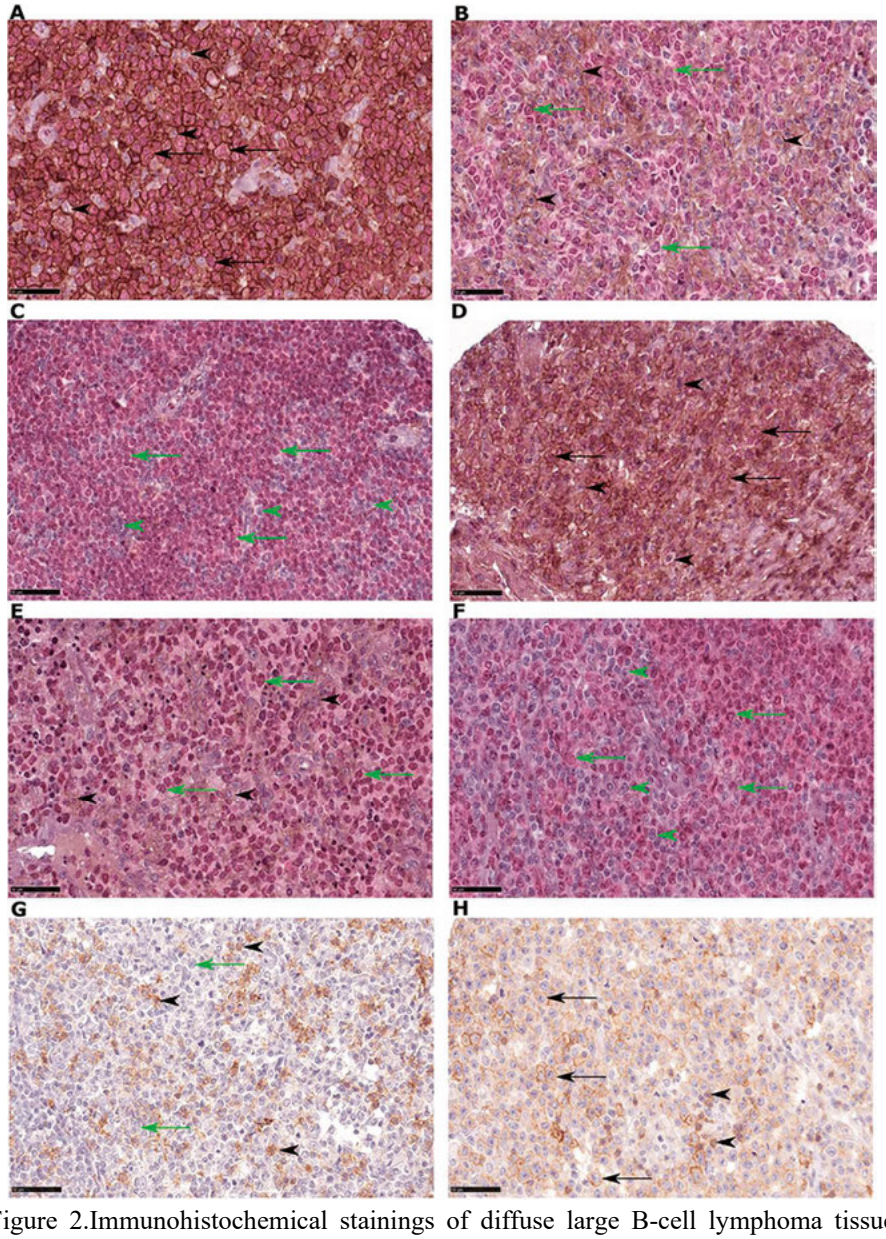

Figure 2.Immunohistochemical stainings of diffuse large B-cell lymphoma tissue from patients with pre-existing rheumatoid arthritis, showing PD-1, PD-L1/PAX5, and PD-L2/PAX5 expression. (A) High amounts of PD-L1+ tumor cells and TILs. (B) A high amount of PD-L1+ TILs and a low amount of PD-L1+ tumor cells. (C) Low amounts of PD-L1+ tumor cells and TILs. (D) High amounts of PD-L2+ tumor cells and TILs. (E) A high amount of PD-L2+ TILs and a low amount of PD-L2+ tumor cells. (F) Low amounts of PD-L2+ tumor cells and TILs. (G) A high amount of PD-1+ TILs and no PD-1+ tumor cells. (H) PD-1+ tumor cells and a few scattered PD-1+ TILs. Magnification: 400× in all images. Legends: Black arrow = PD-1+/PD-L1+/PD-L2+ tumor cells; Black arrowhead = PD-1+/PD-L1+/PD-L2+ TILs; Green arrow = PD-1-/PD-L1-/PD-L2- tumor cells; Green arrowhead = PD-1-/PD-L1-/PD-L2- TILs.

Proteomic methods

Protein quantification methods

In Study I, 12 cytokines and chemokines were measured in plasma using a custom 12-plex Luminex assay. In Study IV, the majority of proteins (n=181) were analyzed using Olink "Inflammation" and "Cardiovascular III" protein panels, utilizing the proximity extension assay (PEA) technique at the SciLifeLab Affinity Proteomics Infrastructure Unit in Uppsala, Sweden. Additionally, four proteins previously identified as potential biomarkers for active AAV or pulmonary disease, not included in the panels, were analyzed using Luminex at SciLifeLab.

PEA technology uses pairs of antibodies that specifically target a protein of interest. Each antibody is linked to an oligonucleotide uniquely matching the specific protein. When both antibodies in a pair bind to the same target protein, the probes are brought close enough to interact, allowing a DNA polymerase to extend the two oligonucleotides and form a DNA sequence that acts as a unique surrogate marker for the specific protein. This marker is quantified using quantitative real-time PCR, with the amount of DNA generated directly reflecting the concentration of the target protein in the sample [100]. (Figure 3)

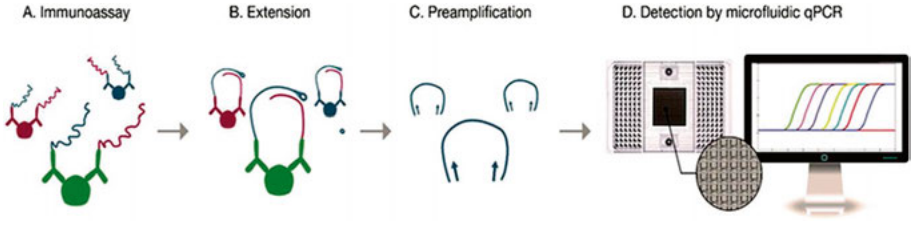

Figure 3. Steps of the Proximity Extension Assay. (A) Antibodies bind to the target protein (immunoassay). (B) Proximity allows DNA hybridization and extension. (C) Amplification of the DNA surrogate marker. (D) Detection and quantification by microfluidic qPCR. Image courtesy of Olink AB. Used with permission.

Protein interaction and pathway analysis

To explore interactions between proteins and their shared roles in biological systems, we utilized publicly available resources that are widely used in proteomic research and considered high quality sources of information. For protein-protein interaction (PPI) analysis, we used the STRING database, which contains data on protein interactions integrated from various sources, including curated experimental studies, pathway databases, computational predictions, and large-scale data sets [101]. STRING assigns confidence scores to each interaction by combining evidence from different sources, enabling

evaluation of data reliability. To further investigate the functions and roles of the studied proteins within biological systems and signaling pathways, we used the Gene Ontology (GO), KEGG, Reactome, and WikiPathways databases. These resources include and compile extensive information mapping the involvement of proteins in various biological processes, pathways, and functions. The data is integrated from various sources, including curated experimental studies, published literature, and computational predictions, such as gene co-expression. While each database varies slightly in focus and content, together they provide complementary and comprehensive resources for investigating the potential involvement of proteins in signaling pathways and biological systems [102].

Statistical analyses

Statistical analyses across the studies included Chi-square or Fisher's exact tests for comparing frequencies of categorical variables. Mann-Whitney U-tests, Kruskal-Wallis tests, and one-way ANOVA were used to compare continuous variables between groups. Survival analyses were conducted using Kaplan-Meier curves and log-rank tests. In Studies II and III, multivariable Cox regression models were also applied to assess predictors of survival outcomes.

In Study I, Spearman correlation analysis was performed to investigate associations between cytokine and chemokine levels, with results visualized as a correlation matrix.

In Study II, receiver operating characteristic (ROC) analysis combined with Youden's index was used to objectively determine the optimal cut-off values for PD-1 and PD-ligands in lymphoma tissue. This method identifies thresholds (cut-offs) that maximize the combined sensitivity and specificity for distinguishing between outcome groups [103]. Death at 12 months was chosen as a clinically relevant outcome measure, reflecting early disease progression and treatment failure. The cut-off value thus represents the expression level with the best combined sensitivity and specificity to differentiate between patients more likely to survive versus those more likely to die within 12 months.

In Study IV, multivariate partial least squares discriminant analysis (PLS-DA) was used to complement the univariate analysis of protein differential expression. This method identifies proteins most important for distinguishing between groups while also taking correlations between proteins into account. Briefly, PLS-DA simplifies complex, multidimensional data by reducing it into a smaller number of components that best explain the variance in the predictor variables (protein expression) while being most relevant to the response variable (group affiliation) [104]. PLS-DA assigns scores to proteins, providing a ranking based on their importance in driving group separations.

Results and discussion of findings

Study I

Study I analyzed cytokines and chemokines central to B-cell biology, free light chains (FLCs) as markers of B-cell activity, and a broad panel of RA-associated autoantibodies in RA patients with DLBCL. Comparisons were made with control groups, including RA patients without DLBCL and population controls.

The RA-DLBCL group exhibited significantly higher median levels of the B cell-associated proteins CXCL13 (796 vs. 199 pg/mL, $p < 0.0001$) and APRIL (1912 vs. 1514 pg/mL, $p = 0.03$), as well as the pro-inflammatory cytokines CXCL9 (1200 vs. 647 pg/mL, $p = 0.001$), TNF (36 vs. 14 pg/mL, $p = 0.002$), and IL-8 (12 vs. 6 pg/mL, $p = 0.02$). Spearman correlation analysis revealed distinct patterns in the RA-DLBCL group, with generally weaker correlations compared to RA and population controls, suggesting altered regulatory pathways in this group. Of the specific proteins analyzed, CXCL13 is particularly noteworthy as it exhibited the most pronounced elevation in RA-DLBCL patients compared to RA controls and for its impact on B-cell biology, relevant to both RA and lymphomagenesis. CXCL13 is a B cell chemoattractant with a central role in recruiting and organizing B cells within lymphoid tissue and driving the formation of tertiary lymphoid structures resembling germinal centers [105]. In RA, CXCL13 promotes the formation of tertiary lymphoid structures in synovial tissue, contributing to local B-cell activation, immune dysregulation, and chronic inflammation [106]. The influence of CXCL13 on B-cells, including activation and survival, has also been implicated in the pathogenesis of NHLs including DLBCL [107]. The involvement of CXCL13 in the disease mechanisms of both RA and lymphomagenesis suggests a possible contributory role in linking these conditions.

Both RA-DLBCL patients and RA controls exhibited elevated levels of free κ and λ light chains compared to population controls, reflecting increased B-cell activity. However, no significant differences in FLC levels were observed between RA-DLBCL and RA controls, providing no evidence, by this method, of heightened B-cell activity specifically in the RA-DLBCL group.

A high proportion of RA-DLBCL patients (94%) were seropositive for IgG CCP2 and/or IgM RF, the hallmark antibodies of RA, compared to 79% of RA controls ($p=0.2$). When additional autoantibody tests were included, all RA-DLBCL patients and 93% of the RA controls were seropositive. Furthermore, the extended antibody panel revealed no significant differences in ACPA fine-specificities or reactivity patterns to other AMPAs. Thus, our results cannot confirm differences in autoantibody frequencies or the presence of distinct autoantibody profiles associated with DLBCL development in RA among the antibodies analyzed in this broad panel.

Patients with the most advanced lymphoma (Ann Arbor stage IV) exhibited significantly higher IL-8 levels compared to those with less advanced stages (I–III), aligning with previous studies suggesting a potential role for IL-8 in DLBCL tumor burden and progression. [108]. Similarly, RA-DLBCL patients with the non-GCB subtype, which is overrepresented in RA-DLBCL, displayed elevated levels of IL-6 and IL-10 compared to GCB patients ($p=0.004$ and $p=0.03$, respectively). IL-6 and IL-10 have been implicated in the pathogenesis of non-GCB DLBCL, albeit in only a subset of cases, where they are suggested to drive tumor progression through STAT3 and NF-κB signaling [109]. Given that both cytokines are upregulated in RA, it could be speculated that the inflammatory milieu in RA, including increased IL-6 and IL-10 levels, might contribute to the overrepresentation of non-GCB DLBCL in RA patients.

To conclude, key findings in Study I include elevated levels of proteins relevant to B-cell activation and regulation in RA-DLBCL, along with altered protein correlation patterns suggestive of dysregulated regulatory pathways. These findings may help inform future research, particularly regarding CXCL13, which warrants further investigation as a potential contributor to the link between RA and lymphomagenesis.

Study II

Study II consists of two parts. The first part compared the expression of PD-1 and PD-ligands in lymphoma tissue across various subtypes from patients with pre-existing RA, SLE, or SjD. The second part focused specifically on DLBCL, comparing PD-1 and PD-ligand expression in DLBCL from patients with pre-existing RA or SLE, respectively, to a control group of DLBCL without rheumatic disease. In RA-DLBCL, findings were further analyzed in relation to RA disease characteristics, lymphoma-related features and survival outcomes.

In the first part, comparisons were made of the median proportions of PD-L1-positive and PD-L2-positive tumor cells and TILs, as well as the number of PD-1-positive TILs, across different lymphoma subtypes. The highest median proportions of PD-L1 positive tumor cells and TILs were observed in cHL (16% and 20%, respectively), followed by DLBCL (2% and 10%, respectively). PD-1 expression in TILs was most frequent in follicular lymphoma (FL) (median 236 cells/HPF), aligning with previous studies demonstrating high PD-1 expression in germinal center follicular cells in FL. Comparisons with previous studies of PD-1 and PD-ligand expression in lymphoma are challenging due to variations in immunohistochemistry methods and the frequent classification of results as positive or negative rather than using quantitative measures, with no standardized thresholds for defining positivity. However, our findings on the relative expression patterns of PD-1 and PD-ligands among lymphoma subtypes align with previous reports in non-

rheumatic contexts, as subtypes previously associated with high or low expression showed no clear deviations.

Notably, in cHL, the median expression of PD-L1 in tumor cells and TILs, as well as PD-1 in TILs, was higher in this study compared to a previous study from our research group that included cHL patients irrespective of pre-existing rheumatic diseases. This finding suggests a potentially higher expression of PD-1 and PD-L1 in cHL patients with pre-existing rheumatic diseases, but given the small number of cHL cases in this study, these results should be interpreted with caution.

In the second part, focusing on DLBCL, the expression of PD-1 and PD-ligands was categorized as high or low based on cut-off values determined using ROC analyses for each marker, as previously described. When comparing the proportions of cases with high versus low expression in RA-DLBCL and SLE-DLBCL, respectively, with DLBCL controls, no significant differences were observed. However, high expression of PD-L1 and PD-L2 in TILs was significantly less common in RA-DLBCL patients compared to controls (51% vs. 77%, $p < 0.001$, and 23% vs. 74%, $p < 0.001$, respectively).

When correlating RA-related disease characteristics with high or low PD-1/PD-ligand expression in RA-DLBCL patients, high PD-L1 expression in tumor cells was significantly more common in those with the highest cumulative RA disease activity compared to those with lower disease activity (5/25, 20% vs. 5/74, 7%, $p = 0.04$). No significant differences in other analyzed factors were identified between these groups that could explain or influence this correlation (table 4). It could be speculated that the inflammatory milieu in RA, characterized by elevated proinflammatory cytokines that may influence PD-L1 expression, contributes to the increased PD-L1 levels observed in DLBCL of patients with more severe RA

Furthermore, RA-DLBCL patients with high PD-L1 expression in tumor cells had significantly shorter overall survival compared to those with low PD-L1 expression (univariate hazard ratio [HR] 2.43, 95% confidence interval [CI] 1.07–5.50), an association that remained statistically significant in the multivariable analysis (HR 4.62, 95% CI 1.55–13.71), which included all significant variables from the univariate analysis. In contrast, high PD-1 expression in TILs was associated with superior overall survival in univariate analysis (HR 0.58, 95% CI 0.35–0.98) but lost significance in the multivariable analysis. High PD-L1 expression in tumor cells may contribute to poor survival through immune evasion, whereas high PD-1+ TILs may reflect an immunologically active tumor microenvironment that is more responsive to chemotherapy. These findings align with previous studies on DLBCL in patients without rheumatic diseases, demonstrating an association between high PD-L1 expression and inferior overall survival [110]. However, caution is warranted when interpreting the survival correlations, as the RA-DLBCL cohort was diagnosed relatively far back in time, influencing available treatment

regimens and potentially contributing to the generally poor survival outcomes observed in this group.

In conclusion, this study was the first to investigate PD-1, PD-L1, and PD-L2 expression in lymphoma patients with pre-existing rheumatic diseases. Our findings suggest an association between more severe RA and increased PD-L1 expression in DLBCL tumor cells, with potential mechanistic implications for RA-related lymphomagenesis. This association may also have prognostic significance and raises the question of whether DLBCL patients with severe RA could represent a subgroup that benefits more from PD-1 inhibitors. These findings warrant further investigation also in other chronic inflammatory conditions to determine whether they reflect a broader mechanism related to chronic inflammation.

Table 4. RA- and lymphoma-related characteristics, including high PD-L1 tumor cell expression, in RA-DLBCL: Comparison of the highest and lower RA disease activity groups.

	RA highest disease activity group	RA lower disease activity group	P-value*
Entire group, n (%)	25 (100)	74 (100)	
Women	19 (76)	39 (53)	0.04
Year of lymphoma diagnosis, median (range)	1987 (1967-1995)	1987 (1966-1997)	0.67**
Age at lymphoma diagnosis, median (range)	71 (47-84)	71 (31-87)	0.59**
High PD-L1 in tumor cells, n (%)§	5 (20)	5 (7)	0.04
non-GCB subtype, n (%)	18 (78)	47 (66)	0.28
Ann Arbor stage, n (%)			
I-II	7 (28)	24 (29)	0.57
III-IV	18 (72)	46 (66)	
EBV positive, n (%)	2 (8)	6 (8)	0.99
Active RA treatment, n (%)†	10 (40)	29 (39)	0.94
Any DMARD, n (%)	6 (24)	17 (23)	0.92
Corticosteroids for RA, n (%)	5 (20)	20 (27)	0.48

RA, rheumatoid arthritis; DLBCL, diffuse large B-cell lymphoma; GCB, germinal centre B-cell like; EBV, Epstein-Barr virus; DMARD, disease modifying anti-rheumatic drugs; *According to chi-square or Fischer's exact test, unless otherwise indicated; **According to Wilcoxon signed rank-test; §High PD-L1 expression in tumor cells = ≥17% of all tumor cells; †Active RA treatment=DMARD and/or corticosteroids

Study III

In Study III, the focus was to characterize the subtypes, localization, and clinical features of lymphomas arising in patients with pre-existing GPA, along with key features of their underlying rheumatic disease.

All except one of the 23 GPA-lymphoma patients, who had localized disease, exhibited severe, generalized GPA with systemic features. The organs most commonly affected by GPA were the ENT region (n = 18), followed by

the lungs (n = 14) and kidneys (n = 14). All patients had been treated with corticosteroids and at least one other immunosuppressive drug, with CYC used in 18 patients (78%) for remission induction. Additionally, 11 patients (48%) received CYC continuously as maintenance therapy for extended periods. The median duration of CYC treatment was 28 months (range 1–170).

The distribution of lymphoma subtypes is presented in Table 5. B-cell lymphomas predominated (83%), with DLBCL being the most common subtype, consistent with the distribution in the general population. Mantle cell lymphoma was identified in 13% of GPA patients compared to 4% in the Swedish Lymphoma Register; however, the small sample size makes the significance of this finding uncertain. Overall, no clear indication of associations between GPA and any specific lymphoma subtype was observed.

Despite a generally high degree of immunosuppression, we found no evidence implicating EBV as a major contributor to lymphomagenesis in GPA, as only one of 18 examined lymphomas tested positive for EBV by EBER in situ hybridization.

Notably, there was no evidence of enriched local lymphomagenesis in inflammation-affected target organs in GPA, such as the ENT region, where only one lymphoma occurred.

Survival outcomes after lymphoma diagnosis were markedly poor, with a median survival time of 4 months (range: 0–66 months). In most cases (n = 14; 74%), the cause of death was directly related to lymphoma or its treatment. Older age and advanced lymphoma stage at diagnosis, along with outdated lymphoma treatment regimens may have contributed to this poor survival. However, among those treated with curative intent (n = 17, 74%), the majority were treated with more modern regimens, including CHOP-based therapy (cyclophosphamide, hydroxydaunorubicin, oncovin, and prednisone) (n = 13), with seven patients receiving additional rituximab (R-CHOP). Furthermore, our results indicated a negative impact of prolonged CYC treatment, as worse overall survival was associated with cumulative CYC doses exceeding 25 g given as part of GPA treatment before lymphoma diagnosis.

In conclusion, the study did not reveal any clear association between GPA and any specific lymphoma subtype, although the frequency of mantle cell lymphoma warrants further attention. Additionally, no evidence was found for lymphoma development in typical sites of chronic inflammation in GPA. This study fills a knowledge gap by being the first to provide detailed information on lymphoma development in patients with pre-existing GPA, a challenging task considering the rarity of GPA and the difficulties in assembling a cohort of GPA patients who subsequently develop lymphoma.

Table 5. Distribution of lymphoma subtypes and stage among the GPA patients

Lymphoma subtype, WHO classification	Number (%)	Ann Arbor stage III-IV, n (%)
All patients	23 (100)	15 (75)
B-cell lymphoma in total	19 (83)	13 (81)
Diffuse large B-cell lymphoma	6 (26)	5 (83)
GC type	3 (50) *	3 (100)
Non-GC type	3 (50) *	2 (67)
Chronic lymphocytic leukemia	3 (13)	…..[1]
Mantle cell lymphoma	3 (13)	2 (67)
High grade B-cell lymphoma, unclassifiable	4 (17)	4 (100)
Low grade B-cell lymphoma, unclassifiable	1 (4)	1 (100)
B-cell lymphoma, unclassifiable	2 (9)	1 (50)
T-cell lymphoma in total	2 (9)	0
High grade T-cell lymphoma, unclassifiable	1 (4)	0
Peripheral T-cell lymphoma	1 (4)	0
High grade malignant lymphoma unclassifiable	1 (4)	1 (100)
Non-Hodgkin lymphoma unclassifiable	1 (4)	1 (100)

GC, Germinal center; *Percentage of all diffuse large B-cell lymphoma; [1]Patients with Chronic lymphocytic leukemia (CLL) excluded since staging according to Ann Arbor generally is not used for CLL

Study IV

In study IV, plasma proteins distinguishing active AAV from population controls, as well as PR3-AAV from MPO-AAV, were identified through univariate differential analysis and PLS-DA. Based on these results, distinct protein sets were defined as frameworks for further exploration of shared and unique mechanisms, through protein-protein interaction and functional enrichment analyses:

- **Shared AAV proteins (n = 21):** This set represented shared mechanisms common to both AAV subtypes and included proteins significantly upregulated in both PR3-AAV and MPO-AAV compared to population controls, supplemented by additional top differentiating proteins identified through PLS-DA.

- **PR3-AAV proteins (n = 35) and MPO-AAV proteins (n = 33):** These sets encompassed all proteins upregulated in each subtype compared to population controls, along with additional subtype-

specific top PLS-DA proteins. They were outlined to reflect the overall pathogenetic mechanisms active in each subtype, including both shared and subtype-specific processes.

- **PR3-AAV-specific (n = 17) and MPO-AAV-specific proteins (n = 23):** These sets included proteins uniquely upregulated in each subtype relative to population controls, as well as those differentially expressed in the direct comparison of PR3-AAV and MPO-AAV, and top PLS-DA proteins distinguishing the subtypes. These sets were defined to highlight key differentiating processes and pathways between PR3-AAV and MPO-AAV, providing insights into their molecular distinctions.

In the functional analysis of activated immune signaling pathways, shared findings across both AAV subtypes included broad inflammatory and immune-related processes, such as cytokine and chemokine activity, immune cell migration, chemotaxis, and STAT signaling. Among individual key differentiating proteins shared by both AAV subtypes relative to population controls, IL-6, TNF-R1, and TIMP-1 emerged as particularly significant across univariate analysis, PLS-DA, hub protein identification, and exploration of associated biological processes.

Results from the functional analyses of the PR3-AAV-specific and MPO-AAV-specific protein sets are presented in Table 6. Among the PR3-AAV-specific findings, a key result from was the enrichment of the “Expression of STAT3-upregulated extracellular proteins” pathway. This pathway emerged as the most significantly enriched in the PR3-AAV-specific protein set and ranked highly in the broader PR3-AAV protein set, suggesting a significant role not only in distinguishing PR3-AAV from MPO-AAV but also in the broader pathophysiology of PR3-AAV. STAT3 signaling is involved in essential immune processes, including granulocyte function, B cell antibody production, and memory T cell development, and its dysregulation has been linked to various autoimmune diseases [111]. Notably, a recent transcriptomic analysis comparing GPA and MPA, identified STAT3 as a putative upstream regulator of a GPA-specific signature distinct from that of MPA [112].

Among individual proteins in the PR3-AAV-specific set, MMP-1, MMP-9, and Oncostatin M (OSM) consistently emerged as key distinguishing proteins in comparisons with MPO-AAV, identified through univariate analysis, PLS-DA, and hub protein analysis. They also appeared in several top pathways in the functional analyses, including the STAT3-related pathway. Upregulated in PR3-AAV also compared to RA and SLE, OSM is a pleiotropic cytokine in the IL-6 family that primarily signals through STAT3. It induces endothelial cell chemokine production, which in turn promotes neutrophil and monocyte recruitment, and facilitates neutrophil adhesion to endothelial cells [113, 114]. Additionally, the "Oncostatin M signaling pathway" emerged as

one of the top enriched pathways in the PR3-AAV-specific set, indicating a potential regulatory role for OSM. Notably, OSM can induce MMP production and activation, a process implicated in granuloma formation in other granulomatous diseases, including tuberculosis, sarcoidosis, and granuloma annulare [115-117], as well as in bone erosive mechanisms in RA [118]. Similarly, in PR3-AAV/GPA, MMPs produced by nasal fibroblasts have been linked to the bone erosive capacity of granulomatous inflammation in the ENT region [119].

A key finding in the MPO-AAV-specific protein set was the overrepresentation of TNF-related proteins. Functional enrichment analyses further highlighted TNF-related pathways in MPO-AAV, with several ranking among the top enriched terms. Notably, the "TNFR2 non-canonical NF-κB pathway," along with additional related functional annotations, was among the top pathways identified. This pathway plays a fundamental role in immune regulation, including its influence on B and T cell function and survival. Dysregulated activation of non-canonical NF-κB signaling has been reported to support the survival and differentiation of autoantibody-producing B cells and has been implicated in the pathogenesis of autoimmune diseases, including RA and SLE [120].

Aside from TNF-related proteins, those linked to T-cell activity, CD8+ T cells in particular, were enriched in the MPO-AAV-specific protein set. CD8a, CD5 and TNFRSF9 were upregulated in MPO-AAV; CD5 is primarily expressed on T cells and is released upon their activation [121], while CD8a enhances antigen recognition of CD8+ T cells and TNFRSF9 supports their activation and effector functions [122]. These findings suggest a potentially greater involvement of CD8+ T cells in MPO-AAV pathophysiology.

To conclude, key findings in Study IV suggest enhanced STAT3 signaling in PR3-AAV compared to MPO-AAV, with OSM as a potential contributor to this activation. Furthermore, OSM-driven STAT3 signaling may underlie the increased MMP expression observed in PR3-AAV, which is relevant to its pathophysiology and characteristic granulomatous inflammation. In contrast, MPO-AAV was marked by prominent TNF signaling, with a specific role suggested for the TNFR2 non-canonical NF-κB pathway and indications of a potentially more pronounced involvement of CD8+ T cells compared to PR3-AAV.

Table 6. Functional enrichment analysis of PR3-AAV-specific and MPO-AAV-specific protein sets, highlighting all identified processes and pathways for PR3-AAV and the top 15 for MPO-AAV.

PR3-AAV-specific protein set		MPO-AAV-specific protein set	
Enrichment term	P_{adj}	Enrichment term	P_{adj}
Expression of STAT3-up-regulated extracellular proteins	$5.1x10^{-11}$	Viral protein interaction with cytokine and cytokine receptor	$1.3x10^{-14}$
Interleukin-4 and Interleukin-13 signaling	$2.5x10^{-9}$	TNFR2 non-canonical NF-kB pathway	$1.5x10^{-14}$
IL-17 signaling pathway	$1.1x10^{-7}$	TNFs bind their physiological receptors	$5.7x10^{-12}$
Malaria	$6.3x10^{-7}$	cIAP1.2 ubiquitinates NIK in cIAP1.2:TRAF2::TRAF3:NIK	$5.2x10^{-10}$
Lung fibrosis	$1.4x10^{-6}$	K63polyUb-cIAP1.2 ubiquitinates TRAF3	$5.2x10^{-10}$
Oncostatin M signaling pathway	$1.5x10^{-6}$	TRAF2 ubiquitinates cIAP1.2 in cIAP1.2:TRAF1:TRAF2:TRAF3:NIK	$5.2x10^{-10}$
IL10 negatively regulates extracellular inflammatory mediators	$5.7x10^{-6}$	Interleukin-10 signaling	$1.6x10^{-8}$
Hepatitis C and hepatocellular carcinoma	$9.7x10^{-6}$	death receptor activity	$2.5x10^{-8}$
Photodynamic therapy-induced NF-kB survival signaling	$1.3x10^{-5}$	LTA trimer binds TNFR1.1B.14	$3.6x10^{-8}$
Bladder cancer	$1.6x10^{-5}$	Protesomal degradation of K48polyUb-TRAF3	$5.9x10^{-8}$
Interleukin-10 signaling	$1.6x10^{-5}$	TNF receptor superfamily (TNFSF) members mediating non-canonical NF-kB pathway	$5.3x10^{-6}$
		Neuroinflammatory response	$7.8x10^{-6}$
		Prostaglandin signaling	$2.1x10^{-5}$
		Inflammatory response pathway	$2.1x10^{-5}$
		Chemokine receptors bind chemokines	$4.1x10^{-5}$

AAV = ANCA-associated vasculitis; PR3-AAV = proteinase 3 ANCA-positive AAV; MPO-AAV = myeloperoxidase ANCA-positive AAV; P_{adj} = adjusted P value.

General discussion

One major challenge in studying the mechanisms underlying the link between rheumatic diseases and increased lymphoma risk is identifying patients with a pre-existing rheumatic disease who later develop lymphoma. The Swedish healthcare registers, with their high completeness and the possibility to link

different registers, offer a significant advantage for identifying these patients. Furthermore, this allows case identification in a population-based setting, facilitating a representative selection of cases for analysis and reducing the risk of selection bias. The cases in Studies II and III were identified through this register-based approach. However, as diagnoses in these registries are based on ICD codes, their accuracy can vary due to potential coding errors or misclassifications. To address this, medical record validation of ICD-coded diagnoses was conducted in Studies II and III to ensure that included cases met the appropriate diagnostic criteria. This validation process reduced the risk of misclassification, strengthening the reliability of our findings and highlighting the importance of diagnostic confirmation in register-based research. In addition to this retrospective method, the prospective and population-based design of the Auto-Lymphoma study enabled the identification of a well-defined cohort of RA patients at the onset of DLBCL in Study I. Importantly, it also allowed for the prospective collection of blood samples and lymphoma tissue, a prerequisite for robust and reliable analyses. Furthermore, the longitudinal design allowed for systematic follow-up and resampling. Given the rarity of such study populations, the well-defined and validated cases of rheumatic diseases and subsequent lymphoma, identified through both register-based and prospective population-based approaches, constitute a strength of the lymphoma-related studies in this thesis.

While these methodological strengths were central to the lymphoma-focused studies, similar challenges exist in research on rare autoimmune diseases such as AAV, particularly in establishing adequately sized, well-characterized cohorts. In this regard, Study IV, which also employed a prospective design allowing for blood sampling near the time of diagnosis, highlights the importance of multi-center collaboration in overcoming this difficulty.

Another major challenge in conducting a proteomic study in AAV is the acute and life-threatening nature of the disease, which makes it difficult to include patients and obtain blood samples before the initiation of potent immunosuppressive treatment. Such treatment can significantly alter protein expression, affecting the reliability of findings. A strength of Study IV in this regard was that, although corticosteroid treatment for up to four days was permitted and had been administered to approximately half of the cases, no other immunosuppressive therapy had been given before sampling, thereby reducing the risk of significant treatment-related effects on the results.

There are limitations to theses studies. While the register-based approach in Studies II and III provides a major advantage in identifying cases of rheumatic disease and lymphoma, the inherent limitations of retrospective methods must be acknowledged. These include the risk of incomplete or missing data, potential data entry errors, and challenges in controlling for confounding factors. As discussed above, using registers to identify cases also carries a risk of coding errors or misclassification. However, medical record reviews and diagnosis validation helped reduce this risk in our studies. In the proteomic

studies (I and IV), technical challenges such as variations in sample preparation cannot be ruled out as influencing the results. However, efforts were made to minimize these effects. Samples were handled according to customary recommendations for storage and processing, and quality control measures were implemented to ensure data reliability. Internal controls were included to account for technical variability, ensure measurement consistency, and minimize potential biases. For Study III, the lack of control groups (for both GPA and lymphoma) impairs the ability to draw conclusions about features of GPA that might serve as risk factors for lymphoma development, as well as the distribution and characteristics of lymphoma subtypes. Sample sizes in Studies I, III, and IV were relatively small, limiting statistical power and, consequently, the ability to make certain interpretations and draw conclusions. Additionally, in the AUTO-LYMPHOMA study, there is a possibility that some severely ill patients were not included because they were considered inappropriate to approach for participation. Furthermore, for some of the most severely ill patients, sampling before the initiation of urgent lymphoma treatment may have been unfeasible, leading to their exclusion from Study I. This could have introduced a selection bias, with potential underrepresentation of the most aggressive cases.

As for future perspectives, further research building on the findings of this thesis, integrating complementary approaches, could refine these results. Methods such as single-cell analyses and transcriptomics could help elucidate the roles of specific immune cells, particularly B-cell regulation and activation in RA-associated lymphoma. Additionally, expanded investigations into the observed subtype-specific alterations in STAT3 and TNF signaling in PR3-AAV and MPO-AAV could clarify their immunopathological significance and their contributions to the distinct disease characteristics of these AAV subtypes. Such research could inform clinically relevant advances, including biomarker discovery and the development of novel therapeutic options tailored to each AAV subtype, ultimately improving patient care.

Concluding remarks

The lymphoma-related studies in this thesis approach the association between rheumatic disease and lymphoma from different perspectives and contribute to the knowledge of this link, including B-cell-related factors in RA-DLBCL, the role of the PD-1 pathway in lymphoma associated with rheumatic diseases, and, for the first time, the characteristics of lymphoma in GPA. The proteomic study on PR3-AAV and MPO-AAV adds to the understanding of disease mechanisms that may contribute to the differences in clinical characteristics between these subtypes. These studies may also guide further research on immune dysregulation and the mechanisms linking immune-mediated diseases to lymphomagenesis, as well as pathogenic processes in AAV. Together such

knowledge has potential implications for enhancing diagnostic methods and advancing treatment development, ultimately contributing to clinically relevant improvements in patient care for these conditions.

Sammanfattning på svenska

Patienter med vissa autoimmuna och inflammatoriska sjukdomar, inklusive flera reumatologiska tillstånd som reumatoid artrit (RA) och granulomatös polyangit (GPA), har en ökad risk att utveckla malignt lymfom. Det finns också en känd koppling mellan vissa reumatiska sjukdomar och en särskilt ökad risk för specifika typer av lymfom, vilket tyder på att det kan finnas gemensamma underliggande orsaker och sjukdomsmekanismer. De exakta orsakerna till detta samband är inte klarlagda, men faktorer som långvarig stimulering av immunsystemet, aktivering av B-celler i en kronisk inflammatorisk miljö och gemensamma genetiska riskfaktorer har föreslagits kunna vara bidragande. Att öka kunskapen om dessa processer är viktigt på flera sätt. Det kan ge nya insikter i hur både reumatiska sjukdomar och lymfom utvecklas, förbättra möjligheterna att tidigt identifiera patienter med hög risk för lymfom och bidra till utvecklingen av förebyggande åtgärder för att minska lymfomrisken hos patienter med reumatiska sjukdomar.

Tre av avhandlingens studier undersöker sambandet mellan reumatiska sjukdomar och lymfom ur olika perspektiv. De två första studierna fokuserar på molekylära mekanismer som kan vara av betydelse, med särskilt fokus på RA och diffust storcelligt B-cellslymfom (DLBCL). Den tredje beskriver lymfom som utvecklats hos patienter med GPA, något som inte studerats i detalj tidigare.

Avhandlingen inkluderar även en proteomikstudie (arbete IV), där ett stort antal proteiner med känd koppling till inflammation och hjärt-kärlsjukdom undersöktes hos patienter med ANCA-associerad vaskulit (AAV), inkluderande undergrupperna GPA och mikroskopisk polyangit (MPA). Sjukdomsmekanismerna vid AAV är till stora delar okända, liksom orsakerna till de skilda sjukdomsyttringarna vid GPA och MPA. En djupare förståelse av dessa processer kan bidra till utvecklingen av mer specifika diagnostiska verktyg och behandlingsstrategier anpassade för respektive undergrupp.

I arbete I undersöktes B-cellsrelaterade mekanismer vid RA-associerat DLBCL ur olika perspektiv. Ett viktigt fynd var förhöjda nivåer av flera inflammations- och B-cellsrelaterade proteiner jämfört med både RA- och populationskontroller. Särskilt intressant med tanke på en möjlig bidragande roll i lymfomutvecklingen vid RA var den förhöjda nivån av CXCL13, ett protein med en viktig roll för B-cellernas funktion.

I arbete II analyserades uttrycket av ”programmed cell death” protein 1 (PD-1) och dess ligander PD-L1 och PD-L2 i lymfomvävnad från patienter med bakomliggande reumatiska sjukdomar, med särskilt fokus på RA-associerat DLBCL. Det framkom inga tydliga generella skillnader i uttryck jämfört med lymfom utan reumatisk sjukdom. Ett viktigt fynd var dock tecken på att hög RA-relaterad sjukdomsaktivitet skulle kunna bidra till ett ökat uttryck av PD-L1 i DLBCL-tumörceller.

Arbete III undersökte lymfom hos patienter med underliggande GPA, med fokus på lymfomtyper, deras lokalisation samt kliniska kännetecken för både lymfomen och den reumatiska sjukdomen. Patienterna identifierades genom nationella hälsoregister på ett populationsbaserat sätt, vilket ger ett urval representativt för GPA-patienter i Sverige. Det framkom inga tydliga tecken på att någon särskild typ av lymfom skulle vara överrepresenterad vid GPA. Det fanns heller inte belägg för en ökad lymfomrisk i organ som ofta påverkas av kronisk inflammation vid GPA. Endast ett fall av lymfom var lokaliserat till de övre luftvägarna.

I Arbete IV identifierades centrala proteiner, biologiska funktioner och signalvägar som var både gemensamma och specifika för undergrupperna av AAV, vilka delades in utifrån ANCA-antikroppstyp i proteinas 3 (PR3)-AAV och myeloperoxidas (MPO)-AAV. Bland de viktigaste fynden fanns tecken på en större betydelse för STAT3-signalering i PR3-AAV och en mer framträdande roll för TNF-signalering i MPO-AAV. Sammantaget tyder resultaten i Arbete IV på att både gemensamma och skilda immunologiska signalvägar är aktiverade i det akuta skedet av PR3-AAV och MPO-AAV.

Sammanfattningsvis lyfter studierna i denna avhandling fram nya aspekter av sambandet mellan autoimmuna sjukdomar och lymfom samt bidrar till att utveckla förståelsen för både gemensamma och skilda sjukdomsmekanismer vid AAV.

Acknowledgements

This thesis was carried out at the Department of Medical Sciences, Faculty of Medicine, Uppsala University. I would like to thank all of you who have helped and supported me in various ways during these years and contributed to this thesis. In particular I would like to acknowledge:

All the patients and blood donors who participated in this research, making it possible.

Eva Baecklund, my main supervisor for the majority of my PhD journey. My deepest gratitude to you for inviting me to participate in your research, for your continuous encouragement, and for always being there to offer guidance and support. I am truly grateful for the knowledge you shared, your patience, and your belief in me, especially during challenging times. Your constant support throughout has been invaluable; without it, I couldn't have managed.

Johanna Dahlqvist, who took over as my main supervisor in the final year of my PhD. Thank you for patiently guiding me through the last study of my thesis and for helping me navigate the administrative tasks necessary to complete my PhD, a particularly challenging task considering my unstructured and absent-minded personality. I truly appreciate your guidance and support in helping me cross the finish line.

Ann Knight, my co-supervisor, for sharing your invaluable clinical and research knowledge. For being an inspiring role model, not only professionally but also personally. Your dedication as both a doctor and researcher has been a continuous source of motivation for me. Thank you for always making time to listen, offer guidance, and provide constant encouragement and support

My co-supervisor, *Gunilla Enblad,* for your support and contribution of expertise in oncology

Peter Hollander, for your patient guidance and assistance with the execution and authorship of the PD-1-related study.

Nora Euler, Vivianne Malmström, and *Caroline Grönwall,* for the nice collaboration and for allowing me to include our collaborative study in my thesis.

Carin Backlin, Alina Johansson and *Rezvan Kiani Dekhordi* for your help with coordination and logistical support.

All collaborators in the *AUTO-LYMPHOMA study group.* Especially *Christer Sundström,* for your expert review of the lymphoma tissue.

All collaborators and co-authors in the ANCA vasculitis collaboration: *Annette Bruchfeld, Per Eriksson, Sophie Ohlsson, Ewa Berglin*, and *Solbritt Rantapää-Dahlqvist.* A special thanks to *Iva Gunnarsson,* who not only gave me the opportunity to begin my career as a resident in rheumatology at the rheumatology department at Karolinska University Hospital back in the days, but has also been a constant role model and source of inspiration for me ever since.

All other co-authors of the papers, not already mentioned. *Daniel Molin, Erik af Klint, Monika Hansson, Anders Larsson,* and *Vincent van Hoef.*

Professors *Lars Rönnblom* and *Gunnel Nordmark,* who, with overall research responsibility, contributed to making my PhD studies possible.

All friends and colleagues at the Department of Rheumatology for your support and for taking care of my patients during my research time, making my dissertation possible. *Karin Hjorton, Johan Back, Maria Lidén, Lilian Vasaitis, Dag Leonard, Efthymia Sidiropoulou, Ágnes Szentpétery, Hanna Lindberg, Cecilia Fugmann, Shamisa Adeli, Ioanna Giannakou, Bjarni Thorsteinsson, Elisabeth Skoglund, Parisa Dadfar, Karin Bolin, Nikolas Malliotis, Nils Holmqvist, Emrah Seyhoglu, Berglind Árnadóttir, Ólafur Sturluson, Antonios Chrysovalantis Tsakiris,* and *Edward McHale Sjödin.*

All the staff at the rheumatology clinic and the research group at the Rudbeck Laboratory.

My parents, *Margareta* and *Ulf*, for always being there for me. *Lars*, for your support and for introducing me to synth music back in the 80s, which has been frequently played during the work on this thesis.

Caroline, for all your support, your admirable patience and endurance with me. *Ebba, Carl*, and *Ellen,* for putting up with a sometimes grumpy and tired dad.

This study was supported by grants from Uppsala Regional Research Council (ALF), the Swedish Cancer Society, the Swedish Society of Rheumatology, King Gustaf V's 80-year Foundation, the Lions Cancer Research Foundation in Uppsala, the Agnes and Mac Rudberg Foundation, the Swedish Kidney Foundation and Uppsala University Hospital

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