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Autoantibodies in rheumatoid arthritis and inflammatory bowel disease

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Abstract

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Autoantibodies are key features of several immune-mediated inflammatory diseases and may serve as biomarkers for diagnosis, prognosis, and disease stratification. The overall aim of this thesis was to investigate the role of autoantibodies for diagnosis and prognosis of rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), with emphasis on their associations with age, sex, disease phenotype, severity, and preclinical disease development.

In Study I, the occurrence of RA-associated autoantibodies was analysed in relation to age at diagnosis and sex. Anti-cyclic citrullinated peptide 2 (anti-CCP2) positivity was associated with younger age, whereas IgA rheumatoid factor (RF) was associated with higher age at diagnosis. These findings demonstrate that demographic factors influence serological phenotypes and should be considered in studies of RA.

In Study II, individual autoantibodies and their combinations were examined in relation to clinical features at RA diagnosis. Anti-citrullinated protein/peptide antibodies (ACPAs) were associated with lower swollen and tender joint counts, while RF was associated with elevated inflammatory markers in an ACPA-dependent manner. No significant associations were observed for the composite DAS28 score, indicating that individual DAS28 components should be analysed separately when evaluating serological phenotypes.

In Study III, the diagnostic and prognostic potential of IgG anti-integrin αvβ6 autoantibodies (anti-αvβ6) was investigated in newly diagnosed IBD. Anti-αvβ6 demonstrated high diagnostic accuracy for ulcerative colitis (UC) and was associated with greater disease extent and inflammatory activity. Although prognostic discrimination between indolent and aggressive UC was modest, persistent antibody levels were linked to a more severe disease course.

In Study IV, the predictive ability of anti-αvβ6 for future UC was evaluated in population-based cohorts. Anti-αvβ6 was detectable years before clinical diagnosis, with predictive performance increasing closer to disease onset. Elevated levels were also observed in early life, indicating loss of tolerance long before clinical manifestation.

Together, these findings demonstrate that autoantibody profiles reflect biologically meaningful heterogeneity in both RA and IBD, and support measurement of autoantibodies for risk stratification and biomarker-guided approaches in immune-mediated inflammatory diseases.

Keywords: autoantibodies, rheumatoid arthritis, inflammatory bowel disease

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To all mothers in science —
who nurture curiosity at work and at home,
who balance experiments and bedtime stories,
and who prove every day that brilliance and love can grow side by side

List of Papers

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

I. **Pertsinidou E**, Manivel VA, Westerlind H, Klareskog L, Alfredsson L, Mathsson-Alm L, Hansson M, Saevarsdottir S, Askling J, Rönnelid J. (2021) Rheumatoid arthritis autoantibodies and their association with age and sex. *Clin Exp Rheumatol.*, 39(4):879–882

II. **Pertsinidou E**, Saevarsdottir S, Manivel VA, Klareskog L, Alfredsson L, Mathsson-Alm L, Hansson M, Cornillet M, Serre G, Holmdahl R, Skriner K, Jakobsson PJ, Westerlind H, Askling J, Rönnelid J. (2024) In early rheumatoid arthritis, anticitrullinated peptide antibodies associate with low number of affected joints and rheumatoid factor associates with systemic inflammation. *Ann Rheum Dis.*, 83(3):277–287

III. **Pertsinidou E**, Salomon B, Bergemalm D, Salihovic S, Hedin CRH, Ling Lundström M, Keita ÅV, Magnusson MK, Eriksson C, Bengtson MB, Grännö O, Aabrekk TB, Movérare R, Rydell N, Ekoff H, Rönnelid J; BIO-IBD consortium; D'Amato M, Detlie TE, Huppertz-Hauss G, Opheim R, Ricanek P, Kristensen VA, Öhman L, Söderholm JD, Kruse R, Lindqvist CM, Carlson M, Repsilber D, Høivik ML, Halfvarson J. (2025) Anti-integrin αvβ6 IgG antibody as a diagnostic and prognostic marker in ulcerative colitis: A cross-sectional and longitudinal study defining a specific disease phenotype. *J Crohns Colitis*, 19(5): jjaf062

IV. **Pertsinidou E**, Grännö O, Bergemalm D, Salomon B, Salihovic S, Eriksson C, Lindqvist CM, Movérare R, Rydell N, Lanka V, Magnusson PKE, Hultdin J, Repsilber D, Grip O, Ludvigsson J, Karling P, Halfvarson J. Preclinical anti-integrin αvβ6 autoantibodies in ulcerative colitis and colonic Crohn's disease: early-life emergence, environmental modifiers, and co-abundance with inflammatory proteins in large population-based cohorts. Manuscript.

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Contents

Introduction ... 11
- The human immune system ... 11
- Autoimmunity ... 13
- Autoantibodies ... 15
- Rheumatoid Arthritis (RA) ... 16
  - Epidemiology, genetics and aetiology ... 16
  - Diagnosis, autoantibodies and serology ... 17
  - Management of RA ... 21
- Inflammatory Bowel Disease (IBD) ... 22
  - Epidemiology, genetics and disease characteristics ... 22
  - Diagnosis and available biomarkers ... 26
  - Integrin αvβ6 ... 28
  - The Preclinical phase of IBD ... 29
  - Management of IBD ... 29
- RA and IBD ... 31

Aims ... 32

Materials and Methods ... 33
- Subjects ... 33
- Autoantibody measurements ... 37
- Protein analysis ... 37
- Statistical analysis ... 38

Results and Discussion ... 40
- Study I ... 40
- Study II ... 41
- Study III ... 44
- Study IV ... 46

Conclusions and future directions ... 49
- Studies I and II ... 49
- Studies III and IV ... 49
- Concluding remarks ... 49

Acknowledgements ... 51

References ... 55

Abbreviations

5-ASA	5-Aminosalicylate
ACCA	Anti-Chitobioside Carbohydrate Antibody
ACPA	Anti-Citrullinated Protein/Peptide Antibody
ACR	American College of Rheumatology
ALCA	Anti-Laminaribioside Carbohydrate Antibody
AMCA	Anti-Mannobioside Carbohydrate Antibody
AMPA	Anti-Modified Protein Antibody
ANCA	Anti-Neutrophil Cytoplasmic Antibody
Anti-CarP	Anti-Carbamylated Protein Antibodies
Anti-Cbir-1	Antibody against bacterial flagellin
Anti-CCP2	Antibody against Cyclic Citrullinated Peptide 2
Anti-I2	Antibody against bacterial DNA fragment I2
Anti-OmpC	Antibody against outer membrane porin C
Anti-αvβ6	Antibody against integrin αvβ6
ASCA	Anti-*Saccharomyces cerevisiae* Antibody
AUC	Area Under the Curve
C-ANCA	Cytoplasmic ANCA
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CDR	Complementarity-Determining Region
hsCRP	High-sensitivity C-Reactive Protein
DAMPs	Damage-Associated Molecular Patterns
DAS	Disease Activity Score
DMARD	Disease-Modifying Antirheumatic Drug
EIM	Extraintestinal Manifestation
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
EULAR	European alliance of associations for rheumatology (formerly European League Against Rheumatism)

FC	Faecal Calprotectin
FDR	False Discovery Rate
FEIA	Fluorescence Enzyme Immunoassay
GH	Global Health
GI	Gastrointestinal
GP2	Glycoprotein 2
GZMB	Granzyme B
HAQ	Health Assessment Questionnaire
HBI	Harvey-Bradshaw Index
HLA	Human Leukocyte Antigen
IBD	Inflammatory Bowel Disease
IBS	Inflammatory Bowel Syndrome
IC	Immune Complex
ICD	International Classification of Diseases
ICI	Immune Checkpoint Inhibitor
IIF	Indirect Immunofluorescence
IVD	In Vitro Diagnostic
JAK	Janus Kinase
LoD	Limit of Detection
MMP1	Matrix Metalloproteinase 1
MPO	Myeloperoxidase
NOD2	Nucleotide-Binding Oligomerization Domain-Containing protein 2
NPX	Normalised Protein eXpression
OR	Odds Ratio
P-ANCA	Perinuclear ANCA
PAB	Anti-Pancreatic Antibody
PAMPs	Pathogen-Associated Molecular Patterns
pMayo	Partial Mayo Score
PR3	Proteinase 3
PRRs	Pattern Recognition Receptors
PSC	Primary Sclerosing Cholangitis
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
S1P	Sphingosine 1-Phosphate

SE	Shared Epitope
SJC	Swollen Joint Count
SLE	Systemic Lupus Erythematosus
SRQ	Swedish Rheumatology Quality
SYND1	Syndecan 1
TGF-β	Transforming Growth Factor beta
TJC	Tender Joint Count
TNF	Tumour Necrosis Factor
Tregs	Regulatory T cells
UC	Ulcerative Colitis
VAS	Visual Analogue Scale

Introduction

The human immune system

Human immunity is characterised by its adaptive and innate immune systems. The adaptive immune system mediates antigen-specific immune responses through T and B lymphocytes. These responses are divided into cellular and humoral immunity. Cellular immunity involves activation of T cells that exert cytokine-mediated and cytotoxic effector functions. In contrast, humoral immunity is mediated by antibodies produced by B cells.[1]

Antibodies, also known as immunoglobulins, are antigen-specific glycoproteins that recognize distinct epitopes on target antigens. All antibodies share a conserved core structure composed of two heavy chains and two light chains forming antigen-binding (Fab) regions and an effector (Fc) region (**Figure 1**). Antigen specificity is determined by variability within the complementarity-determining regions, whereas the constant domains define immunoglobulin class and associated effector properties.

Immunoglobulins exist in two forms. Membrane-bound immunoglobulins function as B-cell receptors and initiate B-cell activation upon antigen recognition. Following activation and T-cell help, B cells differentiate into plasma cells that secrete antibodies with identical antigen specificity. Through class switching and affinity maturation, B cells generate high-affinity antibodies of different isotypes and long-lived memory responses.[2] Five major immunoglobulin isotypes are recognised in humans: IgM, IgD, IgG, IgA, and IgE. Secreted IgM typically forms a pentamer, and is a potent activator of complement system, whereas IgA may be present both in serum and, as a dimer, at mucosal surfaces. IgG is the predominant serum isotype mediating most antibody-dependent effector functions. IgE is mainly involved in allergic and antiparasitic responses, and IgD is primarily expressed as a membrane-bound receptor on naïve B cells.

Once secreted, antibodies bind their target antigens and initiate effector mechanisms including neutralisation, activation of the classical complement pathway, Fc receptor-mediated phagocytosis, and antibody-dependent cellular cytotoxicity. These mechanisms contribute to the elimination of pathogens and infected cells.

The immune system must be able to discriminate between self and non-self antigens to function correctly; the ability to avoid attacking the body's own tissues is known as self-tolerance. Central and peripheral tolerance to self-antigens are mechanisms of the adaptive immune system. Central tolerance occurs during the maturation of lymphocytes in the generative lymphoid organs (thymus and bone marrow) and includes mechanisms such as apoptosis, receptor editing (B cells) and development of regulatory T cells (Tregs). In the case that some self-reactive lymphocytes mature and move to the peripheral tissues, peripheral tolerance is needed to prevent their activation. This is performed through anergy, apoptosis, or suppression by Tregs.

Innate immune cells, including neutrophils, macrophages, dendritic cells, and NK cells, provide rapid, non-specific defence against pathogens. To discriminate between self and non-self, these cells recognize molecular structures in the microbes known as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which are identified by pattern recognition receptors (PRRs) on their surface.[3,4]

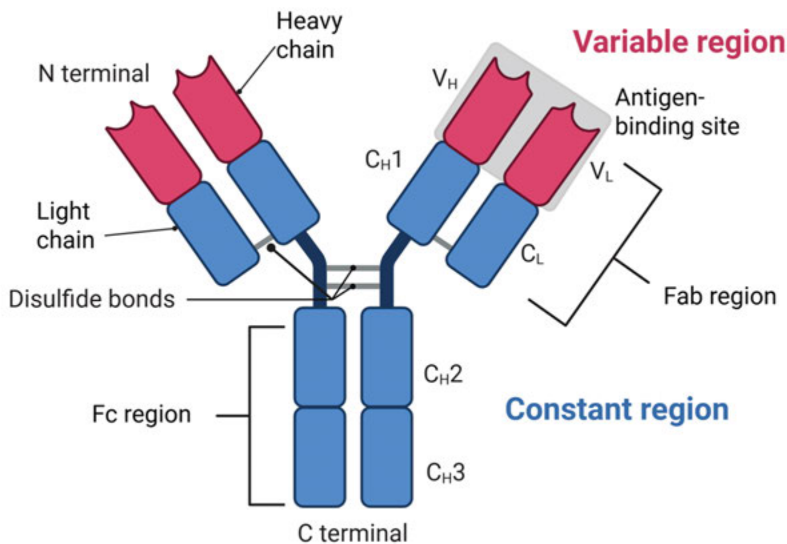

Figure 1. Schematic diagram of a secreted IgG molecule. Created with Biorender.

Autoimmunity

When self-antigens initiate an immune reaction, there is a failure in self-tolerance, which can lead to a broad spectrum of autoimmune diseases. Autoimmunity is characterised by self-reactive immune components and autoimmune disease by autoimmunity plus pathology.[5] Importantly, the presence of autoimmunity does not necessarily imply disease, as self-reactive immune responses or autoantibodies can be present while remaining clinically healthy. Depending on the extent of organ involvement, autoimmune disorders can be classified as organ-specific or systemic. Although several definitions of autoimmunity and autoimmune diseases have been proposed, broadly accepted general criteria are still lacking. This absence of consensus—both in defining autoimmune diseases and determining which conditions fall within this category—continues to hinder research and clinical care. Development of internationally harmonised definitions and classification criteria would help advance the field. Autoimmunity can emerge from a combination of different factors, such as genetic susceptibility and environmental triggers. Through technological advances, many genetic risk factors for autoimmune diseases have been uncovered. However, progress in understanding the far greater influence of environmental factors has been comparatively limited. Both autoimmunity and autoimmune diseases are rising sharply in many regions as suggested by epidemiological studies conducted over recent decades. This trend has been attributed partly to shifts in environmental exposures including changes in our diet, chemical exposures, air pollution, infections, lifestyle habits, stress levels, and climate conditions.[5,6]

It is believed that there are three main phases all autoimmune diseases go through: initiation, propagation, and resolution (**Figure 2**).[7] In the initiation or preclinical phase, individuals have risk factors which predispose them to the disease. This period is usually asymptomatic, and the duration varies a lot between the different diseases. Preclinical autoimmunity can be studied either retrospectively by analysing samples from patients collected as part of longitudinal population-based cohorts or prospectively as part of public health studies which aim to improve disease prognosis. The most efficient approach though, includes cohort studies with high-risk individuals, for example individuals with a family history or presence of the disease in first-degree relatives. Thus, twin cohorts are one of the best types of study cohorts.[8] During the preclinical phase, individuals can be identified through the presence of autoantibodies many years before disease onset.[9-14] For example, in rheumatoid arthritis (RA), several studies have shown that anti-citrullinated protein/peptide antibodies (ACPAs) can antedate the development of the disease by many years. Rheumatoid factor (RF) isotypes are also detectable during

this preclinical phase, and in samples collected more than 15 years before symptom onset, IgA RF has been reported to be more frequent compared with the most frequently appearing ACPA, highlighting the early involvement of RF in the breakdown of immune tolerance.[15] The observation that RA-related autoantibodies and subclinical inflammation can be present years before the onset of clinical arthritis has shifted attention toward the pre-arthritis phase as a potential therapeutic window of opportunity. This phase may represent a period during which the disease process is more amenable to intervention, as early treatment strategies have demonstrated to delay progression to clinically manifest RA. At the same time, timely identification of individuals at high risk raises the possibility that, in the future, true prevention of disease onset may become achievable. Consequently, accurate risk stratification—integrating serological markers with clinical features—is essential to identify those patients most likely to benefit from early intervention while avoiding over-treatment of low-risk individuals.[16]

Most clinical symptoms appear during the propagation phase, where there are signs of increasing inflammation and tissue damage. Epitope spreading is a characteristic phenomenon during this period, where an initial immune response against a particular antigen leads to the subsequent recognition and response against additional epitopes that were not initially targeted. In many autoimmune diseases, new antigenic epitopes are formed and activate more lymphocytes and different immune cells, which are subsequently followed by increased production of cytokines and more tissue damage. Finally, the resolution phase reflects activation of regulatory mechanisms aimed at restoring immune balance, including the expansion of Tregs and engagement of inhibitory receptors that suppress effector responses. However, in most autoimmune

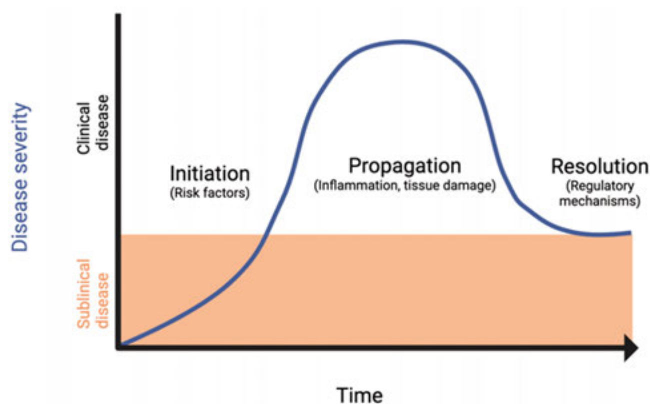

Figure 2. Three major phases of autoimmune diseases. Adapted from Rosenblum et al.[7] Created with Biorender.

diseases this restoration is incomplete and often transient, resulting in a persistent imbalance between effector and regulatory immune responses and contributing to relapsing–remitting inflammation and progressive tissue damage.[7]

Autoantibodies

Autoantibodies result from breakdown of immune tolerance and may develop through direct recognition of self-antigens or via mechanisms such as molecular mimicry. They can be involved in either natural or pathogenic immune responses. Natural autoantibodies are mostly of IgM class and bind to various antigens with low-to-moderate affinity but with high avidity (total binding strength) due to their pentameric structure. The existence of natural autoantibodies is important for the removal of apoptotic cell debris and protection against uncontrolled inflammation. Pathogenic high-affinity autoantibodies of IgG or IgA isotype can emerge as a result of somatic hypermutation and class switching of natural autoantibodies. In autoimmune disorders, pathogenic autoantibodies can directly cause tissue injury either by binding to organ-specific epitopes or by forming immune complexes (ICs), which subsequently activate the complement system and various immune cells, leading to increased inflammation.[17] Some autoantibodies found in different autoimmune diseases are for example RF and ACPAs in RA, anti-dsDNA antibodies in systemic lupus erythematosus (SLE) and the recently discovered IgG antibodies against integrin αvβ6 (anti-αvβ6) in ulcerative colitis (UC).[18]

Autoantibodies can also be used as biomarkers for disease diagnosis or classification. However, the presence of an autoantibody does not per se indicate autoimmune disease. It might appear in high levels in healthy individuals under certain circumstances, like RF during infections or being produced after vaccination.[19,20]

It is critical to develop and use standardised laboratory tests that fulfil the demands for in vitro diagnostic (IVD) assays for clinical use.[8] The progress in autoantigen identification and the introduction of multiplexing technologies have significantly assisted to autoantibody detection for various autoimmune diseases.[21] At present, immunoassays with automation capability are mostly used for serologic testing in clinical laboratories.[22]

In contrast to many routine laboratory parameters in clinical chemistry and haematology, true standardisation of autoantibody assays is today generally considered unattainable. Autoantibodies directed against the same antigen differ between individuals in epitope recognition, isotype distribution, glycosylation, and avidity, resulting in inherent biological variability that cannot be fully aligned across assays. As a result, current expert opinion has shifted from

pursuing strict standardisation toward harmonisation, focusing instead on consensus-based approaches to align on requesting, reporting, and interpretation of autoimmune diagnostics. Even attempts to align assays at comparable diagnostic specificity have not resulted in uniform results across platforms, reinforcing the view that harmonisation rather than strict standardisation is the realistic objective in autoimmune diagnostics.[23-25]

Rheumatoid Arthritis (RA)

Epidemiology, genetics and aetiology

Rheumatoid arthritis is a chronic inflammatory disease affecting 0.5-1% of adults worldwide, with a higher prevalence in women than in men and at older ages.[26] The complexity of disease aetiology and pathogenesis involves a combination of genetic and environmental factors.[27] Many studies have provided strong evidence that HLA-DRB1 alleles carrying the shared epitope (SE), a conserved five–amino acid sequence motif, represent the strongest genetic risk factors for rheumatoid arthritis.[28] Already from the 80s, smoking has been proven to be the major risk factor for the disease, although the mechanism behind it remains to be elucidated. Padyukov et al. and Klareskog et al. have also described a very strong interaction between SE and smoking in RF or ACPA positive RA patients. [29-32] Furthermore, there is growing evidence that RA patients appear to have different composition in their gut microbiota, with decreased diversity than in healthy individuals (**Figure 3**).[33]

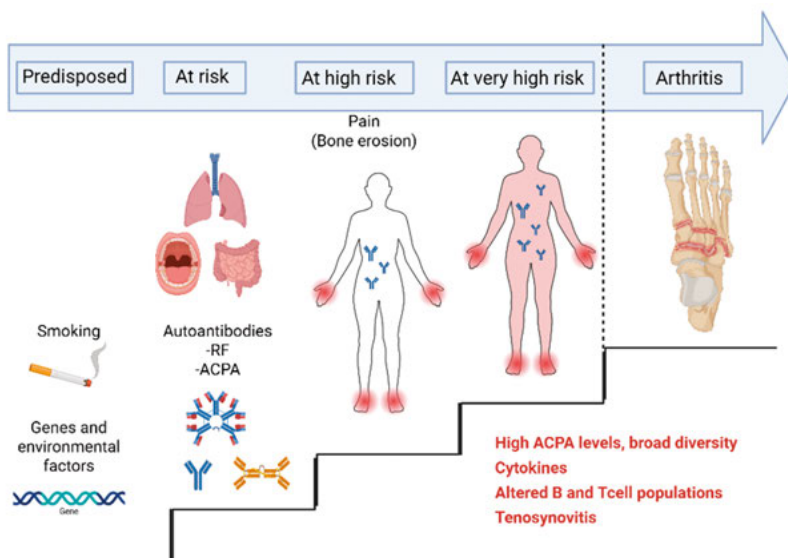

Figure 3. Development of seropositive RA. Created with Biorender.

Diagnosis, autoantibodies and serology

There are two major subtypes of RA, based on the presence or absence of autoantibodies against specific antigens. A distinguishing characteristic of seropositive RA is the presence of these specific autoantibodies in blood and joint fluid. RF is an autoantibody reacting against the Fc part of IgG antibodies and was the first autoantibody described in RA patients.[34,35] Although RF always reacts with IgG Fc, the RF molecule itself might be of any immunoglobulin isotype, and IgM RF is considered most important in RA serology. However, RF has low diagnostic specificity for RA as it can be found also in healthy subjects and in patients with other non-RA diseases.[36] ACPAs are another very important type of autoantibodies in RA that have higher specificity compared to RF. Importantly, both RF and ACPA have been associated with more severe disease, including increased risk of erosive progression and poor prognosis.[37-41]

Citrullination of proteins is an enzymatic process by which arginine residues in proteins/peptides are converted to citrulline residues by peptidyl arginine deiminases. It is estimated that 50-80% of RA patients have RF, ACPAs or both (seropositive patients) and that the occurrence of these autoantibodies can precede disease onset by many years.[13,40,42-44] ACPA positivity becomes more frequent closer to symptom onset,[13,42,43] and thereafter antibody status remains fairly stable during the first 5 years of RA, suggesting that the immunological events determining this phenotype occur before clinical onset. Preclinical studies using multiplex ACPA assays have shown that expansion of the ACPA repertoire precedes symptom onset and is associated with progression to RA.[42] Many studies have discussed the involvement of ACPAs in disease initiation from the lungs and possibly the gums.[45,46] In this context, smoking and other air pollutants (silica, textile dust) seem to trigger citrullination in the lungs, which flags immune system activation.[47,48] Citrulline-containing antigens can also form ICs with ACPAs which in turn activate the complement system.[49] Beyond RF and ACPAs, additional antibody specificities have been implicated as diagnostic and/or prognostic biomarkers in RA. These include antibodies targeting other post-translationally modified proteins and peptides, such as antibodies against carbamylated or homocitrulline-containing proteins (anti-CarP), which are present in approximately 45% of patients with RA, and are associated with poorer radiographic outcomes in early arthritis.[50] Despite high specificity, anti-CarP display relatively low sensitivity.[51] Together with ACPAs and antibodies against acetylated residues, these antibody specificities are collectively termed anti-modified peptide antibodies (AMPAs).[52]

RA is typically defined according to widely accepted classification criteria. In 1987 American College of Rheumatology (ACR) included 7 classification

criteria given in **Table 1**.[53] In these criteria serology was only a minor part, constituting 25% of the number of criteria (4) needed for RA classification.[54]

Table 1. 1987 ACR classification criteria for RA (adapted from Arnett et al. [53]).

1)	Morning stiffness in and around joints lasting at least 1 hour before maximal improvement
2)	Arthritis of 3 or more joint areas with soft tissue swelling or fluid observed by a physician
3)	Arthritis of the hand joints: wrist, metacarpophalangeal or proximal interphalangeal joint
4)	Symmetric swelling (arthritis): as the areas defined in 2 on both sides of the body
5)	Rheumatoid nodules observed by the physician
6)	Abnormal amount of rheumatoid factor in the serum, by any method for which the result has been positive in <5% of normal control subjects
7)	Radiographic changes with erosions and/or periarticular osteopenia in hand and/or wrist joints.

Note: Patient should fulfil 4 or more criteria to have RA, criteria 1 through 4 should be present for at least 6 weeks

Notably, key elements in the 1987 criteria, including rheumatoid nodules and radiographic changes, generally reflect established and quite advanced disease. To date treatment strategies, however, aim to initiate therapy before the development of structural damage, necessitating criteria that enable earlier classification. Accumulating evidence supports the concept of a "window of opportunity" during the early phase of RA, in which initiation of treatment within the first weeks after symptom onset is associated with improved long-term prognosis.[55] Additionally, the limited diagnostic sensitivity of the 1987 ACR criteria as well as significant discoveries, such as the identification of ACPA, led in 2010 to the development of new classification criteria by the ACR and the European alliance of associations for rheumatology (EULAR) (**Table 2**).[56] As part of these criteria both ACPAs and RF should be tested and accounted for in the total score. Six out of ten possible points are needed for RA classification with autoantibodies contributing up to three points. This means that serology may contribute 50% of the criteria needed for RA classification according to the 2010 criteria.[54] However, the use of both RF and ACPAs, combined with level-dependent scoring, exposed important inconsistencies between commercially available assays. Differences in calibration, cut-off definitions, and lack of uniform reference standards can lead to variable classification outcomes for identical patients across laboratories. As a result, RA classification may become assay-dependent rather than biologically driven, limiting comparability between studies and potentially affecting early

diagnosis and treatment decisions.[57] These limitations highlight the need for harmonisation of serological testing and interpretation to ensure consistent and reliable application of the 2010 classification criteria. Improved harmonisation may be achieved through the use of international reference materials, alignment of assay cut-offs based on predefined specificity, and more standardised interpretation of RF and ACPA results. Finally, refinement of serological weighting by considering antibody type (RF vs ACPA), level, and combined positivity may improve the specificity of RA classification.[58]

Table 2. 2010 EULAR/ACR new classification criteria for RA (adapted from Aletaha et al.[56]).

	Category	Point Score
A	Joint involvement (0-5 points) [a]	
	1 large joint	0
	2-10 large joints	1
	1-3 small joints (large joints not counted)	2
	4-10 small joints (large joints not counted)	3
	>10 joints including at least one small joint	5
B	Serology (at least one test needed for classification; 0-3 points) [b]	
	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 one test needed for classification; 0-1 point) [c]	
	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
D	Duration of symptoms [d]	
	<6 weeks	0
	≥6 weeks	1

Note: A total score of ≥6 is needed to classify a patient as having definite RA.
[a] Joint involvement refers to any swollen or tender examination, which may be confirmed by imaging evidence of synovitis. Large joints refer to shoulders, elbows, hips, knees and ankles. Small joints refer to metacarpophalangeal joints, proximal interphalangeal joints, 2nd-5th metatarsophalangeal joints, thumb interphalangeal joints and wrists. [b] Negative means less than or equal to the upper limit of normal (ULN); low positive means between 1-3 x ULN; high positive means >3x ULN. [c] Normal and abnormal are determined by local laboratory standards. [d] Duration of symptoms as per patient's self-report.

RA diagnosis is in practice often based on the combination of classification criteria mentioned above and individualised clinical assessment of the rheumatologist. Synovial joint swelling is a typical clinical characteristic in RA patients, and it is usually presented with morning stiffness and tenderness (**Figure 4**). The inflammatory environment in the synovium comprises a complex cytokine and chemokine network regulated by activated immune cells. Synovial tissue in RA is characterised by infiltration of macrophages, T and B lymphocytes, and proliferation of fibroblast-like synoviocytes, leading to synovial hyperplasia and formation of a thickened lining layer called pannus. Activated synovial cells produce pro-inflammatory cytokines, such as tumour necrosis factor (TNF) α and IL-6, as well as matrix-degrading enzymes that drive cartilage destruction and bone erosion, thereby sustaining chronic joint inflammation.[59] In order to assess disease activity in RA there are different scores that could be used, such as Disease Activity Score (DAS) and its modification which involves 28 joint counts (DAS28) (**Table 3**).[60] Health Assessment Questionnaire (HAQ) is a subjective measure used to assess the disability of the patient.[61,62]

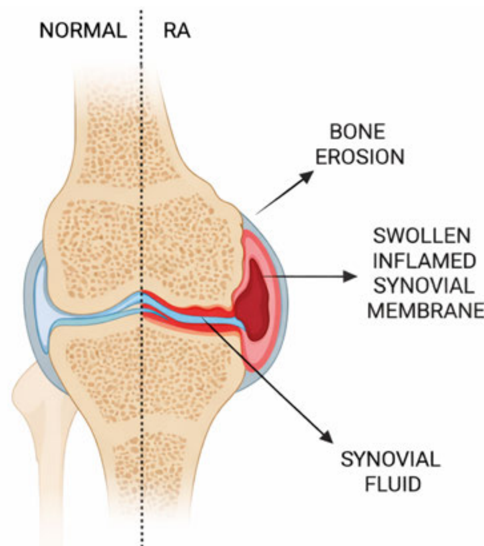

Figure 4. Schematic figure showing the contrast between the joint in normal (left) and RA patients (right). Created with Biorender.

Table 3. Examples of disease activity scores used in RA (adapted from Smolen et al.[61]).

Scoring system	Remission	Low disease activity	Moderate disease activity	High Disease activity
DAS*	≤1.6	>1.6-2.4	>2.4-3.7	>3.7
DAS28**	≤2.6	>2.6-3.2	>3.2-5.1	>5.1

*DAS: Complex formula including the Ritchie index, swollen joint count based on 44 joints (SJC44), erythrocyte sedimentation rate (ESR) and global health (GH)

**DAS28: Complex formula including tender joint count based on 28 joints (TJC28), swollen joint count based on 28 joints (SJC28), ESR (or CRP) and GH

Historically, RF was measured using agglutination tests and more recently nephelometry or turbidometry. All these techniques are non-specific for the immunoglobulin isotype, although they mainly detect IgM RF. As mentioned previously, technological progress has provided a variety of new immunoassays that offer the possibility to test for different isotypes in an automated and more standardised way. In case of ACPA testing, there are a number of different commercially available assays that have been developed, with IgG anti-cyclic citrullinated peptide 2 (anti-CCP2) being the most widely used, in different assay formats. In 2018, Rönnelid et al. showed that using a custom-made microarray for measuring ACPA reactivity to 16 citrullinated peptides and arginine control peptides, they could detect an ACPA-positive subgroup among anti-CCP2-negative patients that had the same genetic and smoking characteristics as anti-CCP2-positive patients.[31,63]

Management of RA

Disease-modifying antirheumatic drugs (DMARDs), mainly methotrexate, is the most common initial treatment for RA. Their role is to reduce joint swelling and joint damage. Another initial treatment strategy is combination of DMARDs with a low dose of corticosteroids. Nowadays, there is increasing interest in the use of biologic agents, such as infliximab, adalimumab and etanercept (TNF inhibitors), abatacept (T cell co-stimulation inhibitor), rituximab (anti-CD20/B cell) and tocilizumab (anti-IL6 receptor). A more recent addition in the treatment options is the use of Janus kinase (JAK) inhibitors which act in the JAK/STAT signalling pathway.[64,65]

Non-drug treatments such as exercise, joint protection, good dietary habits, psychological support and patient’s education are also of major importance.

In recent years, the treatment paradigm in RA has expanded beyond management of established disease toward earlier intervention in individuals at risk, driven by the recognition of a prolonged preclinical phase and the concept of a therapeutic window of opportunity.[55] Preventive and very early intervention trials have explored whether targeted immunomodulation can delay or modify disease onset before clinically apparent arthritis develops. Notably, biological DMARD–based studies such as PRAIRI,[66] ARIAA,[67] and APIPPRA[68] demonstrated that early B-cell or T-cell–directed therapies can delay progression to clinical RA and reduce subclinical inflammation, although definitive prevention has not yet been achieved. These findings support a paradigm shift toward risk stratification and early targeted treatment aimed at altering disease trajectory rather than solely controlling established synovitis.[69]

Inflammatory Bowel Disease (IBD)

Epidemiology, genetics and disease characteristics

Inflammatory bowel disease encompasses a group of immune-mediated inflammatory diseases affecting the gastrointestinal (GI) tract. The disease entity comprises two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC). Although these two diseases have many similarities in their clinical profiles, they also have some important differences which provide evidence for distinct pathological processes.[70] Globally, IBD prevalence is rising in all regions, while incidence has stabilised in many Western countries but continues to increase rapidly in newly industrialised regions, driving a sustained growth in the global burden of disease.[71-73] In Sweden, the prevalence of IBD is 0.65-0.8%[74-76]. CD is most often diagnosed between 15-35 years of age, whereas the age-specific incidence peak for UC is around 20-40 years.[77] In UC, the sex ratio is equal, and for CD, despite the higher incidence in females than in males, there is a trend toward equalisation of sex distribution over time.[77] Studies have shown that paediatric-, adult-, and elderly-onset IBD differ in clinical characteristics, supporting the concept that different ages at onset reflect distinct pathogenic pathways, with implications for patient stratification and therapeutic decision-making.[78]

The familial inheritance is more profound in CD than in UC, as reflected by a greater difference in proband concordance rates between monozygotic and dizygotic twin pairs with CD compared with UC (30% vs 2% for CD and 15% vs 3% for UC).[79] Since the identification of the association of the *NOD2* gene with CD[80,81], more than 370 genetic susceptibility loci have been associated with IBD, the majority of which are shared between CD and UC.[82]

Regarding environmental triggers, smoking is the best studied environmental factor, showing a protective effect against UC, but associated with increased risk for CD.[83]

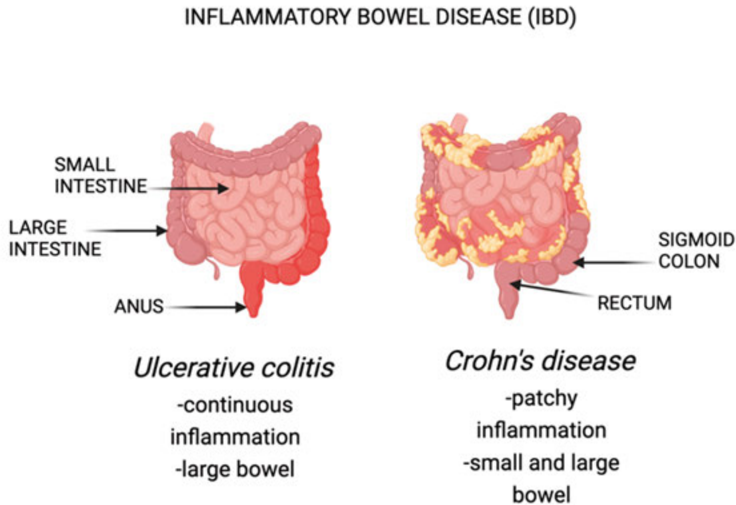

Figure 5. Schematic figure showing the differences between ulcerative colitis (left) and Crohn's disease (right). Created with Biorender.

The pathogenesis of IBD is not completely understood, but studies have shown that genetics and environmental alterations, such as changes in gut microbiota composition and enhanced intestinal permeability, seem to contribute to dysregulated intestinal immunity followed by damage in the GI tract.[84] Extraintestinal manifestations (EIMs) frequently accompany IBD, particularly CD, in which they occur in approximately 40% of patients.[85] These manifestations reflect underlying immune dysregulation and may involve multiple organ systems, including the skin, eyes, joints, and respiratory tract.[86] In UC inflammatory arthropathies and primary sclerosing cholangitis (PSC) represent the most common and clinically relevant EIMs.[87]

Inflammation in CD can appear in any part of the GI, from the mouth to the anus, but most commonly in the terminal ileum and colon, forming characteristic "skip" intestinal lesions (areas of inflammation between normal mucosa) (**Figure 5**). The Montreal classification of CD considers age of onset (A), disease location (L), and disease behaviour (B) as the main phenotypic elements (**Table 4**).[88] Based on this, the inflammation in CD is located in the ileum (CD_L1) in approximately one-third of patients, in the large bowel (CD_L2) in one-third, and one-third have ileocolonic disease (CD_L3) with

inflammation in both the ileum and the large bowel.[88,89] In UC, the inflammation starts in the rectum and may continuously extend to the colon and in some patients it affects the entire colon (**Figure 5**). According to the Montreal classification, UC is classified based on the extent of inflammation and severity (**Table 5**).[88]

Table 4. Montreal classification in CD (adapted from Silverberg et al.[88]).

Parameter	Category	Definition
Age at diagnosis	A1	<17 years
	A2	17–40 years
	A3	>40 years
Disease location	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper disease (can be added to L1-L3)
Disease behaviour	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
	p	Perianal disease modifier (is added to B1-B3 when concomitant perianal is present)

Table 5. Montreal classification in UC (adapted from Silverberg et al.[88]).

Parameter	Category	Definition
Disease extent	E1	Ulcerative proctitis
	E2	Left-sided UC (distal to splenic flexure)
	E3	Extensive UC (proximal to splenic flexure)
Disease severity	S0	Clinical remission
	S1	Mild UC
	S2	Moderate UC
	S3	Severe UC

There is also a difference between CD and UC regarding the depth of the inflammation. In CD, the inflammation often affects all layers of the intestinal wall (transmural inflammation), leading to fibrosis, strictures and fistulas, whereas the inflammation in UC has been regarded as engaging only the mucosa.[90] However, in recent years, the hypothesis that UC is a superficial disease has been questioned, and at least in patients with more severe disease, the inflammation also engages deeper layers of the wall.[91] Already from the '60s, CD diagnosis without ileal involvement (i.e. colonic CD) was introduced but was not broadly accepted before the 70's and early 80's. Since then, many studies have discussed the fact that colonic CD and UC might be difficult to distinguish.

Recently, it has been proposed that ileal and colonic CD should be regarded as separate entities, with UC representing the third main entity of IBD.[83]

Determining disease activity and severity is very important for selecting the appropriate treatment both for CD and UC. The Crohn's Disease Activity Index (CDAI) has been widely used to assess the disease activity in clinical trials on CD patients (**Table 6**). It includes a score of eight clinical or laboratory variables that are summed after adjustment with a weighting factor. The score comprises variables such as the number of soft stools, abdominal pain, general well-being, EIMs, presence of abdominal mass, changes in weight, low haematocrit and opiate use for diarrhea.[92] A more simplified activity score, the Harvey-Bradshaw index (HBI) was developed in 1980 and contains only the clinical parameters mentioned above.[93] In UC, the Mayo Clinic Score is most commonly used for assessing disease activity. It is a composite of four categories that involve bleeding, stool frequency, physician's global assessment and endoscopic appearance, scored from zero to three, with a maximum total score of twelve. A modification of the Mayo Clinic Score, the partial Mayo Score (pMayo), was developed for cases in which endoscopy could not be assessed. It has been shown that pMayo and the full Mayo Clinic Score correlate well.[94]

Table 6. Examples of disease activity scores used in UC and CD.

Tool	Disease	Key components	Output
Mayo Clinic Score	UC	Stool frequency (vs baseline), rectal bleeding, endoscopic activity, physician global assessment	Activity score 0–12
Partial Mayo Score (pMayo)	UC	Stool frequency (vs baseline), rectal bleeding, physician global assessment	Clinical activity score 0–9
Crohn's Disease Activity Index (CDAI)	CD	Stool frequency, abdominal pain, general well-being, extraintestinal manifestations, haematocrit, body weight, antidiarrheal use	Continuous activity index
Harvey–Bradshaw Index (HBI)	CD	General well-being, abdominal pain, stool frequency, abdominal mass, extraintestinal manifestations	Simplified activity score

Diagnosis and available biomarkers

The diagnosis of IBD is based on a combination of clinical symptoms, endoscopic examination, histological features and laboratory tests. The latter includes faecal and blood samples. Faecal biomarkers are very useful due to their specificity for GI diseases. Faecal calprotectin (FC) is a reliable biomarker that is routinely used to measure inflammatory activity in patients with IBD.[95] Calprotectin is a cytoplasmic protein highly abundant in neutrophils and present at lower levels in monocytes. It is released during neutrophil activation or cell death and can be measured in stool. High FC indicates an ongoing gut inflammation.[96] FC has been proven to be a useful screening tool for identifying patients who need to undergo further examination. In patients with CD, FC further correlates with endoscopic activity and can be used for disease monitoring, to assess response to therapy, and to predict clinical flare.[95,97] Another faecal biomarker is faecal lactoferrin, which is an iron-binding protein found inside neutrophils. The marker is primarily used to exclude intestinal inflammation rather than to confirm diagnosis.[77,98]

Other suggested faecal neutrophil markers are myeloperoxidase (MPO) and neutrophil gelatinase-associated lipocalin (also known as human neutrophil lipocalin).[99] Notably, none of these tests alone can confirm the diagnosis of IBD. Oppositely, they are often used in diagnostic algorithms to rule out inflammation.[77] However, stool sampling is poorly accepted by some patients, and adherence to frequent FC testing is fairly low.[100,101] The day-to-day variability between faecal samples is another drawback of this sampling method.[97] Regarding serum/blood biomarkers, CRP is the most commonly used marker to mirror disease activity. Electrolytes, albumin and some vitamins are also examined in patients with IBD due to the risk for hypalbuminaemia and vitamin deficiencies, particularly in patients with extensive small bowel CD.[102]

One of the most common triggers of intestinal immune activation and subsequent IBD pathogenesis is the reaction to normal enteric flora. Many studies have identified a number of emerging microbial components and autoantigens and their corresponding antibodies in IBD patients as a result of this immunological reaction. The presence of antibodies against these microbial peptides may potentially assist in disease classification, diagnosis and in some cases, it can serve as a predictive marker for disease course. Anti-neutrophil cytoplasmic antibodies (ANCAs) are detected in serum samples with indirect immunofluorescence (IIF) on normal peripheral blood neutrophils and are associated with neutrophil-mediated inflammation. IIF is demanding to perform, and the interpretation of the result is subjective and based on the experience of each observer. There are two main ANCA patterns: cytoplasmic (C-ANCA) and perinuclear (P-ANCA), which are used for the diagnosis of vasculitis. C-

ANCAs are most commonly associated with antibodies to proteinase 3 (PR3), whereas P-ANCAs are typically associated with antibodies to MPO. Current international consensus guidelines for ANCA-associated vasculitides recommend antigen-specific immunoassays for PR3- and MPO-ANCA as the preferred initial diagnostic approach rather than routine IIF microscopy when vasculitis is suspected.[103,104] Atypical P-ANCAs are on the other hand more common in patients with IBD and especially UC (prevalence: 40-80%). The target antigen of atypical P-ANCA has not yet been completely identified, but it seems to be associated with the inner surface of the neutrophil nuclear membrane.[105,106] Autoantibodies to PR3 are present in some patients with severe UC.[107]

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs), both of IgA and IgG isotypes, are directed against a 200 kDa oligomannosidic cell wall component of the yeast *S. cerevisiae*.[108] Studies have shown that ASCA can also bind to mannans from *Candida albicans*.[109] ASCAs are detected in approximately 60-70% of patients with CD and tend to remain relatively stable over time. They have also been found in 20–25% of unaffected first-degree relatives, whereas they are uncommon in healthy controls and spouses indicating familial aggregation.[110,111] Twin studies further suggest that although ASCA presence alone may not represent a direct genetic susceptibility marker, genetic factors may influence the magnitude of the antibody response.[112] ASCAs can also be predictive since they can be detected in high-risk healthy individuals before the diagnosis of CD.[11] The role of ANCA and ASCA as diagnostic biomarkers for IBD is questionable due to their moderate sensitivity and specificity as well as their presence in other diseases. The combination of P-ANCA and ASCA has been proposed as a tool for differentiation between CD and UC.[113] However, studies have demonstrated that the increased specificity of this combination is associated with decreased sensitivity (~30-60%).[114-117]

Many other serologic markers have been identified over the years. Anti-OmpC antibody recognizes the outer membrane porin C transport protein (OmpC) of *Escherichia coli.* Anti-OmpC, most commonly of the IgA isotype, have been reported in approximately 55% of CD patients but are uncommon in UC and healthy individuals. The anti-I2 antibody is directed against a bacterial DNA fragment (I2) associated with *Pseudomonas fluorescens*. CD patients appear to have a higher prevalence of anti-I2, predominantly described as IgA responses, in serum (~50%) compared to UC patients (~10%).[118] Another antibody that is present in approximately 50% of CD patients is directed against bacterial flagellin (anti-CBir-1), which induces strong B-cell and $CD4^+$ T-cell responses in colitic mice.[119] Anti-pancreatic antibodies (PABs) are antibodies against the exocrine pancreatic tissue, and glycoprotein 2 (GP2) is the major target autoantigen.[120,121] PABs are present in approximately 30%

of patients with CD and are rare in UC and healthy individuals.[105,122,123] A relatively newer group of antibodies found in CD patients are anti-glycan antibodies, which were first reported by Dotan et al. in 2006.[124] Members of this antibody group are anti-laminaribioside carbohydrate antibody (ALCA) (18-38%), anti-chitobioside carbohydrate antibody (ACCA) (21-36%), and anti-mannobioside carbohydrate antibody (AMCA) (28%).[105]

The rapid development of novel omics technologies has revolutionised biomarker discovery research in IBD and helped elucidate the pathophysiology of the disease. Many recent studies have focused on the measurement of inflammation-related proteins that seem to correlate with changes in clinical parameters and can mirror disease activity.[125]

The proximity extension assay is one of the most commonly used methods for multiprotein profiling. The method is antibody-based and allows multiplexing, using a very low sample volume.[126] Promising results have also shown that proteomic analysis might be used for disease prediction and diagnosis.[127] All these findings are important for guiding future studies with a common goal of personalised treatment and flare prevention.

Integrin αvβ6

Autoimmunity has attracted significant interest in IBD since 1959, when autoantibodies in human UC were first reported.[128] A recent study has shown that anti-αvβ6 are present in the majority of Japanese UC patients and that these autoantibodies might be used to diagnose UC (sensitivity: 92.0%, specificity: 94.8%) and potentially also to monitor disease activity.[18] Integrin αvβ6 is a member of the integrin family, which are transmembrane receptors involved in signal transduction in and out of the cell. Its function is critical for maintaining the integrity of the intestinal epithelial barrier and for immune homeostasis by regulating inflammation via transforming growth factor β1 (TGF-β1) activation.[129,130] Integrin αvβ6 binds to its ligands, such as fibronectin, by recognition of the RGD sequence motif. Recent studies demonstrated that anti-αvβ6 from patients with UC functionally block integrin αvβ6-fibronectin binding and inhibit integrin αvβ6-mediated activation of latent TGF-β1 *in vitro*.[18,131] Based on these findings, the researchers hypothesised that the autoantibody may disrupt the integrity of the colon epithelium, mediate antibody-dependent inflammation, and potentially inhibit the TGF-β1 immune-regulatory pathway, thereby affecting mucosal healing in UC patients.[18] In another study, Rydell et al. confirmed that anti-αvβ6 is significantly higher in Swedish UC patients, compared with patients with IBS as a reference population.[132] Anti-αvβ6 has also been associated with the preclinical phase and can be detected up to 10 years before UC diagnosis.[12] In the same study, the

researchers found that anti-αvβ6 has prognostic properties as it is associated with complicated disease in newly diagnosed UC patients. Recently, anti-αvβ6 was also found in patients with immune checkpoint inhibitor (ICI)-induced colitis exhibiting features similar to those observed in UC.[133] In post-surgical UC, anti-αvβ6 has been reported as a predictor for pouchitis after restorative proctocolectomy with ileal pouch–anal anastomosis.[134] Anti-αvβ6 has also been reported in PSC, including PSC with concomitant IBD, and may correlate with liver disease severity in some cohorts.[135-137]

The Preclinical phase of IBD

It is not yet clear whether antibodies against various microbial and autoantigens play a direct role in the pathogenesis of IBD or are a consequence of mucosal inflammation. A proposed scenario is that in genetically susceptible individuals with exposure to environmental triggers, inflammatory responses to commensal microorganisms are initiated, and this in turn results in a persistent inflammation in IBD patients.[138] The diagnosis of IBD is often delayed, since most patients present with non-specific symptoms and laboratory abnormalities. Moreover, the diagnosis requires invasive investigations, including endoscopy and histological results.[139] Many efforts have been made to shorten this delay, yet there is limited research focusing on the time before the appearance of clinical symptoms.

There is accumulating evidence that a preclinical phase precedes the diagnosis of IBD. Characteristics of this phase include systemic subclinical inflammation, disrupted barrier function, and development of antibodies against microbial antigens and potentially autoantibodies.[12,112,139-141] The identification of predictive biomarkers during this preclinical stage offers a critical window of opportunity for early detection and intervention, wherein the immune dysregulation may still be reversible.[142,143] Together with concurrent changes in intestinal permeability, microbiome composition, and systemic inflammatory markers, the presence of preclinical autoantibodies supports a multistage disease model analogous to other immune-mediated inflammatory diseases and highlights opportunities for early risk stratification and future preventive or disease-intercepting strategies.[144]

Management of IBD

Both CD and UC are characterised by periods of remission and episodes of increasing inflammation, i.e. relapses. IBD management can be a challenge, and inadequate disease control can result in progressive, irreversible bowel damage and long-term complications. Over recent decades, treatment

strategies have evolved toward a treat-to-target approach, with increasing emphasis on objective measures of disease control, particularly mucosal healing assessed by endoscopy.[145] Experts also agree that the ultimate treatment goals should include improving patients' quality of life, which can be measured using different patient-reported outcomes. Since endoscopies cannot be performed frequently in patients, representative biomarkers of inflammation, such as CRP and FC, can be used for monitoring.[146] For mild to moderate UC, 5-aminosalicylates (5-ASA) are administered as first-line therapy. Patients with UC who do not achieve remission on 5-ASA may be treated with corticosteroids, administered either systemically (oral or intravenous) in more severe cases or topically (rectal preparations) in patients with distal disease. In CD, 5-ASA have not demonstrated clear efficacy, and patients with mild CD may be treated with corticosteroids, including budesonide. Patients with moderate to severe CD and UC are typically treated with immunomodulators, biological drugs or small molecules. Anti-TNF agents, such as infliximab, adalimumab and golimumab, were the first class of biologics approved for the treatment of IBD. Subsequently, several additional biologics targeting different inflammatory pathways have been introduced, including ustekinumab (anti-IL12/anti-IL23), vedolizumab (anti-integrin α4β7) and selective IL-23 inhibitors such as risankizumab, mirikizumab and guselkumab. Small molecule therapies include JAK inhibitors and sphingosine 1-phosphate (S1P) receptor modulators. Notably, some of these agents are approved only for one IBD subtype, either CD or UC, potentially reflecting difference in pathophysiology and clinical trial evidence. In both CD and UC, surgery also remains an important treatment option.[147] Studies on the efficacy of faecal transplantation show divergent results, and there is still no evidence to recommend it in patients with IBD outside the context of clinical trials. Diet and lifestyle should also be taken into consideration, although their impact on IBD is not completely understood. Stress management has also been shown to improve symptoms and therefore assistance in mental health can be useful.

Despite an expanding range of available therapeutic options, remission rates remain limited, highlighting a therapeutic ceiling that reflects disease heterogeneity and variability in treatment response. This underscores the growing importance of precision medicine and patient stratification, as clinicians are increasingly required to select the most appropriate therapy for each patient. Consequently, there is a critical need to identify biomarkers capable of predicting treatment response and guiding personalised treatment strategies in IBD.[148-152]

RA and IBD

Many studies have explored whether individuals with one immune-mediated inflammatory disease are at increased risk of developing a second autoimmune disease, and whether certain diseases co-occur more frequently than others.[153] Some observational studies have suggested that RA and IBD may co-occur more frequently than expected.[154] However, the overall evidence remains limited and inconsistent. For example, Mendelian randomisation analyses have reported that genetically predicted risk of RA is associated with an increased risk of IBD, whereas the reverse relationship i.e. IBD influencing the risk of RA, has not been supported.[155] As discussed in a previous section of this thesis, alterations in gut microbiota appear to be a common characteristic of both disease entities. This observation raises the possibility that RA and IBD may share certain inflammatory or immunological pathways, although the underlying mechanism remains incompletely understood. In this context, advances in the conceptual and therapeutic understanding of one disease may provide insights and inform research and clinical strategies in the other.

RA has led the field in defining autoantibody-positive preclinical disease and initiating preventive intervention trials, a paradigm now also emerging in IBD. Conversely, although treat-to-target principles are well established in RA, advances in objective inflammatory monitoring and mucosal assessment in IBD provide complementary insights that may further refine biomarker-guided management strategies across immune-mediated inflammatory diseases. This bidirectional exchange of concepts and strategies provides a rationale for the integrated investigation of RA and IBD undertaken in this thesis.

Aims

The overall aim of this thesis was to investigate the role of autoantibodies as biomarkers for the diagnosis and prognosis of RA and IBD, as well as their association with gender, age at diagnosis, disease severity, and disease phenotypes. More specifically this thesis can be divided into two main disease areas:

Rheumatoid arthritis (Papers I-II):

- The first study aimed to investigate the occurrence of rheumatoid arthritis-associated autoantibodies in relation to age at disease onset and sex.
- The second study aimed to examine how individual rheumatoid arthritis autoantibodies as well as their combinations, were associated with individual clinical signs and symptoms at the time of diagnosis.

Inflammatory bowel disease (Papers III-IV):

- The third study aimed to examine the potential of IgG anti-integrin αvβ6 autoantibodies as diagnostic and prognostic markers in newly diagnosed adult IBD patients.
- The fourth study aimed to evaluate the use of anti-integrin αvβ6 autoantibodies as a biomarker to predict future UC, establish a preclinical cut-off, and examine genetic, environmental, and inflammatory influences on the occurrence and levels of anti-αvβ6 autoantibodies.

Materials and Methods

Subjects

In **study I** and **II**, serum samples were collected from 1600 RA patients aged between 18 and 70 years old, part of the Epidemiological Investigations of Rheumatoid Arthritis (EIRA) case-control cohort in Sweden. Data from EIRA were linked to data from the Swedish Rheumatology Quality registry (SRQ) and other registers, via personal identification numbers, within the framework of an ethics-approved register linkage. All patients were diagnosed according to the 1987 ACR classification criteria[53] within one year of first symptoms between 1996 and 2010. Patients included in these two papers had less than 40 days between the clinical RA diagnosis and inclusion in the EIRA, so that antibody levels and occurrence would match the timepoint when the clinical situation was recorded. EIRA controls were selected randomly from the Swedish population registry and were matched with RA cases for age, sex and residence.[156,157] In total 2934 patients and 624 controls were investigated. Due to the propensities of certain sera (e.g., direct binding to all peptides containing streptavidin) and high background reactivity to the microarray surface, 118 samples (74 patients and 44 controls) were excluded. Two patients and two controls lacked anti-CCP2 data, 24 patients and 13 controls lacked information about HLA-DRB1* alleles and smoking information was missing for 24 patients and 13 controls. A total of 109 patients and 73 controls were excluded, leaving 1600 patients for the final analysis (**Figure 6**).[63] Clinical patient data was obtained through case report forms as well as hospital medical records. Disease activity was determined using DAS28 as previously described.[60] The studies were approved by the ethics committee at Karolinska Institute. All patients and controls gave their informed consent before participation.

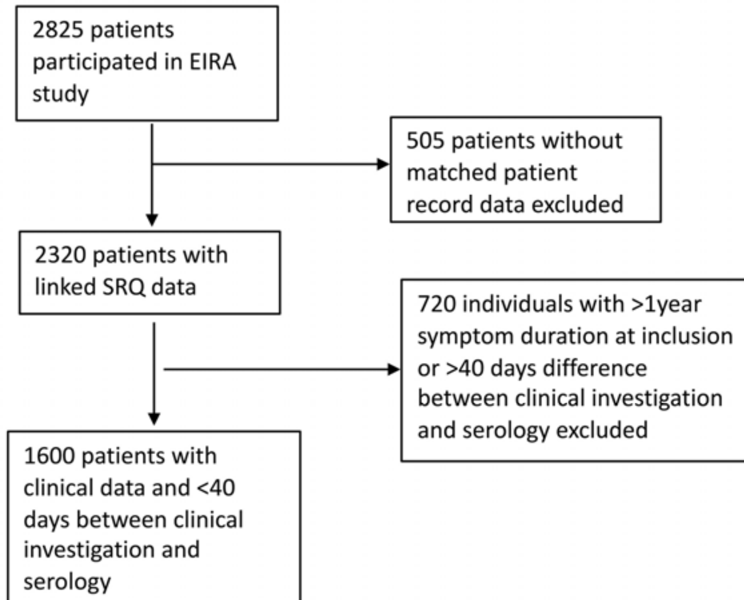

Figure 6. Flowchart showing the study population (N=1600) after applying exclusion criteria. From Paper II. The identical set of patients were used in studies I and II.

For **study III,** serum samples from the Swedish Inception Cohort of IBD (SIC-IBD) and the IBD in South-Eastern Norway (IBSEN) III cohort were analysed (**Figure 7**). In total we included 326 IBD patients, 146 symptomatic controls and 48 healthy controls from SIC-IBD and 366 IBD patients and 204 symptomatic controls from IBSEN III. Additionally, 123 UC patients from SIC-IBD were followed and re-analysed after three months. SIC-IBD, which was our discovery cohort, is a prospective cohort of adult patients aged 18 years or above who were referred to gastroenterology units at eight hospitals in Sweden between November 2011 and March 2021 due to suspicion of IBD. The presence of gastrointestinal symptoms, such as diarrhoea, abdominal pain and blood or mucus in stool, for >2 weeks, indicative of IBD, was the inclusion criterion. Patients who did not meet the diagnostic criteria for IBD were included as non-IBD symptomatic controls.[158] Healthy individuals without a history of chronic gastrointestinal disease or symptoms were also recruited as a second control group through announcements at Örebro University and Örebro University Hospital, Sweden. As a validation cohort, we used patients aged 18 years and above from the IBSEN III cohort. All patients in the validation cohort were recruited from the Norwegian South-Eastern Health Region, Norway, from January 2017 to December 2019.

All patients gave their informed consent before participation. The study was approved by the Regional Ethics Committee (Dnr 2010/313) and the South-Eastern regional Ethical board (2015/946).

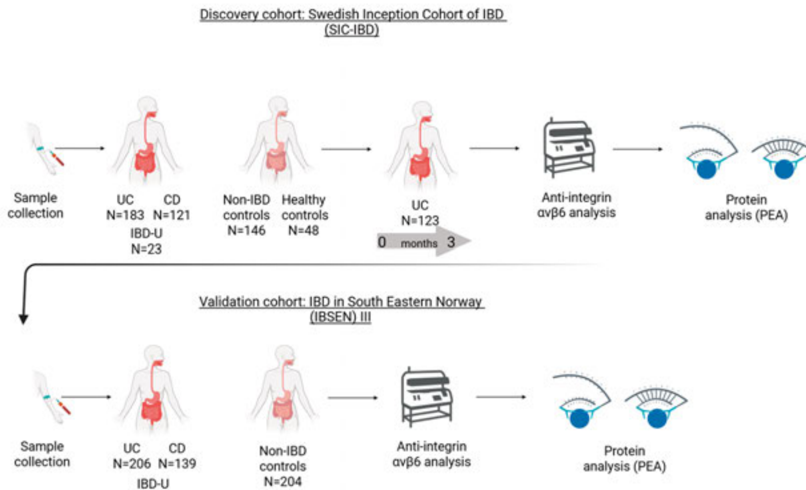

Figure 7. The overall design of study III. Created with Biorender. From Paper III.

For **study IV**, samples from multiple Swedish population-based cohorts were analysed. The Malmö Diet and Cancer (MDC) cohort was used as the discovery cohort for preclinical IBD, with findings validated in the Northern Sweden Health and Disease Study register (NSHDS) cohort (**Table 7**). In both cohorts, individuals were identified through linkage with national and regional health registers, and IBD diagnoses (CD or UC) were defined using a previously validated algorithm based on repeated ICD codes, with additional medical record review for most preclinical cases.[159,160] Each preclinical case was matched to a healthy control free from IBD by birth year, sex, and calendar period at inclusion. To explore early-life and genetic influences, the All Babies in Southeast Sweden (ABIS) birth cohort and the TwinGene study, which is nested within the Swedish Twin Registry (STR), were additionally included. In ABIS, newborns and children later diagnosed with paediatric- or adult-onset IBD and matched controls were identified through register linkage, and serum samples from umbilical cord blood at birth and from venous blood at follow-up were retrieved. Twin pairs, including healthy pairs and pairs discordant for UC, were identified in the STR using the same register-based IBD definition, and serum samples were obtained either through the TwinGene study or directly from participants to assess the impact of genetic and shared environmental factors on anti-αvβ6 levels. The study was approved by the Swedish Ethical Review Authority (2020-07065).

Table 7. Demographics of individuals in the study groups investigated in study IV (adapted from Paper IV).

	n	Age at sampling years	Age at diagnosis years	Female sex n (%)
Discovery cohort (MDC)				
Preclinical UC cases	102	55.5 (50.0-61.3)	69.1 (63.0-74.6)	52 (51)
Healthy controls	102	55.6 (49.6-61.3)	-	52 (51)
Validation cohort (NSHDS)				
Preclinical UC cases	76	50.1 (40.0-59.9)	50.9 (43.0-60.0)	40 (53)
Healthy controls	76	50.1 (40.1-59.9)	-	40 (53)
Birth cohort (ABIS)				
Preclinical UC cases	69	NA	17.9 (16.5-20.6)	34 (49)
Healthy controls	69	NA	-	34 (49)
Twin cohort				
Twin pairs discordant for UC	36	65.9 (61.9-69.8)	52.1 (39.3-61.6) *	21 (58)
Healthy external twin pairs	111	64.9 (60.8-68.7)	-	120 (54)

Age data is presented as median (IQR). *Age at diagnosis refers to the UC twin.

All cohorts included in **studies I-IV** are summarised in **Table 8**.

Table 8. Cohorts included in the present studies.

Cohort name	Study
EIRA (Epidemiological Investigations of Rheumatoid Arthritis)	Study I and II
SIC-IBD (Swedish Inception Cohort of IBD)	Study III
IBSEN III (IBD in South-Eastern Norway III)	Study III
MDC (Malmö Diet and Cancer)	Study IV
NSHDS (Northern Sweden Health and Disease Study)	Study IV
ABIS (All Babies in Southeast Sweden)	Study IV
TwinGene/part of STR (Swedish Twin Registry)	Study IV

Autoantibody measurements

For **studies I and II**, anti-CCP2 was measured with the Immunoscan CCPlus® ELISA (Eurodiagnostica/Svar Life Science, Malmö, Sweden), using 25 U/mL as cut-off corresponding to 98.4% specificity among 551 EIRA controls.

For **study II**, sixteen individual ACPA reactivities were measured with a custom-made in-house multiplex research fluorescence immunoassay based on peptide array technology.[161] The citrullinated peptides represented deiminated sequences from filaggrin, fibrinogen, vimentin, a-enolase, collagen type II and hnRNP A3 as described in detail elsewhere.[63] Fluorescence intensities were normalised and expressed as arbitrary units. For all patients and controls, fluorescence intensities for non-citrullinated control peptides were subtracted, except for CII359-369 (citC1) where the non-deiminated control antigen is in itself a conformation-dependent autoantigen where epitopes are destroyed by citrullination.

IgA, IgG and IgM RF (**studies I** and **II**) and anti-αvβ6 (**studies III** and **IV**) were measured with fluorescence enzyme immunoassays (FEIAs) based on EliA™ technology (Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden).

The EliA RF assays are commercially available. Instead of the manufacturer's recommended cut-off values we used our study research application cut-offs determined as 17.99 IU/mL (IgA RF), 34.32 µg/mL (IgG RF) and 9.96 IU/mL (IgM RF) corresponding to the 98th percentile among 624 EIRA controls respectively.

All RA autoantibody analyses (**studies I** and **II**) had been performed centrally with one technique per analyte.

For **studies III** and **IV**, serum and plasma anti-αvβ6 were measured using a newly developed in-house research FEIA. The research assay of the sandwich-type uses generic standard EliA IgG assay reagents and was adapted from a previously described microplate ELISA for anti-αvβ6.[132] Serum samples were diluted 1:100 prior to analysis. The EliA IgG calibrator system was used to convert response units to serum concentrations (U_A/L). The cut-off for positive results was 400 U_A/L corresponding to the lowest calibrator for IgG (4 µg/L) in the EliA assay and the 96th percentile of 196 healthy individuals.

Protein analysis

For **studies III** and **IV**, the proximity extension assay was used to detect relative levels of 176 proteins in serum with Proseek® Multiplex Inflammation I and Proseek® Multiplex Oncology II panels (Olink Proteomics, Uppsala, Sweden). Normalised Protein eXpression (NPX) values were obtained and levels

below limit of detection (LoD) were reported. In **study III**, proteins and samples with more than 50% of values below LoD or with a quality warning were excluded. Six proteins were common to both panels, and the mean values were used for these proteins. ComBat, which is a data-driven statistical approach that adjusts for batch effects to reduce technical variability between datasets, was used to adjust for batch effects in both cohorts.[162] Principal component analysis was used to control for outliers. In total, data of 173 patients with UC in SIC-IBD and 201 patients with UC in IBSEN III were used. In **study IV**, 29 proteins were excluded, for which >80% of samples from either the discovery or the validation cohorts were below the LoD.

Statistical analysis

In **study I**, t-test was used to compare mean values and χ^2 tests to compare proportions. Univariate analysis was used to evaluate the association between age and sex and RA autoantibodies at diagnosis. This analysis was further extended to multiple regression including all autoantibodies together and adjusted stepwise for different factors including but not limited to age and sex. Stratification analysis confirmed the findings by subsetting of patients. In **study II**, findings from **study I** were used and therefore our analysis was adjusted for age and sex but also for inclusion year and symptom duration. In both **studies I** and **II** the agreement between the occurrence of RA autoantibodies was checked, with the highest kappa value being in 0.63 for IgM RF and anti-CCP2. The same principle was followed, by which the associations in linear regression/univariate analysis were examined (all autoantibodies examined individually) and then confirmed the findings in multivariate regression analysis and stratification analysis. Independent variables (serological markers) were treated as binary variables. Dependent variables (clinical variables) were treated as continuous (SJC, TJC, ESR, CRP, patient global VAS, DAS28, DAS28CRP and HAQ).

For **study III,** the Kruskal-Wallis test was used to compare anti-αvβ6 concentrations across groups, and Dunn's multiple comparisons test was used as a post hoc test. For categorical data a χ^2 test or Fisher's exact test (for expected frequencies <5) was performed, and the Mann-Whitney U test was used for continuous parameters. Receiver operating characteristic (ROC) curves and area under the curve (AUC) with 95% confidence intervals (CIs) were generated to evaluate and compare the diagnostic and prognostic performance of different markers using logistic regression. Comparisons between the AUC values were performed using DeLong's 2-sided test. Linear mixed-effect models were used to investigate differences between baseline and the three-month

follow-up in 123 UC patients within the SIC-IBD. The analysis of protein data was based on a supervised learning model, and significant dysregulated proteins were correlated to anti-αvβ6 levels using the Pearson correlation coefficient.

For **study IV**, associations between anti-αvβ6 levels and future UC diagnosis were evaluated using a logistic regression model in a discovery cohort and subsequently tested in an independent validation cohort. Model performance was assessed using AUC, with optimal cut-off defined by the Youden index in the discovery cohort and applied to the validation cohort to estimate diagnostic performance metrics. Group differences in anti-αvβ6 levels were analysed using non-parametric tests, and correlations with time to diagnosis were assessed using Pearson's correlation coefficient. Model performance was further explored across sampling time intervals and stratified by sex and age, with AUCs compared using DeLong's test. In additional analyses, conditional logistic regression was applied to assess associations at birth, 5, and 8 years of age between cases and matched controls, while intraclass correlation coefficients were calculated in twin pairs to evaluate genetic and shared environmental influences. A sensitivity analysis with adjustment for smoking status was performed. Additionally, an exploratory correlation analysis was conducted, using a composite proteomic score based on proteins previously reported to be associated with preclinical UC. All analyses were performed on log2-transformed data. Anti-αvβ6 values below limit of detection (LoD, 85.6 U_A/L) were substituted with the LoD value divided by the square root of 2.

The statistical calculations in all studies were performed with JMP V.17 (SAS Institute, Cary, North Carolina, USA) and for **studies III** and **IV** we also used R V.4.05 (R Foundation for Statistical Computing, Vienna, Austria). GraphPad Prism V.10 (GraphPad, San Diego, CS, USA) was used for creating graphs. All p values<0.05 were considered significant. In **study III**, the Benjamini–Hochberg procedure was applied to control the false discovery rate in the context of multiple testing.

Results and Discussion

Study I

In this study we investigated the association between RA autoantibodies and demographics in RA patients at the time of diagnosis. Univariate analysis showed that anti-CCP2 ($p<0.001$) and IgM RF ($p=0.004$) associated with lower age (**Table 9**). Moreover, IgA RF ($p=0.005$) and IgG RF ($p=0.009$) associated with male sex, and IgM RF ($p=0.029$) with female sex, in the univariate analysis.

In a multivariate analysis including occurrence of all autoantibodies with no adjustments, the association between anti-CCP2 and younger age persisted ($p<0.001$) and a strong association between IgA RF and higher age at diagnosis appeared ($p<0.001$), whereas the association between IgM RF and lower age weakened ($p=0.04$). When the model was further adjusted for sex, the above-mentioned associations remained, but the association between IgM RF and lower age disappeared (**Table 9**). Further adjustments for smoking, age at inclusion in EIRA and occurrence of SE did not change this pattern.

Table 9. Mean age of 1600 RA patients in relation to the occurrence* of RF isotypes and anti-CCP2 (adapted from Paper I).

	Anti-CCP2 pos/neg	**IgA RF pos/neg**	**IgM RF pos/neg**
n	1020/580	629/908	916/684
Univariate models			
Age (mean, years)	51/54 p<0.001	53/52 p=0.08	51/53 p=0.004
Multivariate models	Std β	Std β	Std β
Age model a[1]	0.148 p<0.001	-0.154 p<0.001	0.073 p=0.04
Age model b[2]	0.145 p<0.001	-0.139 p<0.001	0.056 p=0.113

*Occurrence was determined as above the 98th percentile among population controls for all autoantibodies. [1]Multivariate analysis including occurrence of anti-CCP2, IgA RF, IgG RF and IgM RF. [2]Model a, additionally adjusted for sex.

These results were corroborated in stratification analysis where we looked into the occurrence of individual autoantibodies in relation to age at diagnosis overall, and in subsets of patients defined by the presence or absence of individual autoantibodies.

This study confirms the previously described association between anti-CCP2 positivity and younger age at diagnosis (mean age 51 years vs 54 years for anti-CCP2 negative individuals). In addition, IgA RF positivity was associated with higher age at diagnosis (mean age 53 years vs 52 years for IgA RF negative individuals).[163,164] The latter seems to be characteristic of RA as the opposite has been described previously for Sjögren's syndrome.[165] Our data imply that the previously reported association between IgM RF and young age[164] is secondary, and that the primary association is between ACPA and younger age at diagnosis. Other studies have discussed the presence of erosions in patients with higher age at RA diagnosis but have not investigated the presence of IgA RF.[166,167] An interesting hypothesis that generates from our findings would be that these elderly patients might show more radiological destructions due to presence of IgA RF.[168-171]

Our results highlight the importance of accounting for age and sex in studies examining RA phenotypes in relation to autoantibodies.

Study II

In **study II**, the same 1600 RA patients were included as in **study I**. We evaluated the association between individual RA autoantibodies separately or in combination with individual signs and symptoms at the time of RA diagnosis.

In linear regression, both anti-CCP2, any ACPA peptide reactivity, IgA RF and IgM RF were associated with lower SJC and TJC. Additionally, all autoantibodies were associated with higher ESR at the time of diagnosis (**Table 10**).

Table 10. Associations between anti-CCP2, ACPA, IgA RF, IgM RF and signs and symptoms at the time of RA diagnosis among 1600 EIRA patients. Data presented are least square mean levels from regression analyses (adapted from Paper II).

	Anti-CCP2 pos/neg	**Any ACPA reactivity pos/neg**	**IgA RF pos/neg**	**IgM RF pos/neg**
ESR (mm/1h)	31.3/24.6 $p<0.001$	30.0/23.8 $p<0.001$	33.3/25.4 $p<0.001$	32.4/24.5 $p<0.001$
SJC	9.2/10.6 $p<0.001$	9.4/10.9 $p<0.001$	9.4/10.1 $p=0.012$	9.4/10.2 $p=0.007$
TJC	7.8/9.2 $p<0.001$	8.0/8.7 $p<0.001$	8.0/8.7 $p=0.003$	8.0/8.8 $p=0.007$

All models were adjusted for age, sex, inclusion year and symptom duration.

When we performed multivariate analysis with all RF isotypes and ACPAs, defined as having any ACPA peptide reactivity, IgA and IgM RF associated significantly with higher ESR ($p=0.01$ and $p=0.011$, respectively) and ACPA positivity with low SJC and TJC (both $p=0.0003$). Defining ACPA-positive group as having an anti-CCP2 positive test, yielded similar results.

Using stratification analysis, we confirmed these findings and noted that RF positivity associated with ESR only when ACPAs were present, whereas SJC was significantly lower among ACPA-negative patients when RF was absent. Taken together, in early RA, RF and ACPAs associate with different individual DAS28 components and in opposite directions. In contrast, when the composite DAS28 score was analysed, no significant associations were found.

Lower joint counts in ACPA-positive patients at disease onset suggest that early pathology associated with this phenotype may initially be localised to fewer joints, differing from patients with an ACPA-negative arthritis phenotype. According to previous studies on pre-disease samples from subjects subsequently developing RA, [42,172] the ACPA immune response appears to be initially restricted regarding peptide specificities, but the number of reactivities increases in samples taken close to diagnosis. This increase was particularly prominent close to disease onset. In line with this, we observed a higher number of ACPA reactivities in patients with longer symptom duration within the eligible 1-year patient inclusion period. All analyses were therefore adjusted for symptom duration at the time of sampling.

Previous studies have reported lower DAS28 and fewer swollen and tender joints in newly diagnosed seropositive compared to seronegative patients.[173,174] One of these studies suggested that the increased inflammation in seropositive patients was a result of the 2010 classification criteria.[174]

Importantly, autoantibody data in these studies had been compiled from analyses performed locally at different sites (instead of subject to centralised testing).

The use of 1987 ACR criteria for the classification of our patient cohort allowed us to evaluate the biological importance of these results. When investigating patients with four or more non-RF criteria, that is, fulfilling the ACR criteria without taking autoantibody serology classification item into account, we showed that IgM RF association with ESR ($p<0.001$), and ACPAs with lower SJC ($p=0.047$) and TJC ($p=0.0004$), remained. This is an argument that the conclusions of our observations align with true biological phenotypes associated with individual RA-associated autoantibodies.

Centralised autoantibody testing with standardised cut-offs across all investigated autoantibodies enabled investigation of the independent effects of ACPAs in RF-negative subjects and of RF in ACPA-negative subjects. Moreover, the use of an ACPA multiplex array allowed assessment of the cumulative impact of increasing number of ACPA specificities and identification of a strictly ACPA-negative subset, consistent with reports of residual ACPA reactivity among anti-CCP2–negative individuals.[63] IgM RF was not associated with elevated ESR in ACPA-negative patients but showed a progressive association with ESR as the number of ACPA specificities increased. Across stratified and multivariable analyses, IgM RF was linked to higher ESR at diagnosis only in the presence of ACPAs. Therefore, we proposed an ACPA-dependent proinflammatory effect of RF which is in agreement with data from previous in vitro studies suggesting an ACPA/RF IC-induced inflammation (**Figure 8**).[175,176]

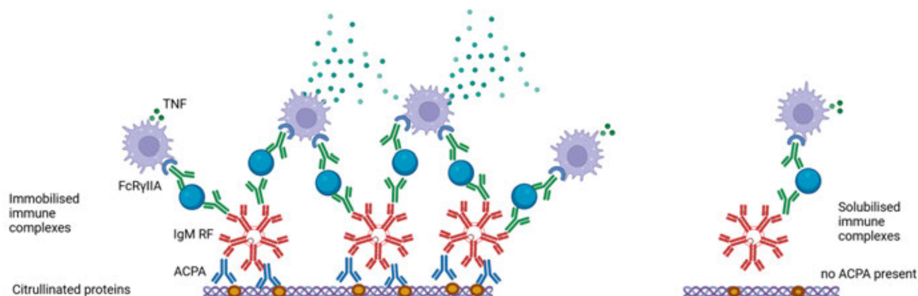

Figure 8. Hypothetical figure showing the ACPA dependency of RF-enhanced production of TNFα after formation of immobilised ICs between ACPA and RF (left) versus after the formation of solubilised ICs with low TNFα-inducing capacity when ACPAs are not present (right). The soluble ICs might or might not contain ACPA. Created with BioRender. From Paper II.

Study III

In this study, we investigated the association between anti-αvβ6 and different clinical phenotypes in two independent inception cohorts of newly diagnosed IBD patients. Additionally, using proteomics data we defined different inflammatory phenotypes in patients with UC and followed their autoantibody levels up to 3 months to examine whether we could prognosticate the disease course. Our study is the first to examine anti-αvβ6 in patients with newly diagnosed colonic CD. We demonstrated that UC patients and colonic CD (CD_L2) appear with significantly higher levels of anti-αvβ6 in comparison to symptomatic and healthy controls in both cohorts, as described in recent studies (**Figure 9**).[12,132]

We reported a sensitivity of 73% and a specificity of 93% for distinguishing UC from symptomatic controls in SIC-IBD, with corresponding values of 79% and 94% in the validation cohort. The diagnostic performance of anti-αvβ6 outperformed hsCRP and FC. Moreover, we examined autoantibody positivity in relation to demographic and clinical parameters in UC and CD patients. Our data showed that anti-αvβ6 positivity was associated with a more severe phenotype of UC in the SIC-IBD cohort, including more extensive colitis ($p=0.008$), increased endoscopic activity ($p=0.005$) and systemic inflammation as shown by increased hsCRP ($p=0.04$) and decreased albumin levels ($p=0.01$). Significantly increased inflammation, as measured by the endoscopic Mayo score was confirmed in UC patients positive for anti-αvβ6 in the validation cohort ($p=0.02$).

In line with these results, significantly higher median levels of anti-αvβ6 were observed with increasing disease extent and endoscopic activity in both cohorts. In addition, higher antibody levels were noted in UC patients with increasing severity of patient reported outcomes in the IBSEN III cohort.

We next identified five proteins that were significantly upregulated in anti-αvβ6 positive UC patients in SIC-IBD and validated two of them—Granzyme B and IL-17A—in IBSEN III. Both have been previously discussed in regard to IBD.[127,177-180]

Finally, to evaluate the prognostic significance of anti-αvβ6, we looked into the difference in disease outcome between autoantibody positive and negative patients and also compared the anti-αvβ6 levels by disease course outcomes. A logistic regression model distinguishing indolent from aggressive UC showed modest discriminative ability of the autoantibody, with an AUC of 0.62 in the discovery cohort and similar discriminative performance in the validation cohort (AUC=0.61). At the optimal cut-off (1100 U_A/L), the model demonstrated a sensitivity of 76% and a specificity of 37% in the validation cohort for predicting aggressive UC. For UC patients in the SIC-IBD cohort,

anti-αvβ6 levels were significantly higher at baseline in patients with an aggressive compared to those with an indolent disease course.

Following anti-αvβ6 levels over time in patients with UC from the SIC-IBD (N=123), we found a significant interaction between disease course and visit (baseline and 3 months) for anti-αvβ6 levels. The levels decreased significantly from baseline to 3 months in UC patients with an indolent disease course (mean log2-change: -1.15; $p<0.001$), whereas patients with aggressive disease showed no significant change in autoantibody levels (mean log2-change: 0.01; $p=0.97$).

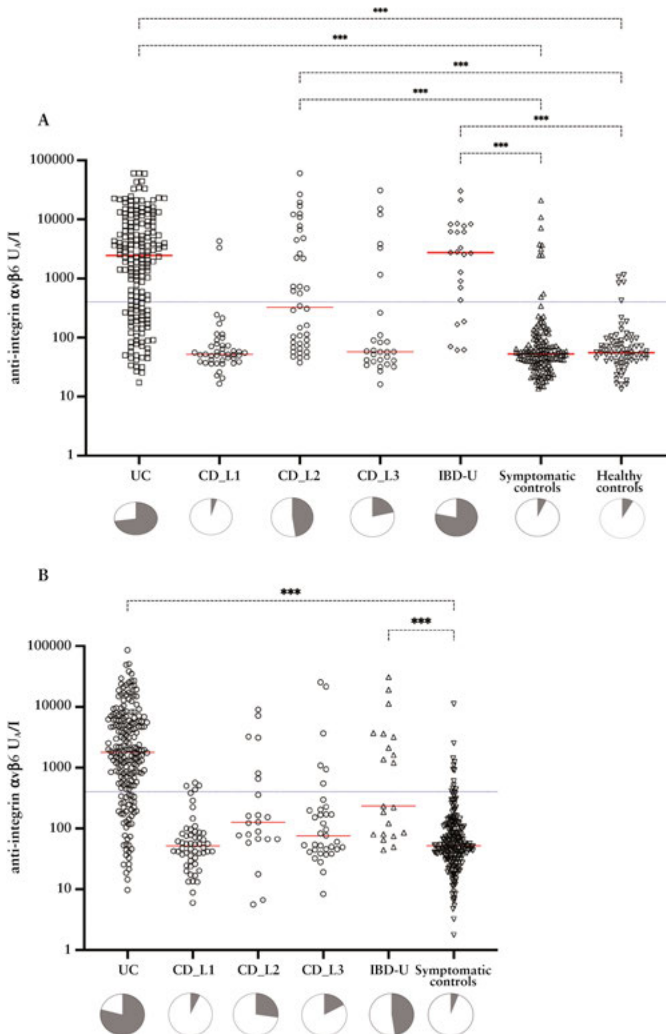

Figure 9. Median levels of anti-αvβ6 differed between disease categories in both the SIC-IBD and IBSEN III cohorts. Anti-αvβ6 levels by disease groups in **A)** SIC-IBD and **B)** IBSEN III. The red line represents the median, and the blue dotted line is set to the suggested cut-off (400 U_A/L). From Paper III.

By examining inception cohorts, we advanced previous knowledge and demonstrated a high diagnostic accuracy of anti-αvβ6 for UC, outperforming established serological markers and inflammatory biomarkers. Diagnostic performance was further improved when combined with FC (in the discovery cohort: AUC=0.92, 95% CI, 0.89-0.96, p=0.02 and in the validation cohort: AUC=0.97, 95% CI, 0.95-0.98, p<0.001), resulting in near-optimal discrimination and enhanced patient reclassification in validation analyses.

Anti-αvβ6 levels were elevated in patients with colonic CD compared with controls in the discovery cohort, although this was not replicated in the validation cohort.

Our findings suggest that increased levels of anti-αvβ6 may reflect a distinct immunological phenotype rather than a nonspecific marker of colonic inflammation.

Study IV

In this study, we evaluated the predictive ability of anti-αvβ6 in two large population-based cohorts. We established a threshold for our research assay (109 U_A/L) for predicting a future UC diagnosis in the discovery cohort (MDC) and validated its performance in an external independent cohort (NSHDS), which resulted in an AUC of 0.79, a sensitivity of 46% and a specificity of 93% (**Figure 10**).

The predictive capacity of the autoantibody increased closer to diagnosis and remained significant even for patients whose samples had been collected more than 20 years before UC diagnosis (AUC=0.67).

As elevated levels of anti-αvβ6 were observed more than two decades before diagnosis of UC, we examined whether the autoantibodies were present already in early life. Autoantibody levels at birth were numerically higher in children who later in life developed UC (OR=2.13; 95% CI, 0.84-5.38). Although only one of the mothers had a diagnosis of UC, these findings most likely reflect transplacental transfer of maternal antibodies. The ORs appeared to increase also after 5 years (OR=2.58; 95% CI, 0.83-8.03) and 8 years (OR=2.50; 95% CI, 0.30-21.00), although the confidence intervals were broad, reflecting the smaller number of available samples at these time points compared with at birth.

Since anti-αvβ6 were observed already in early childhood, we assessed whether genetic predisposition and shared environmental exposures influenced antibody levels in the Swedish twin cohort. When assessing the level of agreement within twin pairs, low intra-class correlation coefficients were

observed regardless of zygosity or disease status, suggesting limited influence of genetic and shared environmental exposures.

Given the limited genetic and shared environmental influences on anti-integrin αvβ6 levels in the twin cohort, we next examined the effect of smoking status, the best-characterised unique environmental risk factor for UC. Overall, anti-αvβ6 levels did not differ across smoking categories. In addition, inclusion of smoking status as a covariate in the regression model did not materially improve the predictive performance in the validation cohort (AUC=0.82).

To explore the relationship between anti-αvβ6 and immune-inflammatory proteins previously associated with preclinical UC,[181] we calculated a composite proteomic score and observed a progressive increase in the score across the lowest to highest quartiles of anti-αvβ6 levels in preclinical UC.

Finally, numerically higher levels of anti-αvβ6 were observed in preclinical colonic CD patients (validation cohort, N=10) compared with matched controls (OR=4.97; 95% CI, 0.40-61.44). ROC analysis showed a moderate predictive capacity to discriminate preclinical colonic CD from controls (AUC=0.62).

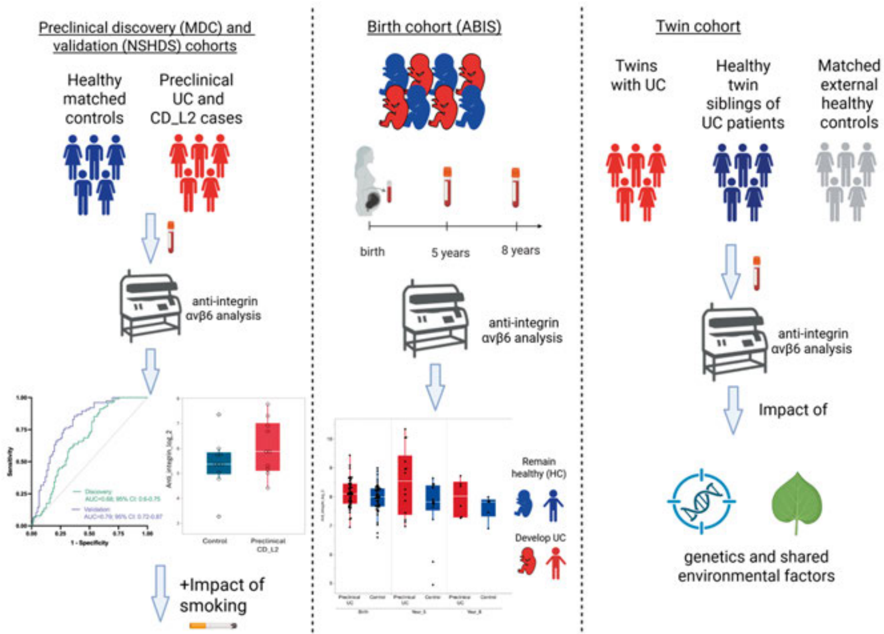

Figure 10. Graphical abstract. Created with Biorender. From Paper IV.

Our study, including both a discovery and a validation cohort, confirms previous studies reporting increased levels of anti-αvβ6 preceding the diagnosis of UC.[12] Moreover, we extended previous work by establishing an optimal

cut-off of 109 U_A/L based on the discovery cohort. Predictive performance of anti-αvβ6 increased closer to diagnosis, with antibody levels correlating with time to diagnosis in the discovery cohort. Longer intervals between blood sampling and diagnosis in our cohorts compared with previous studies by Sawahashi et al. who reported an AUC of 0.89, may partly explain the lower predictive performance of anti-αvβ6 observed in our study.[182]

Previous studies have shown that microbial antibodies are detectable early in life in offspring of individuals with IBD, but anti-αvβ6 was not assessed.[183] In contrast, we observed elevated anti-αvβ6 levels at 5 and 8 years of age in children who later developed UC, suggesting that loss of tolerance to integrin αvβ6 may arise early in life. This early increase likely reflects epithelial barrier stress and is supported by concurrent elevations in immune-inflammatory proteins, indicating parallel development of mucosal immune activation. Collectively, these results support further evaluation of anti-αvβ6 antibodies as a potential marker for preclinical UC risk assessment and may be used to inform the design of future interception and prevention studies.

Conclusions and future directions

Studies I and II

We conclude that future studies which evaluate the impact of individual autoantibodies in RA should take into consideration and adjust their analysis for age and sex. Moreover, we have shown that in early RA, ACPAs associated with lower counts of affected joints and RF with elevated measures of systemic inflammation in an ACPA-dependent manner. Therefore, the impact of different RA autoantibodies should be assessed individually and DAS28 components should be evaluated separately as outcome markers. This approach may increase the precision in algorithms for serology-based treatment decisions in early RA.

Studies III and IV

Collectively, findings from studies III and IV support anti-αvβ6 as a promising biomarker in IBD, particularly UC, with potential utility across diagnostic, prognostic, and preclinical settings. Anti-αvβ6 may enable earlier disease identification, improved patient stratification, and assessment of future disease risk, thereby informing timely treatment decisions and supporting the development of disease interception and prevention strategies aimed at improving long-term outcomes in IBD.

Concluding remarks

In this thesis, RA and IBD are explored as two immune-mediated inflammatory diseases in which heterogeneity in disease development, phenotype, and outcome is closely linked to immunological biomarkers. In RA, we demonstrated that age and sex influence autoantibody occurrence and clinical presentation at disease onset, and that serological subgroups defined by autoantibody positivity represent biologically distinct disease entities. Applying similar principles to IBD, we investigated how age at sampling, sex, and autoantibody status contributes to disease stratification, including distinctions between

antibody-positive and antibody-negative states that may reflect different underlying pathogenic pathways. Importantly, in both RA and IBD, autoantibodies have been shown to precede clinical disease by several years. In RA, the presence of autoantibodies years before clinical onset has been established in several preclinical cohort studies, whereas the present work demonstrates a similar pre-diagnostic autoantibody development in IBD. These findings support the concept of a prolonged preclinical phase and a window of opportunity during which early identification of high-risk individuals may enable disease interception or modification. Together, these findings underscore the value of studying autoantibodies not only as diagnostic markers but also as tools to understand early disease mechanisms and to guide personalised, risk-adapted approaches to prevention and treatment. By integrating demographic factors, serological profiles, and timing of immune activation, these findings may inform future efforts toward more individualised approaches in immune-mediated inflammatory diseases.

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to believe in yourself and follow your own dreams with courage. I love you endlessly.

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