

# Treatment of arthritis with tumour necrosis factor antagonists. Clinical, immunological and biochemical aspects

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From the Department of Rheumatology

# Treatment of arthritis with tumour necrosis factor antagonists. Clinical, immunological and biochemical aspects

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Abstract

The treatment of arthritis has undergone a dramatic change since biological agents targeting specific mediators of the disease process have been introduced. Tumour necrosis factor (TNF) antagonists have been shown to reduce signs and symptoms of disease and to retard the development of tissue damage in the majority of patients.

This thesis focuses on clinical, immunological and biochemical aspects of treatment with TNF antagonists in patients with arthritis. In particular, the studies examine: (i) the feasibility of a structured protocol with central data handling for the prospective monitoring treatment efficacy and tolerability of new treatments in clinical practice, (ii) whether serum levels of cartilage oligomeric matrix protein (COMP) change during treatment with TNF antagonists in a way that corroborates a tissue protective effects of these agents in rheumatoid arthritis (RA), (iii) how different anti-rheumatic treatments modulate the immune response induced by polysaccharide or polypeptide vaccines in patients with RA and (iv) potential predictors of infusion reactions during treatment with infliximab.

All the patients who participated in the studies were monitored according to a standardised clinical protocol of the South Swedish Arthritis Treatment Group (SSATG) developed at the Department of Rheumatology in Lund. We found that such a protocol could be used for monitoring newly introduced anti-rheumatic treatments both at a university department and at other rheumatology units. The performance of TNF antagonists regarding efficacy and safety complied with results of previously published clinical trials.

Serum levels of COMP were measured in RA patients treated with infliximab and etanercept during the initial 6 months of treatment. Serum COMP levels decreased in patients with and without a clinical response, suggesting a damage retarding effect of TNF antagonist treatment.

Altogether, 149 patients with RA participated in studies of the immune response to pneumococcal or influenza vaccination. Patients treated with TNF antagonists and controls showed similar responses to pneumococcal vaccine, whereas methotrexate treated patients showed reduced response to this vaccine regardless of concomitant treatment with TNF antagonists. In contrast, RA patients treated with methotrexate without TNF antagonists had significantly better immune response to influenza vaccination than those receiving TNF antagonists alone or in combination with methotrexate and/or other disease modifying anti-rheumatic drugs.

Possible predictors of infliximab related infusion reactions were studied in a cohort of 213 patients with RA and 76 patients with spondylarthropaties. Infliximab without methotrexate and positive baseline ANA (antinuclear antibodies) were independent risk factors for developing infusion reactions in RA but not in spondylarthropaties.

In conclusion, a structured protocol with central data handling is feasible in clinical practice for documenting the efficacy of and adverse events associated with drugs used for the treatment of arthritis. Serum COMP has the potential to be a useful marker for evaluating tissue effects of novel treatment modalities in RA. Methotrexate treatment in RA reduces antibody response to pneumococcal vaccine, suggesting that RA patients should be vaccinated before the initiation of this treatment. The immune response to influenza vaccination is sufficiently good to warrant vaccination of all RA patients, regardless of treatment. Positive ANA at initiation of infliximab treatment and the use of infliximab as monotherapy is associated with increased risk of infusion reactions in RA.



Key words

Arthritis, rheumatoid arthritis, spondylarthropathies, TNF antagonists, COMP, pneumococcal vaccination, influenza vaccination,infusion reaktion, ANA

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# Treatment of arthritis with tumour necrosis factor antagonists.

## Clinical, immunological and biochemical aspects

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Cover illustration

"Path through the long grass" Pierre Auguste Renoir, 1877, oil on canvas, Musée d'Orsay, Paris, France

Pierre Auguste Renoir (1841-1919) suffered from rheumatoid arthritis. Although severely hampered by arthritis and wheelchair-bound Renoir continued painting until the end of his life.

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**In science, as in life, learning and knowledge are distinct, and the study of things, and not of books, is the source of the latter.**

**T. H. Huxley, 1861**

**CONTENTS**

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# Publications

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

**I** Etanercept, infliximab and leflunomide in established rheumatoid arthritis: clinical experience using a structured follow up programme in southern Sweden.
Pierre Geborek, Meliha Crnkic, Ingemar F Petersson, Tore Saxne
*Ann Reum Dis 2002; 61:793-798*

**II** Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept.
Meliha Crnkic, Bengt Månsson, Lotta Larsson, Pierre Geborek, Dick Heinegård, Tore Saxne
*Arthritis Res Ther 2003; 5:R181-185*

**III** Influence of methotrexate, TNF-blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis .
Meliha Crnkic Kapetanovic, Tore Saxne, Anders Sjöholm, Lennart Truedsson, Göran Jönsson, Pierre Geborek
*Rheumatology 2006; 45:106-111*

**IV** Influenza vaccination as model for testing immune modulation induced by anti-TNF and methotrexate therapy in rheumatoid arthritis patients.
Meliha Crnkic Kapetanovic, Tore Saxne, Jan-Åke Nilsson, Pierre Geborek
*Rheumatology 2006, in press*

**V** Predictors of infusion reactions during infliximab treatment in patients with arthritis.
Meliha Crnkic Kapetanovic, Lotta Larsson, Lennart Truedsson, Gunnar Sturfelt, Tore Saxne, Pierre Geborek
*Arthritis Res Ther 2006 July 26; 8(4):R131.*



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# Abbreviations

| | |
|---|---|
| ACR | American College of Rheumatology |
| AKA | antikeratin antibodies |
| ANA | antinuclear antibodies |
| Anti-CCP | anti-cyclic citrullinated peptide |
| APC | antigen presenting cell |
| APF | antiperinuclear factor |
| AS | ankylosing spondylitis |
| ASAS | Assessments in Ankylosing Spondylitis working group |
| BASDAI | Bath Ankylosing Spondylitis Disease Activity Index |
| BASFI | Bath Ankylosing Spondylitis Functional Index |
| BASMI | Bath Ankylosing Spondylitis Metrology Index |
| COMP | cartilage oligomeric matrix protein |
| CRP | C-reactive protein |
| DAS | disease activity score |
| DMARDs | disease modifying anti-rheumatic drugs |
| ELISA | enzyme-linked immunosorbent assay |
| ESR | erythrocyte sedimentation rate |
| EULAR | European League Against Rheumatism |
| GM-CSF | granulocyte-macrophage colony-stimulating factor |
| HAQ | health assessment questionnaire |
| HI | haemagglutination inhibition |
| HLA | human leukocyte antigen |
| IIF | indirect immunofluorescence |
| IFN | interferon |
| IL | interleukin |
| IL-1Ra | interleukin 1 receptor antagonist |
| MHC | major histocompatibility complex |
| NSAID | non-steroidal anti-inflammatory drug |
| PADI | peptidylarginine deiminase |
| PsA | psoriatic arthritis |
| PsACR | psoriatic arthritis response criteria |
| RA | rheumatoid arthritis |
| RANKL | receptor activator of NF-kappa B ligand |
| RCT | randomised clinical trial |
| RF | rheumatoid factor |
| SDAI | simplified disease activity index |
| SpA | Spondylarthropathies |
| SSATG | South Swedish Arthritis Treatment Group |
| TCR | T-cell receptor |
| TGF | transforming growth factor |
| TNF | tumour necrosis factor |
| VAS | visual analogue scale |
| WHO | World Health Organization |

# Introduction

Chronic inflammatory arthritis is a clinically heterogeneous group of inflammatory disorders including rheumatoid arthritis (RA) and spondylarthropathies (SpA). Arthritis in RA is often symmetric and typically affects the small joints of the hands and the feet. SpA often involves the spine and sacroiliac joints, but enthesophaties and dactylitis are also common symptoms. The clinical symptoms vary between these different types of arthritides but those related to inflammation, i.e. fatigue and inflammatory joint pain, are often present. Since 1999 anti-inflammatory treatment targeting tumour necrosis factor (TNF) has been available in Sweden. Initially, TNF antagonists were approved for the treatment of RA. However, the growing evidence of treatment efficacy in other inflammatory diseases has led to the approval of TNF antagonists for the treatment of other inflammatory conditions, including SpA.

This thesis comprises 5 studies of patients with established RA. The fifth study also includes patients with SpA. The overall purpose of the investigation was to study clinical, immunological and biochemical aspects of treatment with TNF antagonists in patients with established arthritis in clinical practice.

## Rheumatoid arthritis

RA is a chronic inflammatory disease mostly affecting the joints. RA is the most common inflammatory joint disease. It is found worldwide, affecting all ethnic groups with somewhat differing prevalence. A prevalence rate of 0.5-1% was reported in Sweden (Simonsson et al. 1999) corresponding to the prevalence rate in other parts of the Western world. A higher prevalence of about 5% has been found among some North American Indians (Jacobsson et al. 1994a and 1994b, Hirch et al.1998) and the lowest prevalence was reported among rural populations of China, Indonesia and Africa (Silman et al. 1993a, Symmons et al. 2002a and 2002b).

### *Aetiology and pathogenesis*

The aetiology of RA remains unknown but both genetic and environmental factors contribute to the susceptibility to and the severity of the disease. The genetic background of RA is only partly understood, and several genes seem to be involved. Based on data from 2 nationwide studies on twins, the contribution of genetic factors to susceptibility to RA has been estimated to be about 60% (MacGregor et al. 2000). A study on monozygotic twins reported a concordance rate of about 15% (Silman et al. 1993b). Much of the genetic contribution to RA lies within the major histocompatibility complex (MHC) on chromosome 6, and human leukocyte antigen (HLA) class II alleles have been recognised as important genetic risk factors. The association between HLA-DRB1*0401 (DRw1) and RA was initially discovered by Stastny (1978) and association with HLA-DRB1*0404 was reported later (Nepom et al. 1986). Subsequent studies in different ethnic groups found the association between RA and other HLA-class II antigens leading to the shared epitope (SE) hypothesis (MacGregor et al. 1995). The SE is an amino acid sequence on the third hypervariable region of the DR-β chain, near the peptide binding site of the DRB1 molecule. According to the shared epitope hypothesis the SE allows the presentation of arthritogenic peptides to T-cells and is thus involved in the pathogenesis of RA (Gregerson et al. 1987). Certain HLA-DRB1 alleles are found to predispose to more severe disease, but there may also be protective HLA-DRB1 alleles. HLA genes are estimated to contribute 30% to the overall genetic risk of RA, which means that additional gene-environmental interactions and environmental factors must explain the rest.

Significant progress has been made during recent years through studies on genetic influences on disease susceptibility by investigating different candidate genes outside the HLA system. There is now evidence

suggesting that the PTPN22 gene (protein tyrosine phosphatase N22) regulating the activity of both T- and B-cells is associated with RF (rheumatoid factor) positive RA and anti-CCP (antibodies to citrullinated peptides) positive RA (Begowich et al. 2004, Plenge et al. 2005). Also, the PADI4 gene, which encodes citrullinating enzyme peptidylarginine deimidase 4 has been shown to be expressed in synovial tissue in RA and to be associated with anti-CCP positive RA (Yamada et al. 2003, Plenge et al. 2005). The CTLA4 gene encodes cytotoxic T-lymphocytes antigen 4 which is a costimulatory molecule expressed on T-cells acting as a negative regulator of T-cell co-stimulation. Polymorphism within this gene has been shown to influence the risk of developing subsets of RA (Plenge et al. 2005).

The estimated female to male prevalence of RA is 2.5:1 (Lawrence et al. 1998) suggesting that hormonal factors play a role. RA tends to be ameliorated during pregnancy and the relapses often occur during the postpartum period (Silman 2002a and 2002b). Furthermore, RA often starts during the postmenopausal period of life. Women taking oral contraceptives are at decreased risk of developing RA (Silman et al. 2001 and Silman 2002b). Hormone replacement therapy given to postmenopausal women has also been shown to ameliorate inflammation and inflammation-triggered joint destruction (d'Elia et al. 2003a and 2003b, Carlsten 2005).

Smoking is the environmental factor that has been most convincingly shown to be a risk factor for RA (Vessey et al. 1987, Silman et al. 1993c and Silman 1996). Furthermore, studies on gene-environment interactions have shown that the presence of the shared epitope of HLA-DR genes in combination with smoking leads to an even higher risk of developing seropositive RA (Padyukov et al. 2004). Recently, a new model for the aetiology of RA has been proposed, suggesting that smoking triggers HLA-DR restricted immune reactions to citrullinated proteins (Klareskog et al. 2006).

It has been proposed a number of infectious agents including *Epstein-Barr virus, Mycobacterium tuberculosis, Escherichia coli, Proteus mirabilis, retroviruses and parvovirus B19* that are involved in the aetiology of RA. Increased levels of antibodies to some microorganisms led to the hypothesis of molecular mimicry between infectious or other exogenous antigens and autoantigens. However, studies on the role of infections in the direct pathogenic mechanisms that lead to RA have so far been inconclusive. Associations between previous infections, blood transfusion prior to disease onset and obesity have also been discussed as risk factors (Symmons et al. 1997).

As mentioned above, the initiating event(s) of RA is still unknown, but our understanding of the pathogenetic mechanisms is increasing. There is substantial evidence in support of CD4+ T-lymphocytes playing a major role in the initiation and maintenance of synovial inflammation (Harris Jr 1990, Panayi 1995, Hasler 2006). In order to initiate the inflammation, naïve T-lymphocytes must be activated. The activation of T-cells requires 2 signals. First, APC (antigen presenting cells) such as macrophages, dendritic cells or fibroblast-like synoviocytes bearing MHC class II, process the locally resident, still unidentified antigen and present it as a polypeptide to a TCR (T-cell receptor). The second signal includes binding of co-stimulation ligand-receptor complexes such as the CD80/CD86 ligand on the APC with the CD28 receptor on the T-cell. In genetically susceptible individuals, this activation leads to cellular immune responses with infiltration of the synovial tissue by T-lymphocytes, B-lymphocytes and macrophages. T-lymphocytes interact with other inflammatory cells and activate them to produce different cytokines and other signal molecules such as growth factors and proteases (Figure 1).

Inflammation in the joint first involves the synovial membrane. In a normal joint, the synovium covers the inside of the joint apart from the cartilage. It consists of 1-3 superficial cell layers (lining) and a sublining containing an extracellular matrix rich in collagen fibrils and proteoglycans, blood vessels, fat tissue and fibroblasts. During inflammation, cells in the lining layers proliferate and different inflammatory cells infiltrate the synovial tissue. A thickened, congested and oedematous tissue rich in inflammatory cells forms a pannus. The pannus

has the ability to attach to cartilage, particularly at the cartilage-bone junction, and invade the extracellular matrix. The release of proteolytic enzymes leads to the destruction of periarticular cartilage and bone (Mor et al. 2005).

The role of B-lymphocytes in the pathogenesis of RA has been modified several times during the past 50 years. Activated B-cells can act as APC and present antigen peptides to T-cells (Kotzin 2005), produce proinflammatory cytokines (TNF-alpha, IL-6) and immunoregularoty cytokines (IL-10), or can differentiate to plasma cells and produce antibodies such as RF and anti-CCP (Vossenaar et al. 2003). Antibodies against cyclic citrullinated peptides including fillagrin, fibrin, fibronectin and collagen type I might be involved in B-cell mediated tissue damage. The efficacy of the recently introduced B-cell depleting treatment in RA has re-established the important role of B-cells in the pathogenesis of the disease (Edwards et al. 2004a and 2004b).

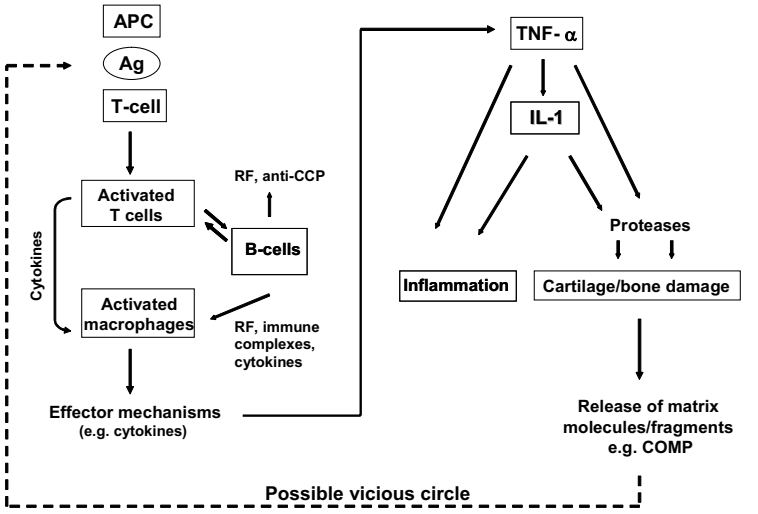


Figure 1: Simplified scheme of the pathogenesis of RA

### *Clinical symptoms, diagnosis and classification*

There is no pathognomonic symptom or test that confirms the diagnosis of RA, and the diagnosis is based on the presence of typical signs and symptoms. During the early course of the disease, many patients present symptoms of undifferentiated polyarthritis (Dixon and Symmons 2005) that may develop into RA or some other arthropathy, resolve spontaneously or remain undifferentiated. Early identification of patients with a possible diagnosis of RA and rapid referral to a rheumatologist for definite diagnosis is essential in order to achieve optimal treatment efficacy. Emery at al. (2002) developed recommendations intended to serve as clinical guidelines for primary care physicians for early identification of patients likely to have RA. Referral to a specialist is recommended if any of the following symptoms is present: three or more swollen joints, involvement of metacarpophalangeal or metatarsophalangeal joints or morning stiffness lasting longer than 30 minutes.

Recently, an EULAR (European League Against Rheumatism) expert committee presented a set of 12 key recommendations for the management of early arthritis (Combe et al. 2006). These recommendations are based on recent

research evidence and expert consensus. The first four recommendations focus on early diagnosis of RA and include: early referral (patients with arthritis in more than one joint should be referred to a rheumatologist, ideally within 6 weeks), diagnosis of early synovitis (detected mostly by clinical examination), a minimum set of diagnostic procedures in order to exclude other diseases mimicring RA (disease history, clinical examination, laboratory tests) and assessment of predictors of persistent and erosive arthritis (tender and swollen joint count, CRP/ESR, RF, anti-CCP and joint erosions evidenced by radiography).

The American College of Rheumatology (ACR) has developed a set of classification criteria for the diagnosis of RA (last revision by Arnett et al. 1988). These criteria are aimed at the selection of RA patients for participation in clinical studies and not for managing individual patients. According to the ACR revised criteria for classification of RA, 4 out of 7 of the criteria must be present during a period of at least 6 weeks for a patient to be classified as having RA, Table 1.

**Table 1: The American College of Rheumatology revised criteria for RA (1987)**

| |
|---|
| Morning stiffness in and around joints lasting at least 1 hour before maximal improvement |
| Soft tissue swelling (arthritis) of 3 or more joint areas observed by a physician |
| Swelling (arthritis) of the proximal interphalangeal, metacarpophalangeal, or wrist joints |
| Symmetric swelling (arthritis) |
| Rheumatoid nodules |
| Presence of rheumatoid factor |
| Radiographic erosions and/or periarticular osteopaenia in hand or wrist joints |

*Co-morbidity*

Co-morbid conditions are common in patients with RA. In a group of 288 randomly selected patients with RA, 54% of the patients reported at least one additional chronic disease (Berkanovic et al. 1990). In a cohort of 450 RA patients, Gabriel et al (1999) found that almost 60% of the patients had other medical conditions, compared with 49% in age- and gender-matched controls without RA. Cardiovascular disease, malignancy, peptic ulcer disease and chronic lung diseases were the most common co-occurring diseases in this study. Recently, several studies have reported increased cardiovascular morbidity in patients with RA (del Rincon et al. 2001, Turesson et al. 2004). The issue of increased risk of malignancies in RA patients, in particular risk of lymphoma, has been addressed in several studies (Prior et al. 1984, Hakulinen et al.1985, Gridley et al. 1993, Baecklund et al. 1998, Thomas et al 2003, Ekström et al. 2003). Baeklund et al (1998) found that the risk of lymphomas was associated with disease activity. RA patients with the highest inflammatory disease activity had a substantially increased risk of lymphoma, whereas treatment with common DMARDs itself was not associated with a higher risk. Furthermore, treatment with DMARDs in RA does not increase the risk of developing lymphoma in patients with high disease activity (Baecklund et al. 2006).

Askling et al. (2005a) conducted a study on the risk of developing solid cancers using the Inpatients Register Cohort of patients with RA in Sweden, and found a marginally increased overall risk for smoking related cancers and non-melanoma skin cancers but a decreased risk for breast and colorectal cancers. The risk of cancer in RA patients treated with TNF antagonists was rather similar to those of RA patients not receiving TNF antagonists. Hyrich et al. (2006) studied baseline co-morbidity in RA patients starting treatment with biological agents, and found that 58% of the patients had at least one co-morbid condition and 25% more than one. Most common were hypertension, depression, peptic ulcer disease and respiratory disease. On the other hand, treatment with TNF antagonists was reported to be associated with a lower incidence of a first cardiovascular event in RA (Jakobsson et al. 2005).

Infections also cause significant morbidity and mortality in RA. Patients with RA exhibit increased incidence of infection, including those affecting the respiratory tract, compared with age-matched subjects without RA (Doran et al. 2002a). The highest incidence rate was found for pneumonia in that study. Possible explanations of the increased infection risk in RA include

immune dysfunction associated with the disease itself, co-morbidity and/or concomitant medication such as immunosuppressive drugs (Mitchell et al. 1986, Doran et al. 2002a and 2002b, Cunnane et al. 2003). The use of DMARDs, including concomitant treatment with glucocorticoids has been found to be associated with increased risk of infection in patients with rheumatic diseases (Segal 1997). However, in the study by Doran et al. (2002b), including patients on traditional anti-rheumatic treatment before the TNF inhibitor era, the use of long-term systemic glucocorticoids, but not DMARDs, predicted infections in RA. The introduction of TNF antagonists has contributed to a somewhat changed pattern of infection in RA (Keane et al. 2001, Lee et al. 2002, Mohan et al. 2003). Upper respiratory tract infections have been described as common adverse events and reasons for withdrawal of TNF antagonist therapy in clinical trials as well as in observational studies (Cunnane et al. 2003). The rate of serious bacterial infections has also been found to be increased (Kroesen et al. 2003, Askling et al. 2005c, Askling et al. abstract 182, EULAR congress, 2006). Furthermore, cases of severe opportunistic infections have been reported during treatment with TNF antagonists (Lee et al. 2002, Cunnane et al. 2003, Mohan et al. 2003). A recently published meta-analysis of 9 randomised clinical trials suggests an increased risk of serious infection during treatment with infliximab and adalimumab (Bongartz et al. 2006), which is in contrast to findings based on the Swedish National Biologics Register, where the risk of infection was only modestly increased (Askling et al. abstract 182, EULAR congress, 2006). TNF plays an essential role in the immune-mediated response to infection, especially against intracellular pathogens and also in granuloma formation (Crum et al. 2005). A two-fold increase in the risk of tuberculosis has been reported in RA patients compared with the general population (Carmona et al. 2003, Askling et al. 2005c). This risk is increased four times in Swedish RA patients treated with TNF antagonists. However, the patients who developed tuberculosis were few, indicating that the risk is still very low (Askling et al. 2005c). Reports of tuberculosis and other severe infections with intracellular agents in those being treated with TNF antagonists have raised concerns about the effect of such treatments on cellular immunity.

## Spondylarthropathies

Spondylarthropathies (SpA) were originally described as a group of "seronegative arthropathies" with similar clinical, radiological and genetic features distinguishable from RA (Moll and Wright 1973, Moll et al. 1974). SpA include ankylosing spondylitis (AS), reactive arthritis, arthritis associated with psoriasis or inflammatory bowel diseases and undifferentiated spondylarthropathy (Khan 2002). In addition, juvenile spondylarthophaties, isolated acute anterior uveitis and spondylitic heart disease associated with the genotype HLA-B27 may be classified within the group but are not discussed in this work. SpA share many common clinical symptoms. Enthesitis, defined as an inflammation of the attachments of tendons, ligaments, joint capsule or fascia to bone, is the hallmark that distinguishes SpA from other arthritides. Other characteristic features of SpA are involvement of the spine, peripheral arthritis and extra-articular manifestations such as eye inflammation, skin involvement, dactylitis ("sausage digits") and/or cardiac involvement.

There is growing evidence that the prevalence of SpA, as a group, is higher than previously thought, and it is believed to be approximately as high as that of RA (Khan 2002).

### *Aetiology and pathogenesis*

The aetiology of SpA is not known. These disorders show familial aggregation and genetic factors play an important role in susceptibility. Concordance rates in AS were found to be 63% in identical twins and 23% in non-identical twins (Brown et al.1996, Brown et al.1997, Brown et al. 2000).

There is a strong association between spondylarthropathies and HLA genes of the MHC, in particular HLA-B27 (Calin et al. 1998). This association is widely recognised, although varying in different ethnic groups and for various spondylarthropathies (Khan 1995, Brown et al.

**Table 2: The association of spondylarthropaties with HLA-B27 in Western European populations**

| | Prevalence of HLA-B27 (%) |
|---|---|
| Ankylosing spondylitis | >90 (Schlosstein et al. 1973, Brown et al. 2002) |
| Reactive arthritis | 40-80 (Ekman et al. 2000) |
| Psoriatic arthritis | 40-50 (Brown et al. 2002) |
| Arthritis associated with inflammatory bowel disease | 35-75 (Reveille and Arnett 2005), Brown et al. 2002) |
| Undifferentiated spondylarthropathy | 70 (Khan 2002) |

1996, Alharbi et al. 1996, al-Arfaj 2001), Table 2.

Based on results from family studies, the contribution of HLA-B27 to the overall genetic risk of SpA has been estimated to be only about 37% (Brown et al. 1997 and Brown et al. 2000, Allen et al. 1999). The exact molecular mechanisms underlying the link between HLA-B27 and susceptibility to SpA have not been elucidated (Granfors et al. 2002). HLA-B27 plays a role in presenting putative arthritogenic peptides to APC. Furthermore, the HLA-B27 molecule has a tendency to misfold and accumulate within the endoplasmatic reticulum. This might lead to an endoplasmatic reticulum stress response and subsequently the secretion of proinflammatory cytokines such as TNF (Colbert 2000). It has also been suggested that impaired intracellular killing of bacteria may be involved in the pathogenesis of SpA (Reveille and Arnett 2005, Granfors et al. 2002).

Certain infections, such as urogenital or intestinal ones, can act as triggers of reactive arthritis. Microorganisms found to be associated with reactive arthritis share some common features. They can all invade mucosal surfaces, are able to live intracellularly and have lipopolysaccharides in their outer membrane. Furthermore, it is generally accepted that the interaction between HLA-B27 positive hosts and triggering bacteria is abnormal (Granfors et al. 2002). The high frequency of asymptomatic microscopic intestinal inflammation reported in SpA (Mielants et al. 1991, Leirisalo-Repo et al.1994) has led to the hypothesis that damage of the gut-blood barrier could be an initiating or perpetuating event in SpA (Reveille and Arnett 2005).

*Clinical symptoms, diagnosis and classification*

The diagnosis of SpA is based on clinical grounds. The European Spondylarthropathy Study Group (ESSG) has proposed a set of criteria for the classification of SpA (Dougados et al. 1991). These criteria were developed for use in clinical trials and in order to facilitate comparisons of results from different studies, Table 3. When used diagnostically they show varying sensitivity.

**Table 3: The criteria proposed by European Spondylarthropathy Study Group for the classification of spondylarthropathies**

| | | |
|---|---|---|
| Inflammatory spinal pain | OR | Synovitis<br>Asymmetric<br>Predominantly in the lower limbs |
| | AND | |
| One or more of the following:<br>Positive family history<br>Psoriasis<br>Inflammatory bowel disease<br>Urethritis, cervicitis, or acute diarrhoea within 1 month before arthritis<br>Buttock pain altering between right and left gluteal area<br>Enthesopathy<br>Sacroiliitis (bilateral grade 2-4 or unilateral grade 3-4, according to the following radiographic grading system: 0=normal, 1=possible, 2=minimal, 3=moderate, and 4=ankylosis) | | |

Ankylosing spondylitis is the most common form of the SpA, with a prevalence of 0.2-2% (Feltelius 2005, Braun et al. 1998). It is 2-3 times more common in men than in women. The diagnosis of AS is based on a combination of symptoms including chronic, inflammatory low back pain, the presence of enthesitis, and radiographic evidence of sacroiliitis. The classification criteria for AS were developed for use in clinical studies (modified New York criteria) and are not diagnostic criteria, although they can serve as diagnostic aids in daily clinical practice (van der Linden et al. 1984), Table 4.

Psoriatic arthritis (PsA) is by definition an arthritic condition associated with psoriasis. The widely used criteria for different subtypes of psoriatic arthritis are still those proposed in 1973 (Moll and Wright), Table 5.

**Table 4: The Modified New York Criteria (1984) for ankylosing spondylitis**

| |
|---|
| A. Diagnosis |
| 1. Clinical criteria |
| a) Low back pain and stiffness for more than 3 months which improves with exercise but is not relieved by rest. |
| b) Limitation of motion of the lumbal spine in both the sagittal and frontal planes |
| c) Limitation of chest expansion relative to normal values corrected for age and sex |
| 2. Radiological criterion: sacroiliitis grade ≥ 2 bilaterally or grade 3-4 unilaterally |
| |
| B. Grading |
| 1. Definite ankylosing spondylitis if the radiological criterion is associated with at least 1 clinical criterion. |
| 2. Probable ankylosing spondylitis if: |
| a) three clinical criteria are present |
| b) the radiological criterion is present without any signs or symptoms satisfying the clinical criteria (other causes of sacroiliitis should be considered.) |

**Table 5: Subtypes of psoriatic arthritis according to Moll and Wright**

| |
|---|
| Arthritis of distal interphalangeal joints with nail changes |
| Spondylitis with or without peripheral arthritis |
| Asymmetric monoarthritis or oligoarthritis |
| Symmetric polyarthritis |
| Arthritis mutilans |

Since there are no diagnostic criteria for PsA the exact prevalence of PsA is not known. Recently, in a study including Scandinavian patients with psoriasis, 30% of patients had arthritis, corresponding to a 1% overall prevalence of PsA (Zachariae 2003). Men and women are equally affected. In about 75% of patients the skin disease is present before the onset of joint symptoms. In approximately 10% psoriasis appears simultaneously with arthritis and in about 15% of patients arthritis precedes the skin or nail symptoms (Svensson 1997, Sieper et al. 2006). Symmetric polyarthritis has been found to be associated with more severe disease (Svensson et al. 2002).

## Inflammation and joint damage

There are two coexisting processes during the course of RA and other destructive arthritides: synovial inflammation, and cartilage and bone damage. Traditionally, clinical symptoms and joint destruction have been thought to be closely connected in patients with RA. Recently, the coupling between inflammation and joint has been questioned. A prospective study including patients with RA observed over 6 years showed continuing joint destruction in spite of improvement in measures of disease activity (Mulherin et al. 1996a and 1996b). Observations in that study suggested that the pathogenesis of joint destruction may differ from that underlying synovial inflammation. A discrepancy between the progression of erosion seen on magnetic resonance scans of the wrist and improvement in swollen joint count (SJC), tender joint count (TJC), health assessment questionnaire (HAQ), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was also reported in patients with early RA (McQueen et al. 1999).

In clinical studies including RA patients treated with TNF antagonists an improvement of signs and symptoms was seen, but also inhibition of joint damage progression (Maini et al.1999, Lipsky et al. 2000, Bathon et al. 2000, Weinblatt et al. 2003, Klareskog et al. 2004). Importantly, a reduction of joint damage progression was observed radiographically regardless of response (Lipsky et al. 2000). Further analysis of the results presented in that study including patients without any evidence of clinical improvement according to the ACR20 response criteria and disease activity score using 28 SJC and 28 TJC (DAS28) confirmed that treatment with infliximab in combination with MTX inhibited the progression of joint damage regardless of the effects on inflammatory signs

and symptoms (Smolen et al. 2005).

Furthermore, Molenaar et al. (2004) demonstrated that clinically relevant progression of joint damage occurred in RA patients in remission after 2 years of follow-up. In RA patients participating in the TEMPO trial of etanercept, CRP >15 was associated with most pronounced joint damage progression in patients treated with methotrexate (MTX). However, the addition of etanercept to MTX inhibited joint damage progression regardless of CRP levels. This uncoupling between the joint damage progression and inflammation was also observed in patients treated with etanercept as monotherapy (Landewé et al. abstract 867, ACR congress 2005a). Also, adalimumab used for treatment of RA is reported to retard the progression of the joint damage despite a high level of disease activity measured by CRP and/or DAS28 (Landewé et al. abstract 266, ACR congress 2005b). Patients with early RA treated with MTX showed more severe joint damage progression than patients who exhibited the same level of clinical response but were treated with adalimumab combined with MTX. These differences were detected after 6 months and increased during 2 years of treatment (Genovese et al. abstract 1178, ACR congress 2005a). Interestingly, results from the AIM trial of abatacept, a CTLA4 inhibitor, show that abatacept in combination with MTX inhibits structural damage progression regardless of the responses according to the ACR20 response criteria (Genant et al. abstract 1991, ACR congress 2005).

Taken together, these studies support the hypothesis that inflammation and joint destruction are not closely related in patients with RA.

### *Molecular markers of joint damage*

Cartilage and bone are dynamic tissues. A characteristic of these tissues is continuous remodelling. Breakdown and rebuilding of components of cartilage and bone occur continuously and these processes are in balance under physiological circumstances. In RA, as discussed above, the balance is disturbed and in established disease degradation predominates. During remodelling (both physiologically and in disease) fragments of joint tissues are released from the cartilage and bone matrix into the synovial fluid and may reach the circulation mainly via lymph vessels. Measurement of such markers of cartilage and bone turnover in body fluids provides information about processes affecting these tissues (Saxne et al. 2006, Kraus 2005). Several macromolecules originating from joint tissues can be detected in body fluids e.g. various aggrecan epitopes, different epitopes of type II collagen, matrilin-1, and COMP (Heinegård et al. 2005, Saxne et al. 2006).

Potentially, biomarkers for cartilage or bone involvement in RA could be used as diagnostic tests, tools for monitoring tissue damage, predictors of tissue damage and also for evaluation of response to treatment (Heinegård et al. 2005, Saxne et al. 2006). In the present work, COMP, one such marker was studied in relation to treatment with TNF antagonists.

COMP is a 434 kDa, non-collagenous glycoprotein composed of five subunits, and was first identified in cartilage (Hedbom et al. 1992, Saxne and Heinegård 1992), but it is also present in the synovium, tendons and the meniscus in small amounts. COMP acts as a catalyst governing the formation of type II collagen fibre (Heinegård et al. 2005). The concentration ratio cartilage/synovial fluid/synovium is approximately 100/10/<1 (Skiöldebrant et al. 2001, Saxne and Månsson, unpublished). Thus, the bulk of circulating COMP in normal individuals and in patients with arthritis most likely originates from cartilage.

In studies of experimental arthritis, serum levels of COMP have been shown to increase during the development of arthritis and to correlate to cartilage damage as visualised histologically (Vingsbo-Lundberg et al. 1998, Joosten et al. 1999a and 1999b, Larsson et al. 2002). In RA, increased serum levels of COMP were found in patients who exhibited rapid hip joint destruction (Månsson et al. 1995). Increased serum COMP levels were also found to be prognostic for future small joint damage (Lindqvist et al. 2005). Furthermore, treatment with IL-1 antagonists, known to reduce cartilage pathology, but not treatment with TNF antagonosts was shown to reduce levels of serum COMP in a murine type II collagen induced arthritis model, although both treatments

ameliorated the signs and symptoms of inflammation (Joosten al. 1999b). Results from that study support the uncoupling of inflammation and joint damage. Glucocorticoid treatment of experimental arthritis in rats normalised initially increased levels of COMP, correlating tissue response to therapy (Larsson et. al 2004). Also, Weitoft et al. (2005) showed that a single intra-articular glucocorticoid injection used to treat knee arthritis in patients with RA, induced a significant reduction in serum COMP levels within 24 hours, suggesting that this treatment has a protective effect on cartilage. In a recently published study including patients with active RA treated intravenously with high-dose prednisolone, serum levels of COMP decreased significantly, while CRP levels remained unchanged (Skoumal et al. 2006). The findings of the above studies suggest that under these conditions circulating COMP reflects the cartilage process and is not influenced by inflammation.

## Autoantibodies

A variety of autoantibodies are associated with RA. The discovery of RF as an immunoglobulin against the Fc portion of IgG was the first evidence of the autoimmune nature of RA. RF was first described by Waaler in 1939. Rose discovered that the serum of patients with RA agglutinated IgG-coated erythrocytes (1948). Several immunoglobulin isotypes (IgG, IgA, IgM, IgD and IgE) have RF activity. RF is present in 1-2% of healthy people, and the prevalence increases with age (Cathcart and O'Sullivan 1970). Approximately 70-80% of patients with RA are RF positive. RF can be found transiently in some infectious diseases, some neoplastic disorders, viral hepatitis and cryoglobulinaemia, etc. Among rheumatic diseases, RF can be detected in e.g. Sjögren's syndrome, systemic lupus erythematosus (SLE), systemic sclerosis and sarcoidosis. RF may appear months to years before the onset of clinical symptoms in RA (Aho et al. 1985, del Puente et al. 1988). RF positivity in healthy people is associated with increased risk of developing RA, and the risk is highest in those with high RF titres (del Puente et al. 1988). RF positivity at disease onset is associated with more severe disease in terms of the development of joint damage (von Zeben et al. 1993, Eberhardt et al. 1988, Eberhardt et al. 1990, Lindqvist et al. 2003) and extra-articular manifestations (Mongan et al. 1969).

After the discovery of RF, several other autoantibodies were detected in sera from patients with RA. In 1964, Nienhuis and Mandema described antibodies against keratohyaline granules around the perinuclear region of human buccal epithelial cells called antiperinuclear factor (APF). Several years later, antibodies against keratin-like structures in rat oesophageal epithelial cells were detected and termed antikeratin antibodies (AKA) (Young et al. 1979). Subsequent studies demonstrated that the target antigen of AKA was filaggrin (Simon et al. 1993) and its precursor molecule, profilaggrin, was the target protein of APF antibodies. Consequently, APF and AKA are similar autoantibodies (Sebbag et al. 1995).

Peptidylarginine deiminase (PADI) is an enzyme that converts an arginine residue of filaggrin molecules to citrulline, and these citrulline residues are recognised by autoantibodies in RA patients (Schellekens et al. 1998). Anti-CCP are autoantibodies to citrullinated filaggrin and other artificial cyclic citrullinated peptides. A specific ELISA (enzyme-linked immonosorbent assay) initially using natural filaggrin and recently highly reactive peptides as antigen, has been developed for the detection of these antibodies. Anti-CCP antibodies are reported to be highly specific to RA, in the range between 89 and 98.5%, but lower sensitivity 33-87% has been reported in different studies (Simon et al.1993, Schellekens et al. 1998, Schellekens et al. 2000, Goldbach-Mansky et al. 2000, Vincent et al. 2002, Rantapää-Dalqvist et al. 2003). Anti-CCP antibodies were detected in stored samples from blood donors many years before clinical disease onset (Rantapää-Dahlqvist et al. 2003, Nielen et al. 2004) indicating that the immune pathology probably begins several years before the onset of clinical symptoms. The occurrence of these autoantibodies has been shown to predict the progression of undifferentiated arthritis to RA

(van Gaalen et al. 2004). Several studies have also shown that anti-CCP antibodies predict a more erosive course of RA (Schellekens et al. 1998, Rantapää-Dahlqvist et al. 2003, Forslind et al. 2004, Lindqvist et al. 2005). As mentioned above, a new model for the aetiology of RA has been proposed where smoking in certain genetically predisposed individuals can trigger the immune reactions to citrullinated proteins (Klareskog et al. 2006).

Antinuclear antibodies (ANA) are auto-antibodies directed against a variety of nuclear antigens. ANA can be detected in healthy individuals, often in low titre and more often in women. Also, the prevalence of ANA is age dependent. ANA positivity has been reported in approximately 3% of healthy individuals at 1:320 serum dilution and 32% at 1:40 serum dilution (Tan et al. 1997). The presence of ANA is associated with a variety of autoimmune diseases e.g. SLE, Sjögren's syndrome and systemic sclerosis, as well as RA and spondylarthropathies. ANA are usually detected using indirect immunofluorescence (IIF) method on HEp-2 (human epithelioma-2) cells and this is used as a screening test although it has low specificity. Furthermore, ANA occur in different patterns as assessed by immunofluorescence. In RA, ANA positivity has been reported to be between 10 and 70% in various studies (Tan et al. 1997, Aitcheson et al. 1980, Linn et al. 1978). The association between positive ANA and developpment of join damage was addressed in few studies with conflicting results (De Carvalho et al. 1980, Meyer et al. 1997). Conflicting results have also been reported regarding ANA positivity and extra-articular manifestations of RA (Quismorio et al. 1983, Turesson et al. 2000, Caspi et al. 2001). Furthermore, a correlation between ANA positivity and the adverse effects of drugs i.e. some DMARDs has also been reported (Smidt et al. 1978, Ferraccioli et al. 1986, Caspi et al. 2001).

## Cytokines

Cells of the immune system communicate with each other by cytokines. Cytokines are small soluble proteins, or glycoproteins, produced by white blood cells and a variety of other cells (Arend and Dayer 1995). Cytokines function as a self-regulatory network involved in many biological processes, such as growth and differentiation of cells and regulation of the immune system. They may act on the cells that secrete them (autocrine function), on nearby cells (paracrine function), or in some instances on distant cells (endocrine action). The cytokine network plays an important role in the process of inflammation in RA (Arend and Dayer 1995). Both pro- and anti-inflammatory cytokines are present in inflamed synovial tissue but pro-inflammatory ones, such as interleukin 1 (IL-1), tumour necrosis factor alpha (TNFα) and granulocytemacophage colony-stimulating factor (GM-CSF), predominate. There is substantial evidence that IL-1 and TNFα are key cytokines in the pathogenesis of RA (Choy and Panayi, 2001). Both are mainly produced by macrophages and monocytes and can be detected in serum and synovial fluid of patients with active RA (Chikanza et al. 1995; Saxne et al. 1988). The cytokine network in rheumatoid arthritis synovial fluid is shown in Table 6.

**Table 6: Cytokine network in RA**

| | |
|---|---|
| Pro-inflammatory cytokines | TNF, IL-1, IL-8, IL-12, IL-15, IL-17, IL-18, GM-CSF |
| Cytokines with both pro- and anti-inflammatory properties | IL-6, IL-16, IFN-γ, TGF-γ |
| Anti-inflammatory cytokines | IL-4, IL-10, IL-11, IL-13 |
| Cytokine antagonists | IL-1Ra, sIL-1R, sTNF-R, IL-18BP |

TNFα is a powerful cytokine with a variety of functions. It causes necrosis of some types of tumour cells. Prolonged production of TNFα during chronic inflammation leads to cell proliferation, cell differentiation and cell death, acute phase protein release, and cachexia. In bone, TNFα inhibits extracellular matrix deposition, stimulates matrix metalloprotease synthesis, and enhances the production of osteoclastogenic cytokines, such as GM-CSF and RANKL (receptor activator of NF-kappa B ligand). Importantly, TNFα promotes bone resorption both *in vitro* and *in vivo* by enhancing the proliferation and differentiation of osteoclast precursors (Tracey and Zhang 1998). TNFα-dependent bone erosion has been shown to be osteoclast mediated, and the absence of osteoclasts alters TNFα-mediated arthritis from a destructive to a non-destructive condition (Redlich et al. 2002). Initially *in vitro* studies showed, and subsequently *in vivo* studies confirmed, that neutralising of TNFα also inhibits IL-1 and reduces IL-6, IL-8 and GM-CSF pro- duction (Feldmann and Maini 2002). These results suggested that TNFα was a potential therapeutic target in RA. Blocking of TNFα with monoclonal antibodies or with soluble TNF-receptor fusion protein in mice with type II collagen induced arthritis led to an improvement in the joint status (Williams et al. 1992). Subsequent clinical trials in patients with RA confirmed the rapid and sustained improvement using both therapeutic modalities (Elliot et al. 1994, Maini et al. 1998 and Maini et al. 1999, Moreland et al. 1997, Weinblatt et al. 2003). During recent years TNF-blocking agents have been successfully introduced for treatment of other rheumatic diseases such as spondylarthropathies (van den Bosch et al. 2000, Braun et al. 2002a,b, Brandt et al. 2003, Davis et al. 2004, Brandt and Braun 2006).

The IL-1 family consists of IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra) (Dinarello 1996). IL-1α and IL-1β have agonistic effects, but bind to two different cell-surface receptors of which only receptor I participates in intracellular signalling. IL-1Ra is a natural antagonist which binds to IL-1 receptors competitively and acts as an inhibitor of IL-1 action without inducing any biological response. IL-1 plays an important role in the activation of T-cells in response to antigens. Furthermore, IL-1 stimulates the adhesion molecules on endothelial cells to enable transmigration of leukocytes to a site of infection. It acts on the hypothalamus thermoregulatory centre causing fever, and is often called an endogenous pyrogen. The competition between the agonistic effects of IL-1α and IL-1β and antagonistic effects of IL-1Ra may determine the effect on target cells (Arend and Dayer 1995, Dinarello 1996). Experimental studies using animal model of arthritis (van den Berg 1997) and subsequently randomised clinical trials showed that IL-1 blockage was associated with moderate anti-inflammatory effects and a reduction in cartilage and bone damage (Bresnihan 1999).

## Assessment of disease activity and function

*Rheumatoid arthritis*

There is no single "golden standard" for the assessment of disease activity or the prognosis of any rheumatic disease. Instead, a core set of variables that reflect disease activity is recommended for use in both clinical trials and observational studies. Such variables may also be used in daily clinical practice (van Riel and van Gestel 2000, van Riel and Schumacher 2001).

A visual analogue scale (VAS) is represented by a single line (usually 10 cm) anchored at one end, and with descriptors such as "no pain" and "the worst possible pain" at each end. The patients indicate by mark on the line how they estimate their condition. Pain, patient global and physician global VAS scales are the most commonly used scales, both in trials and clinical care (Wolfe et al. 2005).

The disease activity score (DAS) is an index that includes tender joint count, total number of swollen joints, ESR and the patient's general health assessment scored on a VAS. The original DAS was developed using graded tender joint count and a 44 swollen joint count (van der Heijde et al. 1990). A modified DAS using 28 joint count for tenderness and 28 joint count for swelling (DAS28), was found to be as valid as the

original DAS (Prevoo et al. 1995). CRP and ESR have been shown to function equally well when used for the calculation of DAS and a DAS28 formula including CRP instead of ESR has been developed (van Riel et al. 2000, http://www.das-score.nl). The DAS28 score indicates the current disease activity on a scale between 0 and 10. Originally, high disease activity corresponded to a DAS28 score above 5.1; moderate 3.2-5.1, low <3.2 and remission was defined as DAS28 <2.6. Recently, a simplified disease activity index (SDAI) was developed. The SDAI is a numerical sum of 5 outcome variables: tender and swollen joint count (28-joint assessment), patient and physicians's global assessment of disease activity (0-10 cm VAS scale) and CRP (mg/dl) (Smolen et al. 2003).

The Stanford Health Assessment Questionnaire (HAQ) a self-reported questionnaire designed by Fries (1980). It reflects the patient's assessment of their function. The HAQ has been proven to be reliable and valid for the assessment of functional disability in RA patients (Ramey et al. 1992, Bruce and Fries 2003a and 2003b). HAQ-Disability Index assesses patient's usual activities during the past week. There are 20 questions in eight categories of functions which the patient answers on a scale from 0 to 3 where 3 represents the highest degree of disability. The minimal clinical important difference in HAQ score is suggested to be 0.22 (Wells et al. 1993). This questionnaire has been translated into several languages and adapted for use in many countries (Bruce and Fries 2003a,b) including Sweden. The Swedish version was developed and validated in Lund (Ekdahl et al. 1988).

Two different sets of improvement criteria are used in clinical trials: the preliminary ACR improvement criteria (Felson et al. 1995) consisting of seven core set variables and the EULAR response criteria based on the DAS using only 3 or 4 core set variables (van Gestel et al. 1996). According to the ACR20 improvement criteria, treatment response is defined as a 20% improvement in tender and swollen joint count and 20% improvement in 3 out of 5 of the following measures: patient and physician's global assessment, pain, disability and an acute-phase reactant compared with base-line levels. ACR50, ACR70 and ACR90 correspond to 50%, 70% and 90% improvement. The EULAR response criteria include not only changes in disease activity, but also current disease activity. These criteria give information on changes in DAS for individual patients. According to the EULAR response criteria a good response is defined as an improvement in DAS >1.2 and final DAS ≤2.4. A moderate response corresponds to an improvement in DAS >0.6 but ≤1.2 and final DAS ≤3.7 and no response to an improvement in DAS ≤0.6 and final DAS >3.7, Table 7.

Components of the response criteria sets and the disease activity indices are displayed in Table 8.

The performance of the ACR, EULAR (based

**Table 7: EULAR response criteria based on DAS28**

| | Improvement >1.2 | Improvement ≤1.2 and >0.6 | Improvement ≤ 0.6 |
|---|---|---|---|
| DAS ≤ 2.4 | Good response | | |
| 2.4< DAS ≤ 3.7 | | Moderate response | |
| DAS > 3.7 | | | No response |

van Gestel et al. Arthritis rheum 1996; 39:34-40

**Table 8: Components of the response criteria and disease activity indices (Adapted from Gülfe et al. 2005)**

| Criteria set | Tender joint count | Swollen joint count | Patient global VAS | Patient pain VAS | Evaluator's global | HAQ | ESR | CRP |
|---|---|---|---|---|---|---|---|---|
| ACR | + | + | +/- | +/- | +/- | +/- | +/- | +/- |
| EULAR (DAS) | + | + | + | - | - | - | + | - |
| SDAI | + | + | + | - | + | - | - | + |

"+"= Required; "–"=Not required;
in the ACR response criteria, any three of the variables marked "+/–" are required.
ACR=American College of Rheumatology; CRP=C-reactive protein; DAS=disease activity score; ESR=erythrocyte sedimentation rate; HAQ= health assessment questionnaire; EULAR=European League Against Rheumatism; HAQ= health assessment questionnaire; SDAI=simple disease activity index; VAS=visual analogue scale.

on DAS28) and SDAI response criteria, and also improvement in HAQ score at the individual level were recently studied in an observational study of patients with RA treated with TNF antagonists (Gülfe et al. 2005). The agreement in responses according to the ACR20 and EULAR overall (EULAR moderate + EULAR good) and SDAI overall (defined as SDAI minor + SDAI major) was good, whereas the agreement between ACR 50, EULAR good, SDAI major and HAQ was found to be poor.

### *Spondylarthropathies*

The Assessments in Ankylosing Spondylitis (ASAS) working group has formed a core set of domains that are deemed to be important in the assessment of AS symptomatic outcome (van der Heijde et al. 1997). These domains are physical function, pain, spinal mobility, patient's global assessment, stiffness, peripheral joints and entheses, acute phase reactants, fatigue and imaging. Specific instruments for measurement are selected for each domain. Criteria for improvement based on these domains have subsequently been developed (Anderson et al. 2001).

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is a self-administered instrument consisting of six, 10-cm horizontal visual analogue scales that measure fatigue, spinal pain (neck, back or hip), peripheral joint pain, overall discomfort from any areas tender to touch or pressure, level of morning stiffness and duration and intensity of morning stiffness (Garrett et al. 1994).

The Bath Ankylosing Spondylitis Functional Index (BASFI) is a self-assessment instrument consisting of 10 questions to be answered on a 10-cm horizontal visual analogue scale. Eight questions reflect function and 2 questions reflect the ability to deal with everyday life (Calin et al. 1994).

The Bath Ankylosing Spondylitis Metrology Index (BASMI) is an instrument including 5 clinical measurements reflecting axial status: cervical rotation, tragus to wall distance, lateral flexion, modified Schober's flexion test and intermalleolar distance (Jenkinson et al. 1994).

Based on the ASAS core set of variables the Swedish Society for Rheumatology has developed guidelines for the assessment of SpA. The domains included in the assessment of axial and peripheral disease are displayed in the Table 9.

**Table 9: The Swedish Society for Rheumatology guidelines for assessment of SpA. The recommended instrument is shown in brackets.**

| Assessment of axial disease | Assessment of peripheral disease |
|---|---|
| Pain (VAS) | Physician's global assessment (VAS) |
| Patient's global assessment (VAS) | Patient's global assessment (VAS) |
| Disease activity (BASDAI) | Pain (VAS) |
| Physical function (BASFI) | Function (HAQ) |
| ESR/CRP | ESR/CRP |
| Spinal mobility (BASMI)* | SJC (66-swollen joint count) |
| | TJC (68-tender joint count) |

*Not mandatory

A response is defined as 50% improvement in BASDAI compared with baseline value, or 2 steps on the scale (0-10) and expert's assessment. Assessment of peripheral disease includes improvement according to the ACR20 and/or psoriatic arthritis response criteria (PsARC) in the case of psoriatic arthritis. PsARC is a composite measure developed for use randomised clinical trials (RCT) that is based on changes in 68-tender and 66-swollen joint counts, physician's and patient's global assessment. A response is defined as improvement in at least two measures (one joint index measure and one global assessment measure) without deteriorate in any of the 4 measures (Mease et al. 2000).

## Pharmacological treatment of arthritis

*Traditional treatment*

The main goals of treatment in arthritis are to reduce the symptoms of joint pain and swelling, i.e. to reduce inflammation, and to prevent joint destruction (Breedveld and Kalden 2004). Historically, medical treatment of arthritis has been focused on controlling inflammation. The first anti-inflammatory agent used for the treatment of RA was acetylsalicylic acid, isolated in 1829 and introduced as Aspirin 1899 (Rodnan and Benedek 1970). Subsequently, other substances were developed with similar mode of action and were grouped in a category called non-steroidal anti-inflammatory drugs (NSAIDs). These substances reduce inflammation by inhibition of cyclooxygenases (COX-1 and COX-2) which are enzymes involved in prostaglandin synthesis. An alternative name, i.e. coxibs has therefore been suggested for this group of compounds. Although these remedies are effective in reducing the symptoms of inflammation, they do not alter the course of the disease or prevent the destruction of cartilage or bone. Several randomised trials have also demonstrated the efficacy of NSAIDs in the control of pain in AS (Zochling et al. 2006).

Glucocorticoids are effective antiinflammatory and immunosuppressive drugs widely used for the treatment of arthritis. Glucocorticoids act on the inflammatory process by interfering with the cytokine network, inflammatory enzymes, adhesion molecules, permeability factors, cellular function and survival (Bijlsma et al. 2002; Bijlsma et al. 2003). Treatment with glucocorticoids has also been reported to reduce the progression of joint damage in RA (Boers et al. 1997, Svensson et al. 2005, Landewe et al. 2002, Wassenberg et al. 2005). There are no studies showing the benefit of glucocorticoids in SpA for axial disease (Zochling et al. 2006) but intra- or periarticular glucocorticoid injections may be useful in SpA (Maugars et al. 1992). Prolonged treatment with glucocorticoids is, however, associated with serious side effects such as hypertension, peptic ulcer disease, osteoporosis, diabetes mellitus, accelerated atherosclerosis, cataracts, psychological disturbances, myopathy, osteonecrosis, skin atrophy and weight gain. In clinical practice glucocorticoids have traditionally been recommended for short-term treatments, in particular in the period before DMARDs have ameliorated the disease symptoms.

DMARDs have been used for the treatment of arthritis since the 1920s, when intramuscular gold was introduced. Historically, DMARDs were withheld until the patient exhibited evidence of joint destruction on radiographs. This treatment strategy resulted in unsatisfactory outcomes and few patients achieved remission. Several studies have demonstrated that early and aggressive treatment with DMARDs improves long-term outcome in RA (Emery et al. 2002). According to the Swedish Society for Rheumatology's current guidelines, treatment with DMARDs should be initiated early, i.e. preferably as soon as a diagnosis of persistent arthritis has been made. The mechanisms of action of many DMARDs are not fully known. Many DMARDs were initially developed for the treatment of other diseases and have found their way into the treatment of arthritis more or less, by accident. Table 10 shows the most commonly used DMARDs for the treatment of arthritis.

As mentioned above, several traditional DMARDs are effective in controlling inflammation, improving functional status and also in retarding joint damage. There is also substantial evidence that combination treatment is more

**Table 10: DMARDs, dosage and indications relevant to this thesis**

| DMARD | Dosage | Treatment indication | | |
|---|---|---|---|---|
| | | RA | AS | Psoriatic arthritis |
| Auranofin | 3-9 mg daily (orally) | + | - | - |
| Azathioprine | 2.5 mg/kg daily (orally) | + | + | - |
| Chloroqine/ Hydroxychloroquine | 3 mg/kg daily (orally)/ 6.5 mg/kg daily (orally) | + | - | - |
| Cyclophosphamide | 1-2 mg/kg daily (orally) 500-1500 mg/m² (intermittently, intravenously) | extra-articular | - | - |
| Cyclosporine A | 2.5-5 mg/kg daily (orally) | + | - | + |
| Chlorambucil | 0.03-0.3 mg/kg daily (orally) | extra-articular | - | - |
| Leflunomide | 10-20 mg daily following a loading dosage (orally) | + | - | + |
| Methotrexate | 7.5-25 mg weekly in a single dose (orally or parenterally) | + | + peripheral arthritis | + peripheral arthritis |
| Mycophenolate mophetil | 0.5-2 g daily (orally) | + | - | - |
| Na-aureothiomalate | 10 mg as a single dose during the first week, thereafter 25-50 mg weekly (intramuscularly) | + | - | - |
| Penicillamine | max 1500 mg daily in 3 doses (orally) | + | - | - |
| Sulfasalazine | 2-3 g daily (orally) | + | + peripheral arthritis | + |

effective than monotherapy and not necessarily more toxic (Boers et al. 1997). Combination treatment can be used in different ways. The step-up approach means that treatment is initiated with one DMARD and, in the case of inadequate response, another DMARD is added. The continuous approach includes concomitant treatment with ≥2 DMARDs initiated simultaneously. In the step-down approach treatment with several DMARDs is initiated simultaneously and successively discontinued when remission is achieved. In a randomised study (COBRA), Boers et al. (1997) demonstrated the efficacy of the step-down approach in the suppression of inflammation and progression of joint damage seen at radiography. Long-term follow-up of patients given combination treatment during the first six months showed that suppression of joint damage was sustained for up to 4 years regardless of subsequent treatment (Landewe et al. 2002). It should be noted that the patients in the combination group received fairly high doses of glucocorticoids, which makes interpretation somewhat difficult.

*Biological agents*

During the recent decades knowledge of the underlying pathogenetic mechanisms of inflammatory joint diseases has increased dramatically. This has opened up opportunities to develop new pharmaceuticals that target specific cell-surface markers or proinflammatory cytokines involved in the pathogenesis of these diseases.

TNF is a proinflammatory cytokine that plays a pivotal role in the development and progression of RA. There are currently 3 TNF antagonists available: infliximab (a chimeric IgG1 monoclonal antibody that binds soluble and membrane-bound TNFα), etanercept (a completely human, soluble receptor binding both TNFα and TNFβ) and adalimumab (a recombinant human IgG1 monoclonal antibody to TNFα). All 3 TNF antagonists have been shown to be more efficacious in reducing signs and symptoms and in particular in reducing the development of joint damage in RA in combination with methotrexate than as monotherapy (Maini et al. 1999, Weinblatt et al. 1999, Bathon et al. 2000, Lipsky et al. 2000, Weinblatt et al. 2003, Keystone 2002, Klareskog et al. 2004).

Treatment with TNF antagonists also reduces the symptoms of AS and PsA (Mease et al. 2000, Marzo-Ortega et al. 2001, Davis et al. 2004, Braun et al. 2006, Kavanaugh et al. 2006a), but a damage protecting effect on these conditions has to be firmly established. Infliximab has recently been shown to retard the progression of damage in hands and feet in PsA (Kavanaugh et al. 2006b). All three remedies were initially used for the treatment of RA but have now also been approved for treatment of AS and PsA.

Another principle tested for the treatment of RA is blocking of the effects of IL-1 using a recombinant receptor antagonist (anakinra). Treatment with anakinra was reported to be effective in ameliorating inflammation and a beneficial effect on the rate of progression of joint erosion has also been demonstrated (Bresnihan et al. 1999). Anakinra in combination with methotrexate has been shown to be effective in the treatment of RA patients (Cohen et al. 2004), but the efficacy seems to be lower than that of TNF-blockers.

Rituximab is a chimeric monoclonal antibody against a B-cell specific antigen, CD20. There are 3 putative mechanisms of action of rituximab: antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and promotion of CD20+ B-cell apoptosis, but the exact mode of action is not clear (Reff et al. 1994, Edwards et al. 2004a,b, Edwards et al. 2005). Rituximab in combination with methotrexate has been reported to be effective in reducing the signs of inflammation in RA patients who had previously failed to respond to one or more DMARDs and TNF antagonists (Edwards et al. 2004a,b, Emery et al. 2006). Currently available biological agents and indications for their use are given in Table 11.

**Table 11: Biological agents currently in clinical use for the treatment of arthritis**

| Biological agent | Dosage | Treatment indication | | |
|---|---|---|---|---|
| | | RA | AS | Psoriatic arthritis |
| Etanercept | 50 mg weekly (subcutaneously) | + | + | + |
| Infliximab | 3-10 mg/kg every 8 weeks following initial loading dosage (intravenously) | + | + | + |
| Adalimumab | 40 mg every 14 days (subcutaneously) | + | + | + |
| Anakinra | 100 mg daily (subcutaneously) | + | - | - |
| Rituximab | 1000 mg iv; in 2 days (day 1 and day 15) | + | - | - |

Abatacept (CTLA4Ig; cytotoxic T-lymphocyte–associated antigen 4 fused to the constant region of human IgG1) belongs to a group of new anti-inflammatory agents that selectively blocks co-stimulatory molecules. Binding between CD80 and CD86 molecules on antigen-presenting cells to CD28 molecule on T-cells is the second signal required for activation of T-cells (Lenschow et al. 1996). The mode of action of abatacept in RA is blocking of CD28:CD80/86 resulting in downregulation of T cell activation. Treatment with CTLA4Ig in combination with methotrexate leads to significant improvement in the clinical signs and symptoms of RA (Moreland et al. 2002, Kremer et al. 2006). It is noteworthy that this has also been shown in patients refractory to TNF antagonists (Genovese et al. 2005b). Abatacept will soon be available for clinical use.

### *Treatment guidelines*

A EULAR expert committee recommends the following strategy for the treatment of early RA:

- early treatment start in patients at risk of developing persistent or erosive disease,
- remission should be a goal of the treatment,
- use of NSAIDs in order to relieve pain and stiffness,
- use of systemic glucocorticoids and intra-articular glucocorticoids but systemic glucocorticoids only temporarily,
- use of methotrexate as anchor drug,
- non-pharmaceutical interventions should be

employed early, and
- regular monitoring every 3 months until remission is achieved (Combe et al. 2006).

A consensus statement on biological agents for the treatment of rheumatic diseases developed by an international expert group includes the following:
- TNF antagonists are recommended for the treatment of RA, AS and psoriatic arthritis after using another DMARD.
- TNF antagonists can be added to pre-existing therapy or may replace previous DMARDs or other biological agents.
- TNF antagonists are effective in MTX naïve patients and may be considered as the first DMARDs when other DMARDs are contraindicated.
- The use of TNF antagonists as the first DMARD in RA should be limited due to concerns regarding long-term safety, but individual patient need should be considered.
- There is no evidence that any TNF antagonist is more effective than any another, although individual differences may exist (Furst et al. 2005).

The Swedish Society for Rheumatology has also developed guidelines for the treatment of RA (www.srfonline.org), as shown in Figure 2.

Recommendations for the management of ankylosing spondylitis have been developed in collaboration between ASAS international working group and EULAR. ASAS group consists of international experts within the field of AS and a patient with AS. The objective has been to construct evidence-based recommendations for management of patients with AS (Zochling et al. 2006). ASAS/EULAR current recommendations for the treatment of ankylosing spondylitis are shown in Figure 3.

The Swedish Society for Rheumatology has developed guidelines for the management of AS and PsA, as shown in Table 12 (www.srfonline.org).

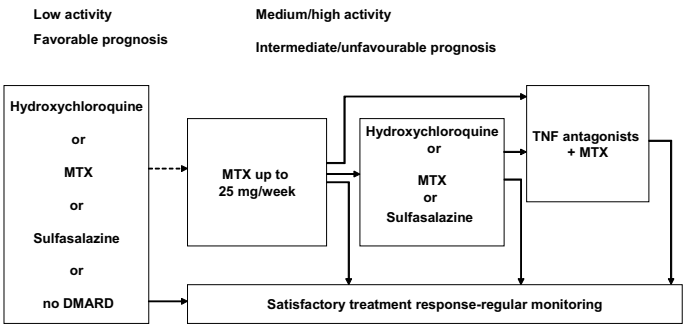


Figure 2: RA treatment, according to the Swedish Society for Rheumatology, 2004

**Table 12: The Swedish Society for Rheumatology's recommendations for the treatment and assessment of ankylosing spondylitis and psoriatic arthritis. Translated from Swedish.**

| Diagnosis | | High activity + unfavourable prognosis | Assessment |
|---|---|---|---|
| Ankylosing spondylitis | Treatment failure to NSAIDs and to 2 and more corticosteroid injections (peripheral arthritis) | Axial disease→TNF antagonists ( ±MTX)<br>Peripheral disease→sulfasalazine (4 months) →TNF antagonists (± MTX) | ASAS |
| Psoriatic arthritis | Treatment failure to NSAIDs and to 2 or more corticosteroid injections (peripheral arthritis) | Axial disease→TNF antagonists (± MTX)<br>Peripheral disease→MTX/cyclosporine/ /sulfasalazine/leflunomide→TNF antagonists | PsaARC<br>ACR20 |

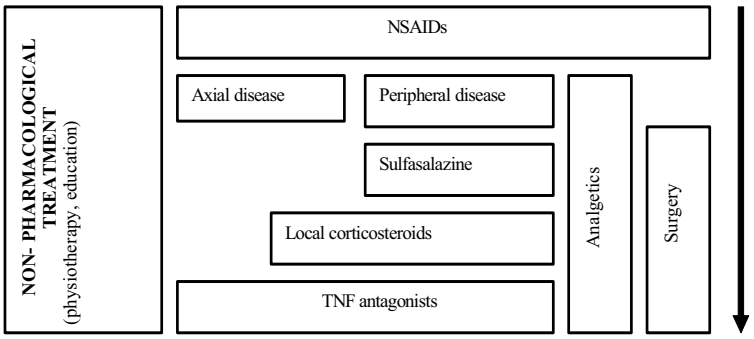


Figure 3: ASAS/EULAR recommendationd for the treatment of ankylosing spondylitis
(Adapted from Zochling et al. 2006)

## Vaccination

In patients with rheumatic diseases, infections cause significant morbidity and mortality. Vaccinations are the most effective measures for preventing these outcames. The Swedish National Board of Health and Welfare recommends pneumococcal and influenza vaccination of all individuals 65 years of age or over, and those suffering from chronic illness with increased risk of severe pneumococcal/influenza infections or their complications (SOSFS 1994, SOSFS 1997). In the majority of patients with rheumatic diseases, should therefore vaccination against pneumococcal infections and influenza be considered. However, vaccination of patients with rheumatic diseases has been somewhat controversial. Case reports on various autoimmune diseases have led to concern regarding the risk of inducing autoimmune diseases by vaccination (Wraith et al. 2003). Sporadic cases of flare of underlying rheumatic disease have been reported following vaccination, although controlled trials could not confirm such findings (Avery et al. 1999). The onset of systemic rheumatic disease, such as vasculitis (Mader et al. 1993), or polymyalgia rheumatica following influenza vaccination (Marti and Anton 2004), and also connective tissue disease and spondylarthropathy following hepatitis A vaccination (Ferrazzi et al. 1997) has been reported. More recently, several studies addressing safety and immunogeneicity of influenza and pneumococcal vaccination in patients with rheumatic diseases have been conducted demonstrating the safety of these vaccines (Chalmers et al. 1994, Francioni et al. 1996, Elkayam et al. 2002a, Fomin et al. 2006, del Porto et al. 2006). Chalmers et al. demonstrated that influenza vaccination was safe in patients with RA and elicited similar immune response to those in healthy controls (1994). However, reduced immunogeneicity following influenza and pneumococcal vaccination mainly due to consequences of concomitant immunosuppressive treatment has been reported in other studies (O'Dell et al. 1992, Mease et al. 2004, Elkayam et al. 2004, Wright et al. 2004, Fomin et al. 2006). Based on the information currently available, the risk of developing autoimmunity after influenza and pneumococcal vaccinations is so far deemed to be substantially lower than the risk of severe infections or complications related to these infections (Wraith et al. 2003).

# Aims of the present investigation

The objective of this work was to study clinical, immunological and biochemical aspects of treatment with tumour necrosis factor antagonists in patients with arthritis. In particular, the aims were:

- to study the feasibility of a structured clinical protocol for prospectively monitoring treatment efficacy and tolerability of new treatment modalities, including TNF antagonists, in patients with arthritis in clinical practice
- to investigate whether serum levels of COMP change during treatment with TNF antagonists in a way that confirms the tissue protective effects of these agents in RA
- to study how different anti-rheumatic treatments modulate immunisation induced by polysaccharide or polypeptide vaccine in patients with RA
- to study predictors of infliximab related infusion reactions in patients with arthritis

# Protocol, study population and methods

## Protocol

All patients who participated in the studies described this thesis were monitored according to a standardised clinical protocol adopted by the South Swedish Arthritis Treatment Group (SSATG). The protocol was developed at the Department of Rheumatology, at Lund University Hospital (Geborek and Saxne 2000). The SSATG protocol has subsequently been approved and used by seven other (and recently two additional) rheumatology units in southern Sweden. The SSATG register covered a population of about 1.3 million inhabitants when the studies were performed. The aim of this protocol was to include and monitor all patients treated with biological agents, regardless of their rheumatological diagnosis. When compared with pharmaceutical sales data the protocol was found to cover over 90% of patients treated with TNF antagonists in the area. Initially, the majority of the patients in the register had RA, but the proportion of patients with diagnoses other than RA has gradually increased (Geborek et al. 2005). The diagnosis, decision to start treatment and treatment goals are determined by the treating physician.

At the initiation of treatment, information regarding diagnosis, disease onset, previous and concomitant DMARD treatment, and systemic prednisolone dosage is recorded (Figure 4). A clinical assessment of structural damage before starting treatment i.e. assessment in expected improvement in HAQ score is made by the treating physician. All patients are evaluated at initiation of treatment and after 3, 6 and 12 months (optionally 0.5, 1.5, and 9 months), and thereafter every 3-6 months.

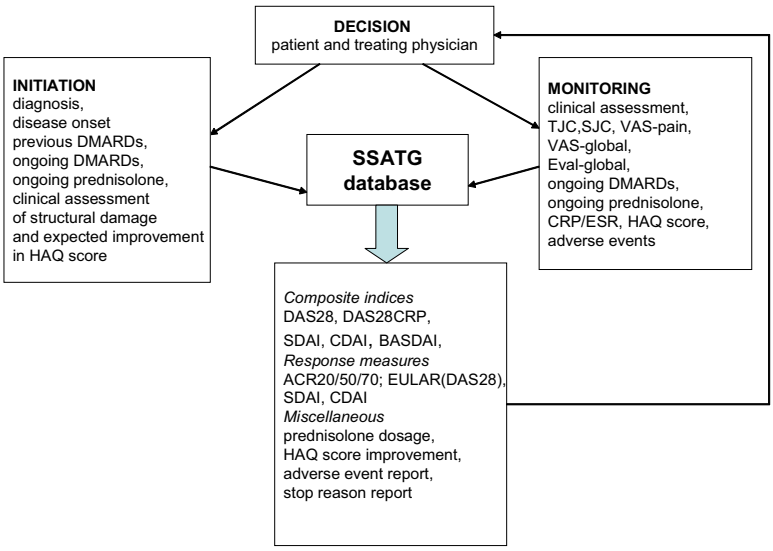


Figure 4: The South Swedish Arthritis Treatment Group (SSATG) protocol

Clinical monitoring includes 28-tender joint count and 28-swollen joint count, a 10 cm, non-anchored horizontal visual analogue scale for pain (VAS-pain) and one for general health (VAS-global), the doctor's global assessment of disease activity on five grade scale (Eval-global) and the validated Swedish version of the HAQ. ESR and CRP are determined at each follow-up. All unexpected events are recorded, and withdrawal from treatment is classified as withdrawal caused by adverse drug reaction, lack of response, or other reason. The computer application calculates the disease activity score for the 28 joint indices (DAS28), improvement defined by the EULAR criteria based on DAS28 and the response according to the ACR20, 50, and 70 response criteria. Furthermore, a reduction in HAQ score and the reduction in oral prednisolone dose by 20 and 50% is recorded.

All adverse events, including infusion reactions, are registered and seriousness graded by one investigator. An infusion reaction is defined as an adverse event occurring during infusion or within 24 hours after the initiation of infusion. The seriousness grades are mild, moderate, serious, and life threatening, where mild is defined as self-limiting and resolved after temporary stop/slowing of infusion, moderate needed closer attention and also extended observation period and often stop of infusion, while serious involved infusion stop, respiratory symptoms/symptomatic blood pressure fall, and need of close monitoring often during a whole day and also occasionally in ward referral. Life threatening involved intensive care treatment.

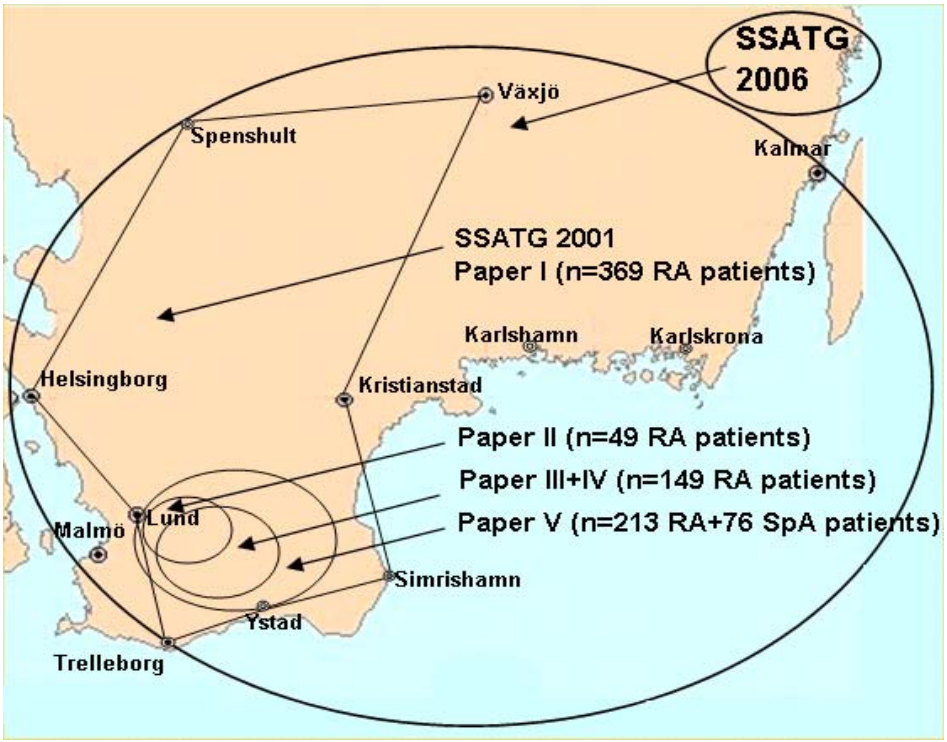


Figure 5: The study population in the different papers

*Study population*

The study population included in the different papers presented in this thesis, and the rheumatology clinics currently participating in the SSATG register are displayed in figure 5.

**Paper I**: All RA patients treated with either TNF antagonists (etanercept or infliximab) or leflunomide between March 1999 and November 2000 at the centres participating in the SSATG at that time (Spenshult, Växjö, Helsingborg, Kristianstad, Lund, Trelleborg, Ystad and Simrishamn) were consecutively included in this study. Altogether, 404 treatments (369 patients) with any of the above drugs started treatment during the observation period. Some patients tried 2 different treatment modalities (n=33) and 1 patient switched between all 3 drugs during the study period. The number of patients receiving etanercept, infliximab and leflunomide were 166, 135 and 103, respectively.

**Paper II**: Patients with RA treated either with infliximab or etanercept at the Department of rheumatology in Lund were included. All patients were monitored in accordance with the structured clinical protocol. Forty-nine patients, of whom 32 received infliximab and 17 etanercept, participated. Only patients without concomitant glucocorticoid treatment or those who were on stable dose of prednisolone (<10 mg daily) were eligible for participation. Patients who had received intra-articular glucocorticoid injections within 3 months prior to the start of treatment were not included.

**Papers III and IV**: Altogether 149 patients with established RA at the Department of Rheumatology in Lund participated in these studies. One hundred and twelve patients received TNF antagonists, of whom 48 were treated with etanercept and 64 with infliximab. A group of 50 patients received TNF antagonists in combination with methotrexate and 62 patients received TNF antagonists alone or in combination with other DMARDs. A group of 37 RA patients were taking methotrexate without TNF antagonists. The number of healthy individuals included in the control group differed between the two studies. Altogether 47 individuals among staff members at the Departments of Rheumatology and Infectious Diseases in Lund received pneumococcal vaccine and participated in the pneumococcal vaccination study as a control group. The corresponding number of healthy controls recruited in a similar fashion for influenza vaccination was 18.

**Paper V**: The study population comprised patients with arthritis who were treated with infliximab at the Department of Rheumatology in Lund between March 1999 and December 2005. Altogether 213 patients with RA and 76 patients with SpA participated in this study.

**Table 13: Some demographic characteristics of the patient populations**

| | | Number of patients | Female (%) | Mean age (years) | Mean disease duration (years) |
|---|---|---|---|---|---|
| Paper I (etanercept/infliximab/leflunomide) | | 166/135/103 | 78/79/82 | 54/55.4/61.3 | 14.9/14.1/14.9 |
| Paper II (etanercept/infliximab) | | 17/32 | 59/78 | 55.3/56.7 | 13.7/12.9 |
| Papers III and IV (etanercept/infliximab/methotrexate) | | 48/64/37 | 79/69/70 | 52.8/53/61 | 18.4/14.1/11.6 |
| Paper V (infliximab) | RA patients | 213 | 73 | 55.9 | 12.6 |
| | SpA patients | 76 | 53 | 45.0 | 13.1 |

*Methods*

### Serum COMP assay

In the study described in Paper II quantification of serum COMP was performed before the initiation of treatment and after 3 and 6 months. Serum COMP was measured by a sandwich ELISA method using two monoclonal antibodies directed against separate antigenic determinants on the human COMP molecule (AnaMar Medical, Lund, Sweden). The detection limit is <0.1 units/l and the intra-assay and inter-assay coefficients of variation are <5%. The assay is not influenced by rheumatoid factors. This assay was developed based on experience gained from use of the original assay (Saxne and Heinegård 1992).

### Vaccination

Pneumococcal and influenza vaccinations were used as models to investigate immune modulation induced by various anti-inflammatory treatments of RA including TNF antagonists (Papers III and IV). All patients were vaccinated with 23-valent polysaccharide pneumococcal vaccine and 3-valent polypeptide influenza vaccine on the same occasion. Blood samples were collected prior to and 4-6 weeks after vaccination. Levels of IgG antibodies to 23F and 6B pneumococcal polysaccharide antigens (Paper III) were measured using the WHO standard ELISA method as previously described (WHO 2005). The lower limit of detection was 0.01 mg/l. All sera were examined on the same occasion in a blinded fashion. The analyses were performed at the Department of Clinical Microbiology and Immunology, Lund University Hospital.

Influenza-virus-specific serum antibody titres were measured using haemagglutination inhibition (HI) assays (Paper IV). HI assays were performed according to WHO standard procedures using haemagglutinin antigens representing the strains of virus included in the vaccine (WHO 1981). The results are given as titres, i.e. the greatest dilution of the serum that achieves complete inhibition of haemagglutination. All sera were titrated on the same occasion in duplicate, blinded with regard to clinical data. HI assays were performed at ViroClinics, Rotterdam, the Netherlands.

### Antinuclear antibodies

ANA were determined prior to initiation of infliximab treatment. In case of missing data, ANA status within a month before treatment start was used. Measurements of ANA were performed using an indirect immunofluorescence assay with HEp2 cells as substrate and anti-IgG conjugates as described previously (Kavanaugh et al. 2000). The analysis was performed using an accredited method at the Department of Clinical Microbiology and Immunology in Lund (accredited according to SS-EN ISO/IEC 17025). Values ≥14 units/ml corresponding to a titre of 400, were considered positive. The reference interval was based on the results of measurements in healthy blood donor controls and the upper limit was determined such that between 1-5% of the controls were positive for ANA.

# Statistical calculations

Non-parametric tests were generally used. Differences between groups were analysed using the chi-squared ($\chi 2$) test for ordinal variables, the Mann-Whitney U test for comparison between groups and Wilcoxon's matched pair test for paired variables. Correlations were calculated using Spearman's rank correlation coefficient. The geometric means of HI titres (GMT) were calculated from log-transformed values, and differences were compared using a paired sample T-test (Papers III and IV). Due to differences at baseline, a binary logistic regression model adjusting for age, gender, disease duration and prednisolone dosage was used (Papers III, IV and V). The data published in Paper III were also re-analysed by applying binary logistic regression model, and these results are presented in this thesis. P-values<0.05 were considered significant.

# Results and discussions

### Etanercept, infliximab, and leflunomide in established rheumatoid arthritis: Clinical experience using a structured follow-up programme in southern Sweden (Paper I)

*The first aim* of this work was to investigate whether a structured protocol is feasible for monitoring new treatments in clinical practice at a university rheumatology department and seven non-university rheumatological units. We specifically examined the efficacy and tolerability of treatment with etanercept, infliximab and leflunomide.

One of the main findings of this longitudinal, observational study was that a structured protocol could be used to monitor newly introduced drugs in clinical practice. The protocol was well accepted by the participating centres and 2 additional rheumatological units have joined the SSATG register since 2001. Patients at the university department in Lund were more comprehensively evaluated but by comparing data from Lund with data from other units it could be shown that such a clinical protocol can also be used at other rheumatology units.

The majority of patients that rheumatologists meet in daily practice are not eligible for clinical trials. A structured clinical protocol, shared by several units, gives the opportunity to rapid collection of information regarding effects and adverse reactions during treatment "in real-life". In contrast, randomised clinical trials provide important information and are necessary to establish treatment efficacy and for the detection of common side effects. However, they are not sufficient to establish long-term efficacy, to reveal possible new therapeutical effects, or long-term and/or rare adverse reactions (Sokka et al. 2003a,b, Pincus and Sokka 2004).

An important advantage of a clinical protocol is the possibility of identifying adverse reactions not previously seen in clinical trials. However, the side effects identified in this study did not differ from those previously reported (Maini et al. 1998, Smolen et al. 1999, Strand et al. 1999, Weinblatt et al. 1999). Continued long-term monitoring of the included patients and the recruitment of new patients into the protocol is necessary to enable evaluation of the full range of side effects.

A further advantage of clinical protocols over most clinical trials is that different drugs can be monitored using the same protocol. The drugs can also be compared in similar types of patients. Therefore, longitudinal, observational studies that include all patients receiving the treatment are important for post-marketing surveillance of newly introduced drugs.

As mentioned above this study had an open character and all patients who started treatment were included in the clinical protocol. This approach may induce a placebo effect and thus a bias favouring a positive response. However, the results we report are no better than the results of clinical trials. Furthermore, the possibility of bias is probably similar for all three remedies, so comparisons are highly relevant.

The treatment efficacy was measured using the ACR20, 50, and 70 response criteria and the EULAR criteria using DAS28. The responses were fairly similar for the two TNF antagonists studied. Both TNF antagonists induced a better clinical response than leflunomide. Furthermore, the continuation of the respective treatment was studied using "survival on drug" analysis. Significantly more patients treated with TNF antagonists than those on leflunomide were still receiving the drug after 20 months (see Figure 6).

All adverse reactions registered during the observation period were graded according to severity by treating physicians. The results are given in Table 14.

Although the treating physicians were encouraged to report all adverse reactions some adverse events may have not been reported. In spite of this potential bias the incidence rates of serious infections and all malignancies in this study are of the same magnitude as those in other, recently published studies (Table 15) (Geborek et al. 2005, Askling et al. 2005a,b, Askling et al. 2006 abstract 0182 EULAR congress, Bongartz et al. 2006).

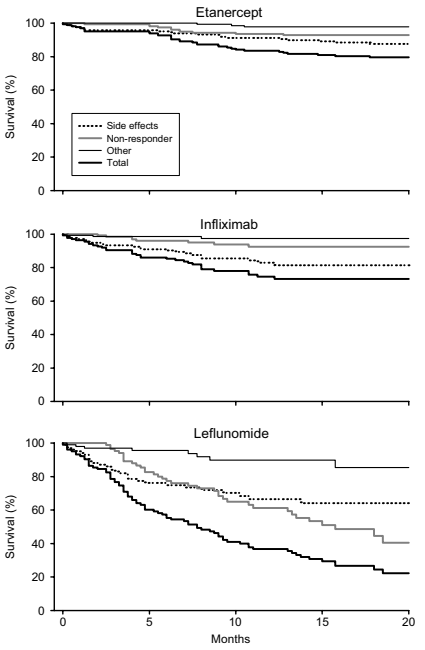


Figure 6: Drug survival of etanercept, infliximab or leflunomide

**Table 14: All fatal, life-threatening and serious adverse reactions registered during the observation period for etanercept, infliximab and leflunomide**

| Severity | Etanercept | Infliximab | Leflunomide |
|---|---|---|---|
| Fatal | 1 gastroenteritis (day 180)<br>1 immunocytoma of the breast (day 220)<br>1 myocardial infarction (day 413) | | |
| Life-threatening | | 1 anaphylactic reaction (day 320)<br>1 mesothelioma (day 42)<br>1 pharyngitis with extremely severe infection of the throat, neck, and upper abdomen (day 480) | |
| Serious | 4 myocardial infarctions (days 41, 63, 130, 501)<br>3 bacterial infections (2 pneumonia and 1 septic arthritis; days 130, 150, 270)<br>2 uterine cervical carcinoma (one in situ; days 160, 413)<br>1 acute myeloic leukaemia (day 440)<br>1 general malaise (day 350)<br>1 leucopenia (day 91)<br>1 Bell's paralysis (day 130)<br>1 cutaneous vasculitis (day 368)<br>1 discoid lupus (recurred on provocation; day 69) | 4 allergic reactions (days 41, 201, 230, 573)<br>2 bacterial infections (one otitis media, one cystitis; days 108, 210)<br>1 Hodgkin's lymphoma (day 129)<br>1 non-Hodgkin's lymphoma (day 180)<br>1 thrombocytopenia (day 250)<br>1 lupus like reaction (day 230)<br>1 discoid lupus (day 20) | 1 leucopoenia (day 108)<br>1 deep vein thrombosis with hypertension and vision impairment (day 226)<br>1 throat pain with swelling of the tongue (day 60)<br>1 clinical polyneuropathy with paraesthesia in feet and shoulders (day 110) |

**Table 15: Incidence rates of adverse events/100 treatment years (95%CI) for different treatment modalities**

| | Etanercept | Infliximab | Leflunomide |
|---|---|---|---|
| Treatment duration (treatment years) | 232.8 | 111.1 | 70.9 |
| Number of patients | 166 | 135 | 103 |
| Adverse events | Incidence (95% CI) | Incidence (95% CI) | Incidence (95% CI) |
| Fatal | 1.3 (0.3-3.8) | - | - |
| Life-threatening | - | 2.7 (0.6-7.9) | - |
| Serious | 6.4 (3.6-10.6) | 9.9 (4.9-17.7) | 5.6 (1.5-14.4) |
| Moderate | 15.5 (10.8-21.4) | 30.6 (21.2-42.8) | 28.2 (17.2-43.6) |
| Mild | 26.2 (20.0-33.7) | 53.1 (40.4-68.5) | 31.0 (19.4-47.0) |
| Not graded | 2.1 (0.7-5.0) | - | 12.7 (5.8-24.1) |
| Infections (lethal+life-threatening+serious) | 1.7 (0.5-4.4) | 2.7 (0.6-7.9) | - |
| Malignancy (all) | 1.7 (0.5-4.4) | 2.7 (0.6-7.9) | - |

## Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept (Paper II)

*The second aim* of this work was to investigate whether serum levels of COMP, a marker for cartilage turnover, changed during treatment with TNF antagonists and whether the changes confirmed the tissue protective effects of these drugs in RA.

The main finding of this work was that serum COMP levels decreased during the first 6 months of treatment with etanercept and infliximab, regardless of the clinical response to treatment and without correlation to changes in CRP.

Serum levels of COMP were measured in 32 infliximab- and 17 etanercept-treated patients with RA during the initial 6 months of therapy. Serum COMP had decreased in both patient groups at the 3-month follow-up ($p<0.001$ and $p<0.005$, for infliximab and etanercept, respectively) and levels remained low after 6 months of treatment compared with baseline values. The decrease in COMP levels was most pronounced in patients with the highest baseline values. Serum levels of COMP decreased regardless of the response according to ACR20 response criteria ($p<0.05$ or better), see Figure 7.

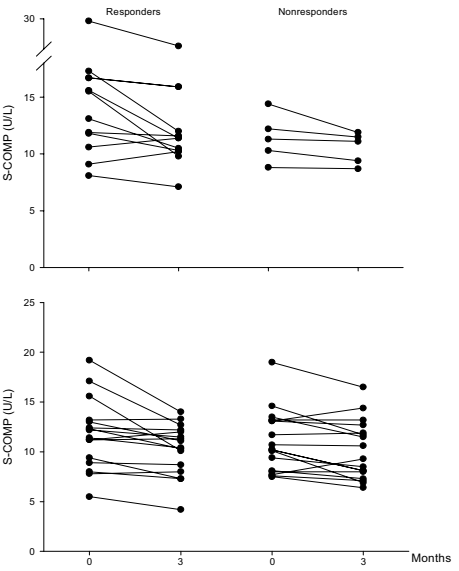


Figure 7: S-COMP at baseline and after 3 months in rheumatoid arthritis patients treated with etanercept (a) or infliximab (b), grouped in responders or nonresponders according to the ACR20 response criteria

The effects of different anti-rheumatic treatments on the progression of joint destruction are often studied by assessing radiographs of the hands and feet. Since the rate of progression is often slow, these studies must be conducted over a long period of time (minimum 1 year). An alternative or complementary approach is to measure levels of molecular markers that reflect the effects of different treatments on tissue, and which may change fairly rapidly after the initiation of the treatment.

Several clinical studies have shown that treatment with TNF blockers, including infliximab, etanercept and, more recently, adalimumab in RA not only reduces signs and symptoms of inflammation, but also prevents the progression of erosive joint changes (Maini et al. 1999, Lipsky et al. 2000, Bathon et al. 2000, Weinblatt et al. 2003, Klareskog et al. 2004). A reduction in progression of joint damage was observed in infliximab treated patients regardless of clinical response (Lipsky at al. 2000). Recently published sub-analysis of these results confirmed that infliximab in combination with MTX inhibited the progression of joint damage in patients without any evidence of clinical improvement according to the ACR20 response criteria and DAS28 (Smolen et al. 2005).

Results from the present study show that TNF antagonists modify the release of COMP from tissue, supporting the interpretation that these treatment modalities retard joint destruction. Levels of COMP decreased in both responders and nonresponders, which is in accordance with the hypothesis that inflammation and tissue destruction are not closely linked. Only patients not undergoing glucocoticoid treatment or those on stable, low-dose prednisolone treatment were included in this study. This approach considerably reduced the number of patients eligible for inclusion. It has been shown that glucocorticoids tend to lower serum COMP levels but the mechanisms governing this effect are not completely known. The lowering of serum COMP by glucocorticoids is not associated with a decrease in CRP or ESR (Saxne et al. 2006, Skoumal et al. 2006), suggesting that the anti-inflammatory effect of glucocorticoids is not the reason. Hypothetically, the effect might be due to the joint protective effect of glucocorticoids (Kirwan 1995, van Everdingen et al. 2002). Recently, moderate doses of glucocorticoids in experimental arthritis have been shown to retard cartilage and bone destruction. This joint protective effect was directly mirrored by decreased serum levels of COMP in that study (Larsson et al. 2004).

One disadvantage of the present study is the lack of radiographs for comparison with the changes in serum COMP levels. As described earlier in this thesis, all patients receiving TNF antagonists are included in the follow-up programme according to the SSATG protocol, and no suitable group was available for comparison. However, efficacy data including these patients (see Paper I) agree with the results of trials that included radiographs (Lipsky et al. 2000, Bathon et al. 2000, Genovese et al. 2002). Thus, results from this study corroborate the proposed joint protective effect of TNF antagonists. Furthermore, the findings support the use of COMP as a noninflammation-related marker of disease processes in RA.

## Influence of methotrexate, TNF-blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis (Paper III)

## Influenza vaccination as a model for testing immune modulation induced by anti-TNF and methotrexate therapy in rheumatoid arthritis patients (Paper IV)

*The third aim* of this work was to investigate whether anti-rheumatic treatment of patients with RA modulate the immune response to pneumococcal and influenza vaccine.

The major findings of the above two studies

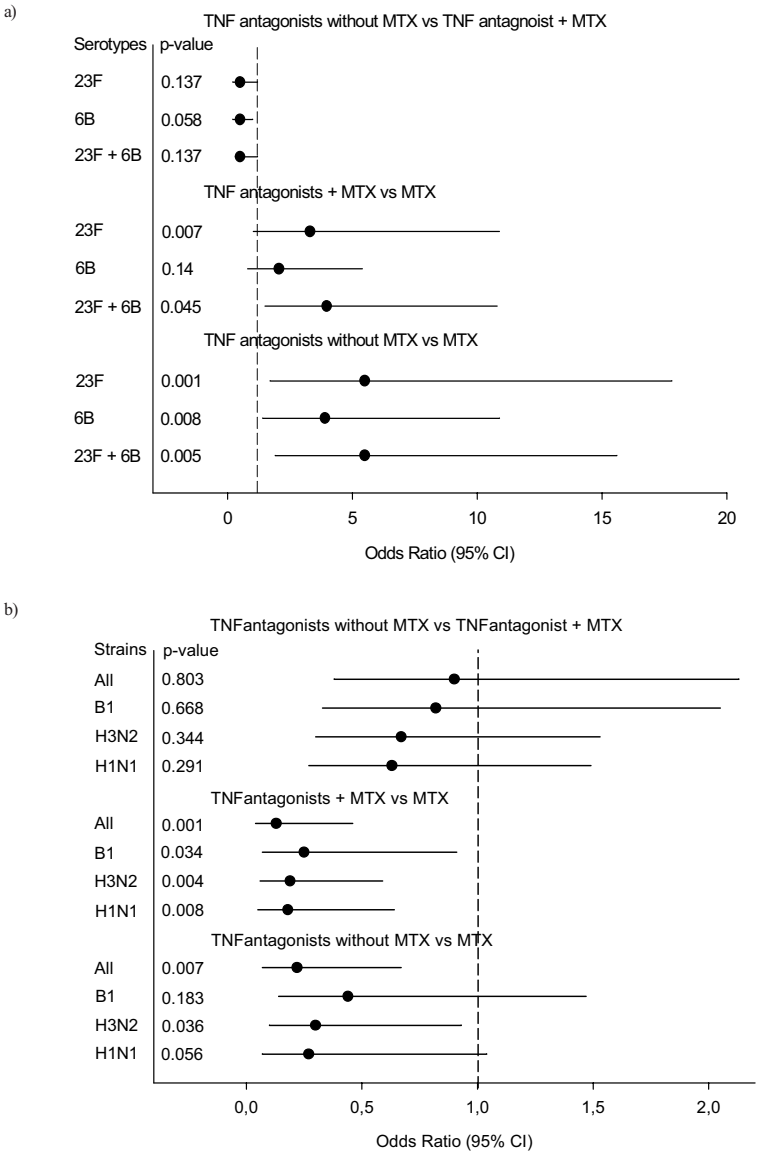


Figure 8: Positive immune response following pneumococcal and influenza vaccination in patients with RA
a) Pneumococcal vaccination. Odds ratio (OR) and 95% confidence interval (95% CI) for patients with positive immune response for 23F and 6B and combination of the serotypes.

b) Influenza vaccination. Odds ratio (OR) and 95% confidence interval (95% CI) for patients with prevaccination titre levels <40 and positive immune response (≥ 4 fold titre increase) to different strains and combination of the strains.

Results are adjusted for age, gender, disease duration and prednisolone dosage.

are that treatment with TNF antagonists and prednisolone in low dosage do not impair the antibody response to pneumococcal vaccination whereas methotrexate reduces the responses. In contrast, RA patients treated with methotrexate without TNF antagonists showed significantly better immune response to influenza vaccination compared to those receiving TNF antagonists alone or in combination with methotrexate and/or other DMARDs.

*Pneumococcal vaccination study in RA*

Positive immune response was defined as ≥2-fold increase in antibody levels to each serotype. Methotrexate-treated patients showed reduced immune response to separate serotypes and the combination of two serotypes when compared with patients treated with TNF antagonists. Controls tended to respond better than patients in the methotrexate group but without reaching significance.

Due to differences in the baseline characteristics between the groups, the results were re-analysed using a binary logistic regression model adjusted for age, gender, disease duration and prednisolone dosage. This confirmed that the immune responses in methotrexate treated group were significantly reduced compared with the TNF antagonist groups, see Figure 8a.

*Influenza vaccination study in RA*

Positive immunisation response to each strain was defined as ≥4 fold increase in prevaccination titres in patients with prevaccination titre levels <40. Prevaccination titres ≥40 were considered protective. The responses to separate strains were generally good in the patient groups but not in controls. High proportion of controls had prevaccination titres above the protective levels (27.8% for H1N1 and H3N2, and 61.1% for B1) making them unsuitable for inclusion in the statistical analyses. A binary logistic regression model including adjustments for baseline differences in age, gender, disease duration and prednisolone dosage was used to compare the immune responses between the groups. As it can be seen in Figure 8b, the positive immune response to combinations of all strains (H1N1+H3N2 +B1) was significantly better in the methotrexate group, even after adjustments to the regression model. The groups treated with TNF antagonists alone or in combination with methotrexate and/or other DMARDs showed a lower number of responders.

In these two studies pneumococcal and influenza vaccination was used to study immune modulation induced by different treatments in established RA. Treatment with TNF antagonists and prednisolone at low doses was not found to impair the antibody response following pneumococcal vaccination, while methotrexate did reduce the response. Reduced serological response following pneumococcal vaccination of RA patients taking methotrexate is in accordance with previous findings (O'Dell et al. 1992, O'Dell et al. 1996, Elkayam et al. 2002a).

In contrast, RA patients treated with methotrexate without TNF antagonists responded better to influenza vaccination than patients receiving TNF antagonists. The underlying mechanisms of these differences are not known. The mode of action of methotrexate in RA is not completely understood, but suppression of immunoglobulin production is one of several proposed mechanisms (Cutolo et al. 2001). Furthermore, there are substantial differences between pneumococcal and influenza vaccine. Pneumococcal vaccine containing 23 types of pneumococcal capsular polysaccharides was used in this study. Influenza vaccine contains haemagglutinin antigens representing 3 strains (two A strains and one B strain) of influenza virus. Polysaccharide antigens are recognised to induce lower immune responses than protein antigens (Fedson et al.1994a,b, Scheifele 2004). Reduced immunoglobulin production, resulting from methotrexate treatment, in combination with decreased immunogeneicity of polysaccharide antigens, might thus be one explanation of the impaired antibody response to pneumococcal vaccination in RA patients treated with methotrexate without TNF antagonists. Since the immune response following influenza vaccination was most satisfactory in the methotrexate group some other mechanisms must be involved.

Antibody response to influenza vaccination in

RA patients treated with methotrexate has previously been reported to be similar to that of age- and gender- matched healthy controls (Chalmers et al. 1994). The immune response to other virus vaccines e.g. hepatitis B vaccine containing purified virus antigen, in patients with RA has been shown to be somehow lower than in healthy individuals but was not affected by methotrexate treatment (Elkayam et al. 2002b). Our results are in accordance with results from these studies, suggesting that the immune response to polypeptide antigens is sufficient in spite of possible impact of methotrexate on the immunoglobulin production. Furthermore, it is generally accepted that antibody production following influenza vaccination is T-cell mediated while responses to polysaccharide antigens are considered to be T-cell independent. Our findings suggest that polysaccharide and polypeptide antigens are processed by different pathways in the immune system.

Regarding treatments with TNF antagonists, antibody responses to polysaccharide antigens were found to be normal or increased in groups receiving these agents. These results are in line with those reported from a study including patients with psoriatic arthritis treated with etanercept (Mease et al. 2004). In that study, both etanercept- and placebo- treated patients showed similar responses to the vaccine, although a subgroup of patients receiving concomitant methotrexate showed lower antibody levels. In contrast, Elkayam et al. (2004) reported lower proportion of responders among RA patients treated with TNF antagonists than age-matched RA patients not receiving this treatment.

Patients treated with TNF antagonists showed clearly lower responses to polypeptide antigens than those on methotrexate. The mechanisms leading to differences in antibody response between these two kinds of treatment are unknown. TNF plays an essential role in the immune-mediated response to infection, especially with intracellular pathogens, but also in granuloma formation (Crum et al. 2005). It is generally believed that the production of influenza-specific IgG is dependent on CD4+ T-cells. TNF antagonists strongly inhibit cell-mediated immunity, which might be relevant in the response to virus antigens such as those presented in influenza vaccine.

The immune responses to vaccination are surrogate markers for protection against the disease. However, provided that good immune response reflects the effectiveness of the vaccine, the current findings suggest that pneumococcal vaccination should be performed prior to methotrexate initiation, whereas TNF-antagonists and a low prednisolone dose do not preclude vaccination during ongoing therapy. Immune response following influenza vaccination was sufficiently good to warrant vaccination of all RA patients regardless of treatment.

## Predictors of infusion reactions during infliximab treatment in patients with arthritis (Paper V)

*The fourth aim* of this work was to study possible predictors of adverse events, in particular infusion reactions, during treatment with infliximab in patients with RA and SpA.

The main findings in this study were that ANA

**Table 16: Odds ratio, 95% CI and p-value for the development of infusion reactions in patients with RA**

| | Patients (N) | Odds ratio | 95% CI | p-value# |
|---|---|---|---|---|
| ANA positivity | 56 | 2.1 | 1.04-4.29 | 0.040 |
| Infliximab without methotrexate | 84 | 3.1 | 1.53-6.29 | 0.002 |
| Infliximab as monotherapy | 46 | 3.6 | 1.73-7.14 | 0.001 |

# Adjusted for age, gender and prednisolone at start of treatment
No predictors of infusion reactions could be identified in patients with SpA.

positivity, use of infliximab without methotrexate, or infliximab as monotherapy are predictors for the development of infusion reactions in patients with RA. The risk of infusion reactions was most pronounced in ANA positive patients treated with infliximab without methotrexate.

ANA were present in 28% of RA and 25% of SpA patients before initiation of treatment. A larger proportion of RA patients (21.1%) than SpA patients (13.2%) developed some kind of infusion reaction during treatment, although these differences did not reach significance. ANA positivity, use of infliximab without methotrexate, or infliximab as monotherapy were associated with increased risk of developing infusion reactions in patients with RA, even after adjustment to a logistic regression model, see Table 16. Lower age at disease onset and longer disease duration were associated with increased risk of infusion reactions whereas age, gender, CRP, ESR, HAQ and DAS28 before starting treatment did not influence this risk in patients with RA. No predictors of infusions reactions were identified in SpA patients.

Infliximab is a chimeric monoclonal antibody comprising 25% murine protein and is thus likely to induce an immune response in humans. Treatment with infliximab is associated with the development of anti-drug antibodies and infusion reactions (Maini et al. 1998, Baert et al. 2003, Bendtzen et al. 2006, in press). However, the infusion reactions are not always typical allergic ones. In this retrospective study the effects of baseline ANA status and concomitant treatment with other immunossuppressive agents on the development of infliximab related infusion reactions were investigated.

The mechanism explaining the association between ANA positivity and infusion reactions is not known. Immunological mechanisms are thought to be responsible for many of the toxic reactions to some DMARDs (Panayi et al. 1978, Smidt et al. 1978, Ferraccioli et al. 1986). Furthermore, Panayi et al. found a positive correlation between HLA-DR phenotypes and toxic reactions to some DMARDs. The findings of increased risk for developing infusion reactions in ANA positive RA patients in our study support the plausibility of underlying immunogenetic mechanisms of drug related side effects. It has been reported that concomitant use of methotrexate reduces the production of the anti-infliximab antibodies (Maini et al. 1998). Our results also point in that direction, suggesting that concomitant treatment with other DMARDs, preferably methotrexate in RA may decrease the immunogeneicity of infliximab and probably also that of other monoclonal antibodies.

# Conclusions

Based on the results obtained from the studies presented in this thesis the following conclusions can be drawn:

- A structured protocol with central data handling is feasible in clinical practice for documenting the performance and adverse events of newly introduced drugs for the treatment of arthritis.
- Serum COMP decreases during treatment with TNF antagonists suggesting a beneficial effect of the treatment on cartilage turnover.
- Serum COMP has the potential to be a useful marker for evaluating tissue effects of novel treatment modalities in RA.
- Methotrexate treatment in RA reduces antibody response to pneumococcal vaccination suggesting that RA patients should be vaccinated before the initiation of this treatment.
- Ongoing treatment with TNF antagonists does not reduce antibody response to pneumococcal vaccination.
- RA patients treated with methotrexate showed significantly better serological response to influenza vaccination than patients receiving TNF antagonists.
- The immune response to influenza vaccination is sufficiently good to warrant vaccination of all RA patients, regardless ongoing treatment.
- Positive ANA at initiation of infliximab treatment and the use of infliximab as monotherapy or without methotrexate is associated with an increased risk of infusion reactions in RA patients.
- Concomitant treatment with DMARDs, preferably methotrexate, should be encouraged before the initiation of infliximab in RA patients in order to reduce the risk of infusion reaction.

# Perspectives for the future

Therapeutic targeting of TNF is a major step forward in the treatment of arthritis. TNF is a cytokine that plays a pivotal role in the pathogenesis of arthritis, but it is also involved in other important physiological functions. Treatment with TNF antagonists has been shown to be effective in reducing signs and symptoms of inflammation and also in retarding tissue damage. Numerous potential cellular targets for immunotherapy are under investigation. However, the long-term consequences of these agents are not known. Continuous surveillance of such novel therapeutic modalities in daily clinical practice is important not only in assessing treatment efficacy but, perhaps even more important, in detecting rare or unexpected side effects. The lesson learned from the introduction of TNF antagonists underlines the need to employ structured clinical protocols for the follow-up of newly introduced anti-rheumatic agents in the future.

In this work, some predictors for the development of treatment-related infusion reactions have been identified. A similar approach may identify predictors of treatment efficacy, treatment failure or adverse events associated with future new treatment modalities.

Increasing knowledge of the mechanisms causing tissue destruction in arthritis has led to development of the molecular marker concept. A number of molecular markers that reflect the process in cartilage and bone have been identified in synovial fluid and serum. COMP is a molecular marker for cartilage turnover that has shown promise as a prognostic indicator in RA, and in this work it was shown that COMP may also be a useful marker for evaluating tissue effects of novel treatments of RA. Further development will lead to new assays that detect selected fragments of molecule possibly only released during pathological processes, thereby increasing the sensitivity and specificity of the technique.

Anti-rheumatic treatment has the potential to modulate the immune response. The impact of treatment on the immune response may have major consequences for patients with rheumatic diseases in terms of compromised immune defence against common and/or rare and severe infections. In this work it was found that immune modulation studied by two kinds of vaccination, is different for different agents but also depends on which antigen the immune system is exposed to.

# Popularized summary in Swedish- sammanfattning på svenska

Reumatoid artrit (kronisk ledgångsreumatism; RA) är en inflammatorisk sjukdom där lederna är det primära målorganet. Spondylartropatier är ett gemensamt namn för ett flertal kroniska inflammatoriska sjukdomar med många gemensamma tecken som till exempel: inflammatorisk ryggsmärta, inflammationer i leder, senskidor, muskelfästen, förekomst av ”korvfingrar/-tår”. Orsaken/er till dessa sjukdomar är fortfarande okänd. Däremot har kunskapen om olika processer under sjukdomsutveckling ökat avsevärt de senaste decennierna. Man har identifierat flera ämnen som spelar en viktig roll vid dessa sjukdomar vilket har lett till utveckling av effektiva läkemedel. Tumör nekrotisk faktor (TNF) är ett protein som anses spela en viktig roll vid kroniska inflammatoriska sjukdomar. Detta är ett normalt förekommande ämne i kroppen med flera viktiga funktioner inom immunförsvaret. Läkemedel som blockerar effekter av TNF, sk TNF antagonister, har visat sig minska tecken på inflammation i kroppen såsom ledsvullnad, ledömhet, morgonstelhet, trötthet men också bromsa utveckling av ledskador (destruktion) hos de flesta patienter med ledgångsreumatism. Så småningom har man kunnat visa att TNF antagonister är effektiva vid andra kroniska ledinflammationer inklusive spondylartropatier. Dessa läkemedel har funnits på marknaden sedan 1999 och effekter av dessa behandlingar på lång sikt är inte kända.

I denna avhandling har vi studerat olika aspekter av behandling med två TNF antagonister (etanercept och infliximab).

I första delarbetet har vi studerat de kliniska aspekterna av behandling med tre antireumatiska läkemedel: leflunomide, etanercept och infliximab. Alla patienter som började behandlas med något av dessa läkemedel vid Reumatologkliniken i Lund eller någon av 6 andra reumatologiska enheter i södra Sverige (Spenshult, Helsingborg, Trelleborg, Simrishamn, Kristianstad och Växjo) mellan mars 1999 och april 2001 inkluderades i denna studie. Patienterna följdes under studietiden enligt ett strukturerat kliniskt protokoll utvecklat vid Reumatologkliniken i Lund [South Swedish Arthritis Treatment Group (SSATG)]. Uppföljning enligt SSATG-protokollet innebär regelbundna besök varvid patienten träffar en läkare, man registrerar antal svullna och ömma leder, eventuella biverkningar eller andra händelser som har inträffat och en bedömning av sjukdomens aktivitet görs både av läkaren och av patienten. Blodprover för kontroll av inflammation tages vid varje besök. Alla data registreras och skickas till Lund för central bearbetning. Syftet med första delarbetet var att studera om ett sådant strukturerat kliniskt protokoll med central databearbetning är användbart för att utvärdera nya behandlingar vid RA. Vi har kunnat visa att ett strukturerat klinisk protokoll är användbart både vid ett universitetssjukhus och vid mindre reumatologiska enheter. TNF antagonister visades ha ungefär liknande effekter som tidigare rapporterats från kliniska prövningar men leflunomide visade sig vara mindre effektivt jämfört med resultat från kliniska prövningar. Även biverkningar som har registrerats motsvarar de rapporterade i de kliniska prövningarna.

I andra delarbetet mättes förändringar i nivåer av ett protein (COMP) i blodet under behandling med etanercept och infliximab (två TNF antagonister). COMP är ett protein som normalt finns mest i brosket men även i mindre mängder i flera andra vävnader i kroppen. COMP frisläpps från brosket in i ledvätska och når så småningom blodbanan vid olika sjukdomsprocesser som drabbar broskvävnad. Syftet med detta arbete var att studera om COMP-nivåerna i blodet ändras på ett sätt som går samman med de ledskyddande effekter som rapporterats vid behandling med TNF antagonister. Vi kunde visa att COMP-nivåerna i blodet sjunker, vilket stödjer hypotesen att behandling med infliximab och etanercept bromsar utveckling av ledskador hos

patienter med RA. COMP är en potentiell markör för evaluering av vävnadseffekter av ny-introducerade behandlingar vid RA i framtiden.

I de tredje och fjärde delarbetena har vi studerat hur olika behandlingar som används vid RA påverkar de immunologiska svaren efter pneumokock och influensavaccination. Vi har funnit att methotrexat, vilket är det mest använda cellhämmande medlet vid RA, minskar immunsvar efter pneumokockvaccination. Däremot, var immunsvaret efter influensa vaccination bättre hos methotrexat behandlade patienter jämfört med de patienter som behandlades med TNF antagonister. Dessa resultat tyder på att pneumokockvaccination borde genomföras före start av methotrexat behandling. När det gäller influensa vaccination, var det immunologiska svaret tillräckligt bra oavsett vilken behandling som gavs för ledsjukdom vilket talar för att alla RA patienter kan vaccineras mot influensa med samma svar på vaccinationen.

I det femte delarbetet studerades faktorer som kan förutsäga utveckling av infusionsreaktioner (överkänslighetsreaktioner i anslutning till tillförsel av preparat) vid behandling med en av TNF antagonisterna (infliximab). I denna studie har både patienter med RA och spondylartropatier deltagit. Det visade sig att vid förekomst av antikroppar mot olika ämnen i cellkärnan i blodet (dvs om man är ANA positiv) löper patienter med RA en högre risk för att drabbas av en infusionsreaktion under behandlingen. Den risken är även ökad om man får infliximab behandling utan samtidig behandling med annat cellhämmande läkemedel. Den största risken att få infusionsreaktion har de patienter som är både ANA positiva före behandlingsstart och får infliximab utan methotrexat. Inga faktorer som kan prediktera infliximab relaterade infusionsreaktioner har kunnat identifieras hos patienter med spondylartropatier.

Erfarenheter från introduktion av TNF antagonister för behandling av artrit sjukdomar understryker vikten av noggrann uppföljning av nya läkemedel genom att använda ett strukturerat kliniskt protololl.

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