Early Rheumatoid Arthritis
Aspects of severity and co-morbidity

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To my family

Table of Contents

Abstract

Background Rheumatoid arthritis (RA) is a systemic progressive destructive joint disease with an increased risk for co-morbidity and premature death if untreated. Cardiovascular disease (CVD) is the main cause of death but also other co-morbid conditions contribute to the patient's shorter life expectancy. Inflammation seems to be important for the development of CVD, but knowledge of its relationship with other co-morbidities is sparse. Early disease modifying anti rheumatic drugs (DMARDs) can suppress disease activity and improve the long-term outcome. The aim of this thesis was to evaluate prospectively aspects of disease activity and severity in a large cohort of patients with early RA. Predictive and prognostic markers, occurring in early disease and with implications for disease outcome and co-morbidity were evaluated.

Methods Patients with early RA (i.e., symptomatic for ≤12 months) according to the 1987 classification criteria) have, since December 1995, been consecutively included in a large survey of prospective and observational studies on the progression of RA and the development of co-morbidity. Autoantibodies, inflammatory, genetic markers and radiographs have been analyzed. In Paper I, 210 RA patients and 102 controls were followed regularly for two years. The predictive value of four different ACPAs in relation to disease activity and radiological progression was evaluated. In Paper II (n = 700) and in Papers III-IV (n =950), patients with early RA from the four northern-most counties of Sweden were followed regularly for 5 years. Data on risk factors and co-morbidity was collected, according to the study protocol, from clinical records and self-reported questionnaires from patients at inclusion into the study cohort and after five years.

The predictive value of traditional and potential disease related risk factors for new cardiovascular events (CVE) was evaluated (II). In Paper III, the impact of age at the onset RA, stratified as being young onset RA (<58 years; YORA) and late onset RA (≥58 years; LORA) on disease activity, severity and chosen treatment, was evaluated. In Paper IV, the development of new co-morbidities after RA onset and their relation to inflammatory activity was assessed.

Results The presence of anti-mutated citrullinated vimentin (MCV) antibodies was associated with a more severe disease course, estimated by disease activity score, erythrocyte sedimentation rate (ESR) and swollen joint count after 24 months, compared with anti-CCP2, anti-CCP3, and anti CCP3.1 antibodies. In Paper II, the incidence of a new CVE during 5 years was explained by several of the traditional CV risk factors, and potentiated by a high disease activity. Treatment with DMARDs decreased the risk. In Paper III, LORA patients were associated with greater disease activity/severity at disease onset and over time compared with YORA patients who were more often ACPA positive. YORA patients were treated earlier with DMARDs, whilst LORA patients were more often treated with corticosteroids and less so with DMARDs early in the course of their disease. In Paper IV, 53% of patients had one or more co-morbidities already at the onset of RA. After 5 years, 41% of the patients had developed at least one new co-morbidity. ESR at baseline and accumulated disease activity (after 24 months) were associated with a new co-morbidity after five years.

Conclusion Early RA patients sero-positive for anti- MCV antibodies appeared to have a higher disease activity over time. The occurrence of a new CVE in early RA patients was predicted by traditional risk factors for CVD which were potentiated by a high disease activity. Treatment with DMARDs decreased the risk. Patients with young onset of RA were associated with a higher frequency of ACPA. Late onset of RA was associated with higher disease activity/severity at inclusion and over time. However, LORA patients were more often treated with corticosteroids and less so with DMARDs early in the disease course. Development of a new co-morbidity during the five years following diagnosis was related to ESR.

Abbreviations

ACPA	anti-citrullinated protein/peptide antibody
ACR	American College of Rheumatology
Anti-CCP	anti-cyclic citrullinated peptide
APC	antigen presenting cell
AUC	area under the curve
CABG	coronary artery bypass grafting
CCI	Charlson Comorbidity Index
CIRS	Cumulative Illness Rating Scale
CI	confidence interval
COPD	chronic obstructive pulmonary disease
COX-2	cyclo-oxygenase-2
CRP	C reactive protein
CV	cardiovascular
CVD	cardiovascular disease
CVE	cardiovascular event
DAS28	Disease Activity Score 28 joint count
DM	diabetes mellitus
DMARD	disease modifying anti-rheumatic drug
DVT	deep vein thrombosis
ELISA	enzyme-linked immunoassay
ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
Ex-RA	extra-articular rheumatoid arthritis
GC	glucocorticosteroids
HAQ	Health Assessment Questionnaire
HDL	high density lipoprotein
HLA-SE	human leukocyte antigen-shared epitope allele
HT	hypertension
IL	interleukin
ICED	Index of Coexisting Disease
LDL	low density lipoprotein
MCP	metacarpophalangeal joint
MCV	mutated citrullinated vimentin
MHC	major histocompatibility complex
MI	myocardial infarction
MRI	magnetic resonance imaging
MTP	metatarsophalangeal
MTX	methotrexate
NSAID	non steroid anti-inflammatory drug

OR	odds ratio
PAD	peptidyl arginine deiminase
PE	pulmonary embolism
PIP	proximal interphalangeal joint
PTPN22	protein tyrosine phosphatase non-receptor type 22
RA	rheumatoid arthritis
RF	rheumatoid factor
ROC	receiver operating characteristics
SD	standard deviation
SIR	standardized incidence ratio
SJC	swollen joint count
SSZ	sulphasalazine
TIA	transient ischemic attack
TJC	tender joint count
TNF-α	tumour necrosis factor-alpha
T0	baseline/inclusion
T5	patients had been followed for 5 years
US	ultrasound
VAS	visual analogue scale

List of publications

The thesis is based on the following papers that will be referred to by the appropriate Roman numeral:

I. Innala L, Kokkonen H, Eriksson C, Jidell E, Berglin E, Rantapää-Dahlqvist S. Antibodies against mutated citrullinated vimentin are a better predictor of disease activity at 24 months in early rheumatoid arthritis than antibodies against cyclic citrullinated peptides. *J Rheumatol* 2008; 35: 1002-1008.

II. Innala L, Möller B, Ljung L, Magnusson S, Smedby T, Södergren A, Öhman M-L, Rantapää-Dahlqvist S, Wållberg- Jonsson S. Cardiovascular events in early RA are a result of inflammatory burden and traditional risk factors: a five year prospective study. *Arthritis Res Ther* 2011; 13: R131.

III. Innala L, Berglin E, Möller B, Ljung L, Smedby T, Södergren A, Magnusson S, Rantapää-Dahlqvist S, Wållberg- Jonsson S. Age at onset determines severity and choice of treatment in early rheumatoid arthritis: a prospective study. *Arthritis Res Ther* 2014;16: R94.

IV. Innala L, Sjöberg C, Möller B, Ljung L, Smedby T, Södergren A, Magnusson S, Rantapää-Dahlqvist S, Wållberg- Jonsson S. Comorbidity in patients with early rheumatoid arthritis. Does inflammation matter ? *In manuscript.*

Sammanfattning på svenska

Reumatoid artrit (RA), ledgångsreumatism är en livslång autoimmun sjukdom. Det är den vanligaste inflammatoriska ledsjukdomen och drabbar ca 0,5-1% av den vuxna befolkningen. Den vanligaste insjuknandeåldern är 55-60 år och ca två tredjedelar av patienterna är kvinnor. Orsakerna till RA är inte fullständigt kända men en samverkan mellan ärftlighet, miljöfaktorer och hormoner tros föreligga. Sjukdomsförloppet varierar och spontan förbättring förekommer men många utvecklar en svårt ledförstörande sjukdom. Ungefär 70-80 % av patienterna har autoantikroppar och de vanligaste och diagnostiskt viktigaste är reumatoid faktor (RF) och antikroppar mot citrullinerade proteiner/peptider (ACPA). En del RA patienter får också extra-artikulära manifestationer d.v.s. symtom utanför lederna från t.ex. hjärta, lungor, nerver mm vilket är ett tecken på sjukdomens systemiska karaktär. Många studier har visat att patienter med RA har en ökad dödlighet och sjuklighet jämfört med allmänna befolkningen. Hjärt-kärlsjukdom är den främsta dödsorsaken, men även andra sjukdomstillstånd bidrar till patientens kortare livslängd. Komorbiditet (samsjuklighet) är sjukdomar som är överrepresenterade men inte direkt relaterade till RA, men som kan vara associerade till RA sjukdomen eller dess behandling och som bidrar till patientens totala sjukdomsbörda.

Inflammation har visat sig kunna ha betydelse för utvecklingen av hjärt-kärlsjuklighet, men kunskapen om inflammationens betydelse vid annan komorbiditet är sparsam. Tidig behandling med olika sjukdomsmodifierande antireumatiska läkemedel (DMARD) kan dämpa sjukdomsaktiviteten och har i många studier setts förbättra det långsiktiga resultatet.

Syftet med detta arbete har varit, att följa en stor grupp RA patienter från tidig sjukdomsdebut och försöka identifiera risk/skyddsfaktorer för sjukdomsutveckling, prospektivt, under 2 respektive 5 år.
Ett annat syfte har varit att utvärdera vilken betydelse sjukdomsaktiviteten har för sjukdomens svårighetsgrad och utvecklingen av komorbiditet.

Denna studie startade i december 1995 och patienterna har följts regelbundet på hemortssjukhusen i Västerbottens, Norrbottens, Jämtlands och Västernorrlands län. Alla patienter uppfyllde kriterierna för RA enligt 1987 års ACR-klassifikation.

I delarbete I inkluderades 210 RA-patienter och 102 friska kontroller i Västerbotten och följdes regelbundet i 2 år. Vi undersökte olika citrullinerade antikroppars (ACPA) betydelse för sjukdomsaktivitet och svårighetsgrad och deras förmåga att förutsäga sjukdomsutvecklingen vid tidig RA. Händer och fötter röntgades vid inklusion och efter 2 år.
I delarbete II-IV ingick patienter med tidig RA från de fyra nordligaste länen i Sverige. Uppgifter om inflammation (sänka), klinisk inflammation (antal ömma/svullna leder, VAS skalor för smärta och allmänt välbefinnande), genanalyser, autoantikroppar och blodfetter analyserades. Uppgifter om traditionella hjärtkärlriskfaktorer och all komorbiditet har registrerats, komplikationer till RA sjukdomen och detaljer om farmakologisk behandling har samlats in, i noggranna journal studier i enlighet med ett studieprotokoll. Patienterna har besvarat enkäter avseende komorbiditet vid inklusion och efter 5 år.
I delarbete II inkluderades 700 patienter varav 442 patienter varit sjuka minst 5år. I denna studie undersöktes förekomsten och betydelsen av traditionella hjärtkärl och sjukdomsassocierade riskfaktorer samt betydelsen av läkemedelsbehandling för utveckling av en ny hjärtkärlhändelse hos patienter med tidig RA.
I delarbete III-IV inkluderades 950 patienter varav 665 (i delarbete III) och 726 patienter (i delarbete IV) som varit sjuka minst 5år.
I delarbete III utvärderades insjuknandeålderns (uppdelad efter medianåldern i tidig sjukdomsdebut <58 år och sen sjukdomsdebut ≥58 år), betydelse för prognostiska faktorer, sjukdomsutveckling och vald farmakologisk behandling.
I delarbete IV undersöktes förekomsten av komorbiditet vid sjukdomsdebuten och under de första 5 sjukdomsåren och om det fanns någon koppling till inflammatorisk aktivitet.

Delarbete I: Patienter med nydebuterad RA som hade den ACPA typ som benämns anti- MCV -antikroppar föreföll ha en högre sjukdoms aktivitet över tid men alla undersökta ACPA-typerna predikterade röntgen progress efter två år.
Delarbete II: Patienter med RA löper ökad risk att insjukna och/eller dö i hjärtkärlsjukdom, de traditionella hjärtkärl riskfaktorerna har inte ensamt kunna förklara detta i tidigare tvärsnitts och retrospektiva studier. I denna prospektiva studie fann vi att de flesta traditionella riskfaktorerna för hjärtkärlsjukdom hade betydelse för framtida ny hjärtkärl händelse och hög sjukdomsaktivitet, föreföll kunna förstärka effekten av de traditionella riskfaktorerna. Behandling med DMARD minskade risken vilket visar att hämning av sjukdomsaktiviteten är av största betydelse inte bara ur ledsynpunkt, utan även ur en hjärtkärl aspekt.

Delarbete III: Patienter med sen sjukdomsdebut (≥58 år) hade högre sjukdomsaktivitet, nedsatt funktion och mer röntgenförändringar vid debuten och över tid jämfört med patienter med tidig sjukdomsdebut (<58 år) som oftare hade ACPA en markör för svårare sjukdom. De yngre patienterna erhöll tidigare i sjukdomsförloppet sjukdomsmodifierande behandling (DMARDs) medan de äldre patienterna oftare behandlades med kortison och mindre med DMARDs tidigt i sjukdomsförloppet. Senare års forskning ger stöd för att DMARDs bör sättas in tidigt i sjukdomsförloppet om inte något hinder av medicinska skäl föreligger för bästa resultat på sikt. **Delarbete IV**: Vid sjukdomsdebuten hade 53 % av RA patienterna en eller flera andra sjukdomar (komorbiditet) och efter fem år hade 41 % av patienterna utvecklat minst en ny sjukdom. Sjukdomsaktiviteten vid insjuknandet i RA var associerad med en ny komorbiditet efter fem år.

Med nuvarande behandlingar som dämpar sjukdomsaktiviteten vid RA kan man se att patientgruppen har förbättrats med tydligt mindre funktionshandikapp och mindre behov av proteskirurgi. Sjukdomsförloppet varierar mellan patienterna och det är viktigt att skilja patienter med lindrig sjukdom och god prognos från dem med svår sjukdom och dålig prognos och ge optimal behandling till varje patient. Den allmänt rådande uppfattningen är att det är angeläget att behandla nydebuterad RA snabbt (inom 3 månader) och kraftfullt för att på sikt minska risken för funktionsnedsättning och andra komplikationer till sjukdomen. Resultaten i detta arbete kan ha betydelse för förståelsen av tidigt insatt inflammationshämmande antireumatisk behandling samt vikten av hjärtkärl skyddandeåtgärder hos RA patienter. Härtill bör man beakta insjuknandeålderns betydelse vid val av antireumatisk behandling samt förekomsten av övrig komorbiditet i det dagliga patient arbetet.

Introduction

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that primarily affects the joints. It is present worldwide with increasing prevalence in the elderly (Rasch et al., 2003). It is the most common inflammatory disease of the joints and is characterized by the destruction of cartilage and bone leading to functional decline and disability if left untreated. The pathophysiology is not completely understood and no single test or gold standard exists to confirm the diagnosis, which is based on the clinical signs and symptoms typical of RA phenotype (Visser et al., 2002). Approximately two-thirds of patients are rheumatoid factor (RF) and/or anti-citrullinated peptide/protein antibodies (ACPA) sero-positive which has prognostic implications. RA is a systemic disease affecting, both directly and indirectly, most organs with the development of extra-articular manifestations (Turesson, 2013). Patients with RA have an increased risk for cardiovascular involvement and other co-morbidities that contribute to a shortened lifespan compared with the general population (Doran et al., 2002a, Smitten et al., 2008, Sodergren et al., 2007). In patients with RA inflammation appears to be important for the development of cardiovascular disease (CVD) and other co-morbidities (Wallberg-Jonsson et al., 1999). It is well known that early therapy with disease modifying anti-rheumatic drugs (DMARDs) can suppress disease activity, improve function and long-term outcome (Leirisalo-Repo, 2013).

Epidemiology

Rheumatoid arthritis (RA) occurs worldwide with various incidence and prevalence among populations (Alamanos et al., 2006, Symmons, 2002). The disease affects both genders but approximately 2/3 of patients are women (Doran et al., 2002c) In Western countries the incidence of RA is estimated to be 10-50 per 100 000 yearly (Alamanos et al., 2006, Eriksson et al., 2013). The incidence appears to increase with age for both men and women.(Alamanos et al., 2006, Eriksson et al., 2013). In a retrospective study Doran et al., who investigated the time trends of RA over forty years, found a significant decreasing incidence from 61.2/100,000 in 1955–1964, to 32.7/100,000 in 1985–1994. In the same inception cohort, the incidence increased with age up to 85, but reached a peak earlier in women compared with men (Doran et al., 2002c). The prevalence of RA is about 0.5-1.0 % in population-based studies in most Western countries (Arnett et al., 1988, Neovius et al., 2011, Silman et al., 2002). In a recent study by Neovius and

colleagues, the prevalence of RA in adults in Sweden was 0.70% (Neovius et al., 2011). The prevalence of RA in Chinese populations was 0.2 %, markedly lower than in Western countries (Zeng et al., 2008). Populations with the highest recorded prevalence of RA, up to 5.3-6.8 %, were seen in Native American Indian populations (Silman et al., 2002). This suggests not only an important genetic influence on the development of RA but also that environmental and lifestyle factors appear to be important.

Aetiopathogenesis

Despite extensive research, the primary cause of RA remains unknown. The clinical heterogeneity of the disease from a self-limiting to progressively chronic disease, and the immunological difference between patients, has made it difficult to identify an aetiological agent. In addition to interaction between several genetic, environmental risk factors, sex hormones, the immune system, stochastic factors have been proposed.

An autoimmune disease such as RA is considered to start with a loss of immunological tolerance, i.e., the ability to discriminate between self and non-self in an immunologically susceptible host. Recognition of an as yet unknown autoantigen by specific T-cells in the synovial membrane has long been considered to be crucial in the pathophysiology of the rheumatoid synovitis (Harris, 1990).

The pathogenesis of RA includes activation of innate immunity (neutrophils, macrophages, monocytes, and fibroblastic synoviocytes) and the adaptive immune system with the involvement of T- and B-lymphocytes. The humoral response is considered to be important for the production of auto-antibodies such as RF and ACPA. The two immune systems interact through specific (cytokines, chemokine and autoantibodies) and non-specific (prostaglandins, complement, protease, nitrous oxide) mediators. Pro-inflammatory cytokines important for the pathogenesis of RA present in the inflamed joint are tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6, all of which are secreted by activated macrophages and fibroblast-like synoviocytes (Harris, 1990, McInnes et al., 2007). A consequence of the synovial inflammation is that the synovium becomes hypertrophic, forming a tumour-like pannus that invades and destroys cartilage and bone, leading to pain and loss of function in the patient (Harris, 1990). Over time, most patients develop erosions within cartilage and bone, narrowing of joint space and peri-articular decalcification usually visualized as radiographic changes consistent with RA (Larsen, 1974).

Several genetic loci have been suggested to be associated with the development of RA but the most important genetic risk factor implicated to date is the presence of the “shared epitope” (Gregersen et al., 1987). The major histocompatibility complex (MHC), a genetic complex located on the short arm of chromosome 6 includes genes encoding for the HLA class II molecules which are presented on the surface of antigen presenting cells (APC; i.e., macrophages, B-cells and dendritic cells). The HLA class II antigens associated with RA are HLA-DRB1*0101/0401/0404/0405/0408, the so-called shared epitope (Gregersen et al., 1987).

The strongest genetic factor associated with RA is the HLA-shared epitope (SE), which accounts for 37 % of the genetic contribution (Deighton et al., 1989). However, this figure may be an overestimate in view of a recent study in which the contribution from SE was estimated to be only a approximately 10% of the total genetic variance for RA (Diogo et al., 2014).

The protein tyrosine phosphatase non-receptor type 22 (PTPN22) is another immune regulatory gene outside the HLA region that has been associated with RA (Begovich et al., 2004, Gregersen et al., 2005). This gene acts on the threshold of T-cell activation as a negative regulator. The risk allele leads to T-cells with a lower threshold for activation (Hasegawa et al., 2004). Currently there are more than a hundred other genetic loci associated with RA and several of these risk genes have a link to pathways and cell types of importance in RA pathogenesis, which could give options for new RA drugs (Okada et al., 2014). Support for a genetic predisposition comes from the family history of patients with RA and studies of twins with approximately 60% of the occurrence of the disease being explained by shared genetic effects (MacGregor et al., 2000). In a recent study the familial risk of heritability was 50% for ACPA sero-positive RA and 20% for ACPA sero-negative RA (Frisell et al., 2013). However, the concordance in monozygotic twins is only 15% indicating that factors other than genetics, such as environmental factors, play roles in the disease onset (Silman et al., 1993).

Pre-clinical RA (i.e., RA before clinical symptoms are apparent) is a period of autoimmunity with the formation of RA related antibodies, e.g., RF and ACPAs, in a genetically susceptible individual that can occur many years before disease onset (Rantapaa-Dahlqvist et al., 2003). Autoantibodies, such as RF and ACPA gradually increase in concentration the closer to the onset of disease (Rantapaa-Dahlqvist et al., 2003). Furthermore, autoantibody characteristics, e.g., isotype, avidity, glycosylation and epitope spreading, change as the time to diagnosis decreases (Brink et al., 2013, Kokkonen et al., 2011). One hypothesis is that antibodies are initially generated outside the joints and that an environmental factor interacts at a tissue surface, i.e.,

“anatomic sites”, such as the periodontium, lung, gastrointestinal mucosa or other tissues leading to autoantibody production. Thus, a local autoimmune mediated inflammatory reaction occasionally develops in a genetically susceptible individual and becomes systemic (Demoruelle et al., 2014).

Tobacco smoking is the most well documented environmental risk factor associated with an increased risk for RA (Krishnan et al., 2003, Silman et al., 1996). Heavy cigarette smoking (Costenbader et al., 2006, Hutchinson et al., 2001), RF sero-positivity (Stolt et al., 2003), and smoking in combination with carriage of the SE genes (Padyukov et al., 2004) are strongly associated with the development of RA. Smoking is also known to predispose for ACPA sero-positive disease and Klareskog et al. have proposed a possible biological hypothesis that explains the interaction between HLA-DR SE genes and smoking for development of ACPA sero-positive RA (Klareskog et al., 2006). They showed that smokers had more citrullinated proteins in bronchoalveolar lavage cells compared with non-smokers (Klareskog et al., 2006, Makrygiannakis et al., 2008). Thus, in a genetically susceptible individual with a history of smoking, both of these risk factors may generate a specific immune reaction in the lung parenchyma and the formation of citrullinated proteins yielding antibodies such as ACPAs (Klareskog et al., 2006 Demoruelle et al., 2014). Also another study showed that RA patients who carried double copies of SE and were heavy smokers had markedly increased risk for ACPA positive RA compared with SE non-carriers (Pedersen et al., 2007). In contrast, Bang et al , showed that a combination of HLA-DR SE genes and having a smoking history was associated with both ACPA/RF positivity and ACPA/RF sero-negative RA (Bang et al., 2010). Furthermore, Fischer and co-workers reported ACPA positivity and chronic lung disease in individuals asymptomatic regarding joint involvement to precede the development of classical RA, giving additional support for airway tissue as a possible potential site of autoimmunity (Fischer et al., 2012).

The role of the oral mucosa has been investigated since chronic periodontitis (inflamed gingival mucosa) has been associated with systemic RA related autoantibodies (Smit et al., 2012). Porphyromonas gingivalis, a microbe commonly involved in periodontitis, has the capacity to produce peptidyl arginine deiminase (PAD) enzyme capable of citrullinating human peptides/proteins (Wegner et al., 2010). Mikuls et al. reported that individuals without clinical arthritis, but having autoantibodies against Porphyromonas gingivalis and being RF and/or ACPA sero-positive were at risk of future development of RA if they have a higher background risk for developing RA based on genetics and/or a relevant family history (Mikuls et al., 2012).

An infectious aetiology for RA has been sought. Molecular mimicry of the shared epitope in association with Proteus mirabilis in the gut mucosa, which can cause genitourinary infections, has been proposed by Rashid and colleagues (Rashid et al., 2007). Furthermore, polyarthritis can be triggered by miscellaneous viruses such as cytomegalovirus (CMV), Epstein-Barr and, Parvoviruses B19 (Carty et al., 2003, Stahl et al., 2000). However, the role of molecular mimicry in the pathogenesis of autoimmune diseases is doubtful due to conflicting results reported in different studies (Albert et al., 1999).

A hormonal and reproductive influence involved in disease susceptibility has been proposed due to the higher incidence of RA in women compared with men. The risk of developing RA is reduced during pregnancy, however the opposite is seen during the first 3 months postpartum. Furthermore, the peak incidence of RA in women occurs 10 years before that in man and coincides with the early years following the menopause when oestrogen levels fall considerably. Various studies of the use of oral contraceptive pills (OPC) and breast feeding as risk factors for RA have yielded inconsistent results (Berglin et al., 2010, Karlson et al., 2004, Oliver et al., 2006, Pikwer et al., 2012).

Antibodies

In RA a number of different disease-associated autoantibodies have been identified. Rheumatoid factor (RF) was the first antibody identified that is still in clinical use for diagnosing RA and it is one of the seven criteria for the classification of RA according to the American College of Rheumatology (ACR) 1987 classification criteria (Arnett et al., 1988). RF is reactive with the FC portion of IgG isotype antibodies and can be of the IgA, IgG or IgM isotype. The sensitivity for RF in patients with established RA is 60-80 %, whilst the specificity is 95%. The prevalence rises with advancing age and about 17 % of healthy elderly individuals are RF sero-positive (Palosuo et al., 2003). Moreover, patients with many other autoimmune diseases and various infectious diseases can also be sero-positive for RF. RF lacks disease specificity and cannot alone explain, and be a specific antigen driving, the destructive inflammatory response that characterizes RA (Newkirk, 2002) (Palosuo et al, 2003). This is quite different compared with ACPA, that is suggested to more specifically be involved in the pathogenesis of RA and in the bone destruction process (Kleyer et al., 2014). Nevertheless, RF is still important as a prognostic marker, since the presence of extra-articular manifestations is associated with RF but not with ACPA to the same extent (De Rycke et al., 2004). However, in another report both ACPA and RF were associated with extra-articular manifestations but high RF level seemed to be

more important than ACPA in patients with active extra-articular RA (Turesson et al., 2007a)

During the past decade interest has focused on the immune response to citrullinated proteins as the true autoantigen(s) in RA. In 1998 Schellekens at al. reported that the antigen recognized by anti-perinuclear factor (AFP) antibodies and anti-keratin antibodies (AKA) autoantibodies, already detected during the 60s and 70s of the last century, contained citrullinated peptides that contained arginine residues post-translationally modified by peptidyl arginine deiminase (PAD) (Schellekens et al., 1998, Vossenaar et al., 2003). This initiated the development of the "antibody CCP2 test" using cyclic citrullinated peptides (CCPs) as the test substrates for detecting anti-citrullinated protein/peptide antibodies (ACPAs) (Schellekens et al., 2000). In the healthy physiology, citrullination is a naturally occurring process present in a variety of cells, tissues but also in the inflamed synovium. Citrullination is a post-translational modification of proteins/peptides and it is not specific for RA. Instead, it is the production of autoantibodies against citrullinated proteins and the humoral response to the citrullinated proteins that is thought to be highly specific for RA (Vossenaar et al., 2004b). The citrullinated antigens expressed in the joint are fibrinogen, vimentin, collagen type II and α-enolase (Burkhardt et al., 2005, Masson-Bessiere et al., 2001, Saulot et al., 2002, Vossenaar et al., 2004a). These four citrullinated proteins/peptides are well established candidate antigens that can be found in the inflamed synovium and may form immune complexes with ACPA resulting in a progression of the inflammatory process (Quirke et al., 2011). Therefore, involvement of ACPA may explain why ACPA positive RA patients are reported to have a more erosive disease course and to be in need of more aggressive treatment compared with ACPA sero-negative patients (Seegobin et al., 2014). A higher concentration of ACPA also appears to be associated with a more erosive disease (Berglin et al., 2006).

Furthermore, ACPA have been shown to be present at all stages of the development of RA, i.e., years before disease onset (pre-patients), at the onset of disease (early RA) and in established disease (Rantapaa et al., 2003). The first generation anti-CCP assay (anti-CCP1) was based on citrulline containing peptides derived from the sequence of filaggrin . This test had a diagnostic sensitivity of 68% and a disease specificity of approximately 96-98% (Schellekens et al., 2000). Because filaggrin is not found in the joint, it was concluded that other proteins, not related to filaggrin, may contain epitopes more appropriate for detection of ACPA (van Venrooij et al., 2008).Thus, to improve the diagnostic sensitivity and specificity, a second generation, the anti-CCP2 assay, was developed. The substrates for this test were sera that contained citrullinated peptides

randomly selected from patients with RA and tested for sequences that gave the best discrimination. The development of an anti-CCP2 enzyme-linked immunosorbent assay (ELISA) increased the diagnostic sensitivity to 70-75% with a specificity of 95-99%. The anti-CCP2 test is widely used in the clinical practice worldwide (van Venrooij et al., 2008). As a further development of the anti-CCP2 test, third generation tests were developed, i.e., an anti-CCP3 test for the detection of IgG antibodies, and anti-CCP3.1 that detects the combination of IgG and IgA antibodies. These tests showed essentially the same sensitivity and specificity as the anti-CCP2 test for RA (dos Anjos et al., 2009). Additional tests have subsequently been developed, including a commercial test that detects antigen known as anti- mutated citrullinated vimentin (anti-MCV) (Dejaco et al., 2006). Thus, the presence of ACPA is a useful diagnostic marker in early disease with a sensitivity equivalent to that of RF, 70-75 %, but with a much higher specificity of approximately 98% (van Venrooij et al., 2008). Since joint damage occurs early in the disease course and ACPA is a more specific diagnostic and prognostic marker compared with RF at disease onset, the presence of ACPA has been included in the 2010 RA classification criteria (Aletaha et al., 2010).

These two autoantibodies, i.e., RF and ACPA, are the most frequently measured in current clinical practice. However, assays for other antibodies are under development and another antibody that is generated as a result of post-translational modification of proteins is/are anti-carbamylated protein antibodies (anti-CarP) (Shi et al., 2013).

Criteria for rheumatoid arthritis

In 1958, the first criteria for the classification of RA were published by Ropes et al and were used for more than 30 years (Ropes et al., 1958). In 1987 these criteria were revised by Arnett et al. and published as “The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis (ARA 1987)” (Arnett et al., 1988). The main purpose of these revised criteria was the classification of disease in epidemiological studies and not for the diagnosis of individual cases. Even so, the seven criteria (Table 1) have been useful in clinical practice as guidelines in arriving at a diagnosis of RA and to distinguish established RA from other rheumatic disorders. The problem with the ARA criteria is that it applies to establish RA and has poor sensitivity and specificity for the diagnosis of early-onset RA. They have, however, been widely used in clinical studies of RA patients around the world to ensure homogeneity among the patients included.

Studies undertaken during the past decade have convincingly shown the importance of a quick diagnosis of RA and initiation of treatment with

disease-modifying anti-rheumatic drugs (DMARDs) for the best possible patient outcome in the long run (Finckh et al., 2006). Consequently, new criteria were developed in 2010 with the purpose to identifying patients with early disease (Aletaha et al., 2010). The 2010, American College of Rheumatology (ACR)/the European League Against Rheumatism (EULAR) new criteria for the classification of RA use a scoring system evaluating four categories (joints (0-5), serology (0-3), symptom duration (0-1) and measurement of acute phase reactants (0-1). Thus, these new criteria also include the presence of anti-citrullinated proteins/peptides (ACPAs) and acute-phase reactants, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Patients are diagnosed with a definite RA if they score 6 or more out of 10 possible points (Aletaha et al., 2010). Recent reports show that the 2010 criteria seem to be "superior" compared with the 1987 ARA criteria for classification of patients earlier in the disease course (Arnett et al., 1988). However, better understanding of the implications of these criteria is needed regarding the long-term outcome, such as disability, radiographic damage and mortality (Humphreys et al., 2013, Mjaavatten et al., 2013).The 1987 ARA revised criteria were used in all studies in the present thesis and early RA are defined as being symptomatic for ≤12 months.

Table 1. The 1987 American Rheumatism Association (ARA) classification criteria. A patient is diagnosed with RA if at least 4 of 7 criteria are satisfied and criteria 1-4 must have been present ≥6 weeks (Arnett et al., 1988).

1. Morning stiffness	Stiffness in and around joints lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joints	Simultaneously, soft tissue swelling or fluid of at least 3 joint areas, observed by a physician*
3. Arthritis of hand joints	At least one area swollen in wrist, MCP or PIP joints
4. Symmetric arthritis	Simultaneous involvement of the same joint areas on both sides of the body (bilateral involvement of PIP, MCP or MTP joints is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces or in juxta-articular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Posteroanterior hand and wrist radiographic changes typical for RA, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

*The 14 possible areas are right or left proximal interphalangeal (PIP) joints, metacarpophalangeal (MCP) joints, wrist, elbow, knee, ankle, and metatarsophalangeal (MTP) joints

Measurement of disease activity and severity

Disease activity assessment

Patients with RA should be seen frequently and closely monitored if the disease is active in order to rapidly instigate the appropriate therapy and control the disease. The Disease Activity Score, 28-jount (DAS28) (Prevoo et al., 1995) is a commonly used and valid tool for the assessment of disease activity and response to therapy (van Gestel et al., 1998). The DAS28 value, together with a clinical assessment, can support the clinician in deciding treatment and is frequently used in research for comparison in clinical trials. The DAS28 includes the number of swollen and tender joints, ESR or CRP and the patient's global assessment of their state of health measured by a visual analogous scale (VAS). Clinical remission is defined as DAS28 <2.6, low disease activity DAS28 <3.2, medium DAS28 from 3.2 to 5.1, and high disease activity has a value of DAS28 >5.1. Response to therapy can be estimated according to certain criteria (described fully in the Material and Methods section below; Table 5) (van Gestel et al., 1998).

Assessement of disease severity and functional capacity

The Health Assessment Questionnaire (HAQ) score for functional capacity is a self-reporting questionnaire which makes it possible to follow the patient's disability progression (Ekdahl et al., 1988). A recent study of HAQ progression over 10 years following diagnosis showed that patients with high stable HAQ score had an increased risk for mortality (Norton et al., 2013b). The presence of extra-articular manifestations is associated with a more severe disease course and co-morbidity. High disease activity and a decline in functional ability are contributing factors (Turesson, 2013). Co-morbidity is common in patients with RA and this adds to increased disability and mortality (Gullick et al., 2011); this aspect will be discussed below. Joint damage, assessed as radiographic progression, is another marker of a more severe disease, a worse prognosis and outcome (Aletaha et al., 2006).

Radiology

Plain radiography (X-ray) imaging is routinely used for assessment of joint destruction and determination of disease outcome. In early disease, radiographic changes are not often apparent but if the inflammation remains high, cartilage destruction and joint erosions can be visualized by X-ray. Ultrasound (US) and magnetic resonance imaging (MRI) are more sensitive methods that can provide information during the pre-radiographic stages by visualizing inflammatory changes in the synovia and detect joint erosions (Ostergaard et al., 2005). Bone oedema, which may precede the development of erosions, can also be visualized by MRI (McQueen, 2013). In a recent study examining early RA patients with MRI the authors proposed that early bone oedema was an important predictor of subsequent radiographic progression (Hetland et al., 2009). Nuclear imaging techniques, such as positron emission tomography (PET), are highly sensitive but are not yet used in rheumatology (McQueen, 2013). Dual energy absorptiometry (DXA) of the hands can detect a change in bone mineral density in early disease. However, the DXA analysis is not yet in routine clinical use (Haugeberg et al., 2006).
Plain X-ray imaging of hands and feet can be interpreted by several scoring systems and is often used to assess joint destruction in clinical studies. The modified Larsen method is based on a global score for both erosions and joint space narrowing in which 32 joint areas are assessed (Larsen, 1995). Details will be described further in the “Material and Methods” section. Another scoring system widely used in clinical studies is the modified Sharp score (van der Heijde, 1996).

Extra-articular manifestation

Extra-articular rheumatoid arthritis (Ex-RA) is associated with a poorer outcome, co-morbidity and mortality (Turesson, 2013, Young et al., 2007a). Patients with RA having a more severe disease appear to suffer more frequently from Ex-RA but Ex-RA can also occur in early RA patients (Turesson et al., 1999). The prevalence and incidence varies depending on the study design, and there is no internationally agreed and unifying classification for Ex-RA manifestations (Young et al., 2007). The Malmo criteria include only severe features (pericarditis, pleuritis, Felty’s syndrome, interstitial lung disease, vasculitis related neuropathy, scleritis /episcleritis, retinal vasculitis, glomerulonephritis, systemic vasculitis and severe cutaneous vasculitis) and the presence of nodules is not included (Turesson et al., 2004). Others use a more broad classification addressing

constitutional findings as well as complications of RA (Fleming et al., 1976). The most common extra-articular manifestations are rheumatoid nodules, approximately 30 % of the patients present over time with such nodules. The incidence of rheumatoid vasculitis has been reported to decline during the last decade, probably due to better management of RA (Myasoedova et al., 2011b, Watts et al., 2004). In a retrospective cohort study of serial cross sectional data of US Veterans from 1985-2006 a decrease in severe Ex-RA was observed. For RA associated lung disease there was, however, an increase from19 to 22 cases per 1000 over a 10-year period (Bartels et al., 2010).

Co-morbidity

A co-morbid disease is a concomitant but unrelated disease, usually used to indicate the co-existence of two or more disease processes in the same individual. The presence of co-morbidity in clinical practice can delay diagnosis, influence treatment decisions and prognosis. Each co-morbid disease may have its own effect on the patient's feeling of well-being, and the effect of co-morbidities is generally related to complications and mortality. Co-morbidity can also play a role in different types of studies; act as an important confounder in epidemiologic studies and a powerful predictor of health care outcomes and costs (Gabriel et al., 1999, Gullick et al., 2011, Norton et al., 2013a).

Co-morbidity indexes

There is no consensus regarding the definition of co-morbidity in the current literature and the various indices have limitations. In a report on the validity among co-morbidity indexes, de Groot *et al.* reviewed 13 different methods to measure co-morbidity (de Groot et al., 2003). They concluded the Charlson Comorbidity Index (CCI), Cumulative Illness Rating Scale (CIRS), Index of Coexisting Disease (ICED) and the Kaplan-Feinstein Index to be valid and reliable methods for assessing co-morbidity in clinical research studies (Charlson et al., 1987, Linn et al., 1968, Greenfield et al., 1993, Kaplan et al., 1974). Co-morbidity can be the cause or the consequence of the index disease (*e.g.*, RA) and the index disease can share the same risk factors as the co-morbid condition as presented by de Groot and in Table 2 of Michaud (de Groot et al., 2003, Michaud et al., 2007). The available co-morbidity measurement instruments differ markedly and choosing the most appropriate is difficult (de Groot et al., 2003, Gabriel et al., 1999). Michaud *el al.* claim that co-morbidity indexes have no role in clinical practice but their ability to predict important outcomes in patients with RA makes them

useful for research (Michaud et al., 2007). The primary approach has been to evaluate co-morbidity for the risk of certain outcomes such as mortality or being hospitalized. A number of researchers use a modified version of the Charlson index (described below) to register the presence of co-morbidity conditions (Gullick et al., 2011).

Table 2. The link between RA and a co-morbid illness can be complex as illustrated by Michaud et al., 2007).

Types of comorbidity

Type	Cause	Direction	Example
I	Unrelated to RA or its treatment	Unrelated	Appendicitis
II	Co-morbidity	From CC to RA outcome	Depression and work disability
III	RA consequences	From RA consequence to CC	Decreased functional status to GI ulceration, herpes zoster
IV	RA illness	From RA to CC	MI, lymphoma
V	RA treatment	From RA to CC	Cyclophosphamide and cancer; corticosteroids and infection
VI	Common external factor	From factor to RA; from factor to CC	Smoking and lung cancer

CC, co-morbid condition; GI, gastrointestinal; MI, myocardial infarction; RA, rheumatoid arthritis

Charlson co-morbidity index (CCI)

The CCI index was developed in 1987 to create “a prognostic taxonomy for co-morbid conditions which singly or in combination might alter the risk of short term mortality”. The original study was based on the mortality of 604 patients 1 year after admission to the medical service at New York Hospital during 1 month in 1984, and was initially validated within a cohort of breast cancer patients. The CCI is based on 17 diagnoses (various later versions are available with 17-22 diagnostic categories including age), each weighted by a mortality risk. Thus, the medical conditions are weighted 1-6 with total scores ranging from 0-37. Weights were based on the relative risk of dying, and were used to indicate that not all co-morbid conditions have the same impact on the total co-morbidity burden (Charlson et al., 1987).

Cumulative Illness Rating Scale (CIRS)

The purpose of the original study developed by Linn *et al.* was to estimate the total medical burden of chronic illness (multi-morbidity) and capacity for patients to survive, and was based on in-patient hospital mortality in a Veterans hospital in 1964. In the original version the rating scale consisted of 13, valid, body systems categorized on a five-point (pathophysiological) severity scale (Linn et al., 1968). CIRS Geriatrics (CIRS-G) is a modified version developed for a geriatric population and resembles CIRS (Miller et al., 1992).

Index of Co-existent Disease (ICED)

This index measures the functional outcome (disability) caused by co-morbidity. It is based on two different dimensions; firstly, the estimated disease severity and frequency of 14 co-morbid conditions, and secondly, the severity level of 11 dimensions of physical and mental impairment. The scores are based on a list of symptoms, signs, and laboratory tests (Greenfield et al., 1993).

Kaplan and Feinstein classification of co-morbidity

This method for classifying co-morbidity was developed to evaluate co-morbid diseases in addition to one specific index disease. It makes a distinction between vascular and non-vascular co-morbidity, and was developed especially for the use in diabetes research. Two forms of classification were used, focusing on the type of co-morbidity and the pathophysiological severity of the present co-morbid conditions (Kaplan et al., 1974).

Cardiovascular co-morbidity

Cardiovascular disease (CVD) is the leading cause of mortality worldwide in the general population. The CVD burden has, however, decreased during the past decades. This is due to better care, prevention and treatment of CVD risk factors (McGovern et al., 2001, Rambiharilal Shrivastava et al., 2013). The so called, traditional CVD risk factors include diabetes mellitus (DM), hypertension (HT), hypercholesterolemia, having a smoking history and obesity. Furthermore, increasing age, being male and, in recent years, high levels of high sensitivity C-reactive protein (hsCRP), and physical inactivity are also associated with increased CVD risk in the general population (Ridker et al., 2004, Wilson et al., 1998) although some controversy

regarding the role of CRP exists (Emerging Risk Factors et al., 2010). In patients with RA cardiovascular illnesses are increased both in terms of morbidity (Maradit-Kremers et al., 2005a, Sodergren et al., 2007, Solomon et al., 2003) and premature mortality (Avina-Zubieta et al., 2008, Sokka et al., 2008, Wallberg-Jonsson et al., 1997, Wolfe et al., 1994) compared with the general population. This has been highlighted more clearly during the past decades and clear-cut research has shown that despite modern anti-rheumatic therapy, the cardiovascular mortality remains higher compared with that of the general population (Avina-Zubieta et al., 2008, Sodergren et al., 2007, Symmons et al., 2011, Wallberg-Jonsson et al., 1997). According to previous reports, the traditional risk factors for CVD cannot fully account for this increased risk in patients with inflammatory rheumatic diseases, more specifically in this context RA (del Rincon et al., 2001, Gonzalez et al., 2008, Maradit-Kremers et al., 2005a). For instance, dyslipidaemia in RA patients, manifest with low levels of total cholesterol, LDL cholesterol (LDL), high-density lipoprotein (HDL) and high triglyceride levels, is proposed to be due to the presence of an underlying inflammatory response (Myasoedova et al., 2011a, Svenson et al., 1987). Nevertheless, dyslipidaemia predicts sub-clinical atherosclerosis in RA patients (Jonsson et al., 2001) indicating a complex relationship between lipid levels and inflammation (Toms et al., 2010). In patients with RA a low body weight has been associated with an increased risk for CVD and mortality. This rheumatoid cachexia may reflect the higher degree of inflammation seen among RA patients (Escalante et al., 2005, Kremers et al., 2004). Likewise, a clear link between RA and insulin resistance, most likely reflecting the observation that insulin resistance is associated with inflammation, has been reported (Chung et al., 2008, Dessein et al., 2003). On the other hand, the incidence of diabetes was not found to be increased (Solomon et al., 2004). Tobacco use is associated with susceptibility for and severity of RA and is an independent risk factor for CVD in RA patients (Costenbader et al., 2006, Maradit-Kremers et al., 2005b, Padyukov et al., 2004). In a recent meta-analysis, Boyer *et al.* summarized that, compared with the general population, CVD risk factors such as smoking, diabetes, and low HDL were more frequent among RA patients but there was a significant difference between studies regarding its registered prevalence of these risk factors (Boyer et al., 2011).

Patients with sero-positive RA were reported to suffer CVD events more often compared with sero-negative patients and aging was suggested to have an enhanced effect on the risk for CVD in sero-positive RA patients (Crowson et al., 2013).

One suggested explanation for the accelerated atherosclerosis in RA patients are the shared immunological features of the pathogenesis of rheumatoid

arthritis and atherosclerosis, *i.e.*, a similar inflammatory response in the synovia and the atherosclerotic plaque involving factors such as macrophages, CD4+ T-cells and pro-inflammatory cytokines (Libby, 2006, Sattar et al., 2003).

Current knowledge suggests that it is important to suppress inflammatory activity early in the disease course since the risk for CVD seems to be increased at the onset of disease and continues to increase with disease duration (Chung et al., 2005, Holmqvist et al., 2010, Kerola et al., 2012, Young et al., 2007b).

Other co-morbidities

In addition to an increased risk of CVD, RA is associated with several co-morbidities and complications such as lung disease, infection, malignancy, gastrointestinal disease, and osteoporosis (fracture risk) that may be associated with the underlying disease but also with the immunosuppressive treatment. It can be difficult to distinguish between disease manifestations of RA *per se*, other co-morbidities and iatrogenic effects of the drugs used to treat the disease. The impact of co-morbid conditions can also make it difficult to treat the patient in accordance with established national guidelines.

Pulmonary disorders

Pulmonary diseases have been reported to be common in RA patients and associated with an increased morbidity and mortality (Gabriel et al., 2009). Over time approximately 50% of RA patients may develop some form of respiratory disorder (Gabbay et al., 1997). Airways, pleura and the lung parenchyma can all be affected. The risk factors implicated are older age at disease onset, male gender, smoking and disease severity (Bilgici et al., 2005, Bongartz et al., 2010). In several recent studies, the authors reported that RA patients had an increased risk for chronic obstructive pulmonary disease (COPD) (Bieber et al., 2013, Shen et al., 2014a) and asthma (Shen et al., 2014b) compared with the general population but there are conflicting data, especially regarding asthma (Rudwaleit et al., 2002). Also interstitial lung diseases (ILD) were reported, in association with RA, to comprise a broad spectrum of diseases with a reported prevalence ranging from 19 to 44% (Dawson et al., 2001). In a recent study of patients with early RA, the authors reported a yearly incidence of 4.1/1000 (95% CI 3.0, 5.4) and the 15-year cumulative incidence was 62.9/1000 (95% CI 43.0, 91.7) for RA

associated ILD. Approximately one third had already developed ILD at baseline, *i.e.*, at inclusion into the study (Koduri et al., 2010). Furthermore, many cases of ILD remain undetected or may be mild or asymptomatic although there is a high mortality on symptomatic ILD with a median survival after diagnosis of 3.5 years (Hakala, 1988). The aetiology of RA associated ILD is unknown, however many DMARDs, *e,g.*, methotrexate (Cannon, 1997), infliximab (Bongartz et al., 2006), leuflunomid (Scott, 2004), injectable gold (Hakala., 1988) and sulfazalasine (Ulubas et al., 2004) have been associated with treatment of lung disease and/or ILD. For infliximab there are some reports of rapidly progressive fatal ILD (Chatterjee, 2004).

Infections

Patients with RA have an increased risk of bacterial, tubercular, fungal, opportunistic and viral infection, with the highest risk of infection being in patients with active and severe disease (Doran et al., 2002a). The high mortality risk from infections in RA patients was reported to increase with disease duration, *e.g.*, SMR 11.1 after 18 years and 14.9 after 27 years of follow-up, respectively (Symmons et al., 1998). Doran *et al*. reported RA patients to have an increased risk for many infections compared with a population based control group and suggested the increased risk to be attributable to either the presence of immune dysregulation in the RA itself or complications to the immunosuppressive treatment (Doran et al,. 2002a). In all, 64% of the RA patients had documented infections and 48% had at least one infection that caused hospitalization. Risk factors predicting infections were older age, corticosteroid use, leukopenia, extra-articular disease and other accompanying co-morbidities. The use of DMARDS was not associated with the increased infection risk after adjusting for these factors (Doran et al., 2002b). RA itself was suggested to be a plausible explanation as well as premature ageing of the immune system, the impact of chronic co-morbidity conditions and immunosuppressive therapy (Listing et al., 2013). The authors also pointed out that current knowledge shows an up to-4-fold increased risk for serious infections with long term glucocorticoid (GCs) use, rising in a dose dependent manner, and that tumor necrosis factor (TNF) blockers increase the risk up to 2-fold with the combination increasing the risk additionally. Furthermore, they suggested that one should be cautious and perhaps avoid combination therapy in elderly RA patients with multiple co-morbid conditions who already have an increased risk for infections (Listing et al., 2013). In accordance with current guidelines, tuberculosis (TB) screening is recommended in patients before starting treatment with biological agents. This is because the risk of tuberculosis

reactivation is well established especially in patients treated with anti-TNF-blockers (Dixon et al., 2010). Vaccination, based on age and the risk of preventable diseases such as influenza and pneumonia (especially in the elderly), should be undertaken before starting a regimen of DMARD or biological agent therapy.

Malignancies

Cancer is the second leading cause of death after CVD in RA patients (Gullick et al., 2011). As a result of a meta-analysis the incidence rate of the overall burden of malignancies did not differ from the general population with a standardized incidence ratio (SIR) of 1.05 (95%CI 1.01, 1.09)(Smitten et al., 2008). There was a decreased risk for breast cancer with an SIR of 0.84 (95%CI 0.79, 0.90) and also for colorectal cancer (SIR 0.77 (95%CI 0.65, 0.90)). However, the risk of lung cancer was increased, with an SIR of 1.63 (95%CI 1.43, 1.87), and a two-fold increased risk of malignant lymphomas, with the highest risk for Hodgkin's lymphoma (SIR of 3.29 (95%CI 2.56-4.22) compared with non-Hodgkin's lymphoma (SIR of 1.95 (95%CI 1.70,2.24)(Smitten et al., 2008). Furthermore, malignant lymphomas in RA patients are strongly associated with chronic inflammation (Baecklund et al., 2006).

Gastrointestinal disorders

RA is associated with different gastrointestinal co-morbidities (Ebert et al., 2011). The increased risk of upper or lower gastrointestinal ulceration and bleeding in patients with RA has been suggested in the first place to be associated with the use of NSAIDs (Magnano et al., 2005). Concomitant use of corticosteroids seems to increase the risk for gastrointestinal ulcers (Garcia Rodriguez et al., 2001).

Osteoporosis

Osteoporosis/osteopenia in RA patients depends on many factors and the increased risk has been attributed not only to the severity of RA but also to age, disability, corticosteroid therapy (Haugeberg et al., 2000, van Staa et al., 2006) and disease duration (Kelly et al., 2002). Furthermore, the relative risk for an osteoporotic fracture in RA patients with no recent glucocorticoid (GC) use was reported to be 1.2 (95%CI 1.1, 2.3) and the risk was more than doubled with recent GCs use, even use of low doses (van Staa et al., 2006).

Thyroid disease

In a recent report, hypothyroid disease was significantly associated with CVD in RA patients after adjustment for traditional CV risk factors. However, there was no difference regarding the incidence and prevalence of thyroid disease in RA patients compared with the general population in that study (McCoy et al., 2012). This is in contrast to a study by Raterman et al. who reported an increased risk of hypothyroidism in female RA patients compared with controls (Raterman et al., 2008). Moreover, Bengtsson et al. found that RA patients taking medication with thyroxin had a doubled risk of both ACPA sero-positive and ACPA sero-negative RA. They also showed that the risk of ACPA sero-positive RA was further increased if the patient was a smoker or carried the shared epitope (SE) (Bengtsson et al., 2013)

Studies reporting on co-morbidity

Co-morbidity studies in patients with early RA have been scarce. However, in parallel with the rheumatologists' recognition of co-morbidity being of large importance for the total burden of disease, studies on co-morbidity have become more frequent lately (Table 3).

Table 3. Studies reporting on co-morbidity in patients with RA

Authors/country	Type of study/ number of RA patients/controls	Co-morbidity instrument used	Most common reported co-existing disease at disease onset	Conclusion
Gabriel et al., 1999 USA	Prevalence population based cohort N=450 Controls N=891	CCI and ICED	CHF 17%, MI 14%, chronic pulmonary disease 12%	RA was a predictor of rise in comorbidity over time
Kroot et al., 2001 Netherlands	Inception cohort, longitudinal N=186	None	CVD 29%, respiratory disease 18%, dermatological disease 11%	Approximately 27% of the early RA patients had at least one chronic co-existing at disease onset
Hyrich et al., 2006 UK	Biological cohort N=7818	None	CVD including HT 22% and IHD 6%. Asthma 10%, COPD 5%, DM 5%	Patients treated with biological agents had high levels of baseline comorbidity approximately 58% before the initiation of biologics
Kapetanovic et al., 2010 Sweden	Inception cohort, longitudinal N=183	None	CVD, including HT 16%, malignancy 6%	More than 40 % of the early RA patients had another disease at inclusion with rise over time
Tiippana-Kinnunen et al., 2013 Finland	Inception cohort, longitudinal N=80	CCI_a	At study entery , had 20% at least one comorbidity, most commonly HT	High baseline CCI_a, showed higher disease activity both in early disease and over time
Norton et al., 2013a UK	Inception cohort, longitudinal study N=1460	CCI_a	HT (SIR)=1.61, IHD (SIR)=1.60, stroke in females (SIR)=1.34, COPD in males(SIR)=1.63	Co-morbidity was related to mortality and functional decline
Dougados et al., 2014 France	International cross-sectional N=4586	None	Depression 15%, asthma 6.6%, CVE 6%, solid malignancies 4.5%,COPD 3.5%	RA patients had high prevalence of co-morbidities. There is great variability among countries in prevalence and also regarding compliance with recommendations for preventing and handle these co-morbidities

CCI= Charlson comorbidity index; CCI_a= the age- adjusted CCI, adds an extra point for each decade of age >50 years (Charlson et al., 1987); ICED= Index of Co-existent Disease (Greenfield et al., 1993); CHF=congestive heart failure; MI=myocardial infarction; CVD= cardiovascular disease; HT= hypertension; IHD= ischemic heart disease; COPD= chronic obstructive pulmonary disease; DM= diabetes mellitus; SIR= standardized incidence ratio;

Pharmacological treatment of rheumatoid arthritis

The progress in understanding the pathogenesis in RA has promoted development of new pharmacological treatments and strategies with improved outcomes. The “window of therapeutic opportunity” states that aggressive management in the early stages of disease is recommended in order to delay and/or prevent future morbidity, joint damage and functional disability (Boers, 2003, Furst, 2004). Disease duration at the time of DMARD initiation strongly affects the response to DMARD therapy, i.e., patients with a longer disease duration do not respond to treatment to the same extent as patients with shorter disease duration (Anderson et al., 2000, Lard et al., 2001, O'Dell, 2004, Rantalaiho et al., 2010). Therefore, DMARD therapy should be initiated as soon as possible after diagnosis and preferably within 3 months from disease onset (Gremese et al., 2013, Lard et al., 2001).

In a accordance with current guidelines on treatment of RA (Singh et al., 2012, Smolen et al., 2010, Svensk reumatologisk förening, 2012) the approach is to initiate aggressive therapy promptly after diagnosis and to step up the therapy, monitored by an assessment of disease activity, to achieve clinical remission. However, there is no cure for RA and the patients respond differently despite equal treatment strategies. Traditional DMARDs, and the new biological agents, provide no or only a partial treatment response in some patients. There is a lack of knowledge as to which patient will respond to a particular treatment, who will suffer side effects due to the inserted therapy and for how long the immunosuppressive treatment should be continued. Sustained remission is rarely achieved and requires ongoing pharmacological therapy (Bykerk et al., 2013, Prince et al., 2012, van den Broek et al., 2011). There is a lack of predictors for a treatment response and a” gold standard measure of remission” does not exist. A low disease activity might be reasonable in many patients (Paulus, 2004, Sokka et al., 2009). However, the treatment options available have increased the last two decades with synthetic DMARDs and diverse biological agents. The best chance to achieve remission is to treat the patient in a “tight control” way, i.e., frequently controlling the clinical response and modifying the treatment by adding or switching therapy if the treatment is deemed insufficient (Leirisalo-Repo, 2013).

Non-steroidal Anti-inflammatory Drugs (NSAIDs)

These drugs are used for symptomatic control of inflammation, stiffness and pain by inhibiting the synthesis of pro-inflammatory prostaglandins. There is no evidence that they alter the natural history of the disease but they are often used early in the disease course. Because of undesirable adverse effects such as an associated gastropathy, renal, liver toxicity and an increased risk of cardiovascular side effects, monitoring for possible toxicity in clinical practice is important, especially in elderly patients (Gullick et al., 2011). The selective cyclooxygenase 2 (COX2) inhibitors that remain on the market are used with caution after rofecoxibe was withdrawn in 2004 because of the cardiovascular side effects reported in a large randomized study (Coxib et al., 2013).

Glucocorticoids (GCs)

Oral GC are often used as bridging treatment in combination with DMARDs during the early stage of the disease course because of the rapid relief from inflammation and symptoms pending the DMARDs effect. In order to control of disease flares, shorter courses of 20-15 mg/day tapering to 5 mg/day over 7-5 days may also be effective. Also treatment of monoarticular synovitis with intra-articular GCs injections is very effective (Hetland et al., 2010). GCs have been reported in several studies to have positive effects with respect to the remission rate and inhibition of radiographic progression (Gorter et al., 2010, Hafstrom et al., 2009, Kirwan et al., 2007) but contradictory results have also been reported (Paulus et al., 2000). For RA patients with severe extra-articular manifestations, such as interstitial lung disease, systemic vasculitis, *etc*., the usage of high intravenous doses of GC is invaluable (Turesson, 2013). However, treatment with GC is controversial and long-term use should be avoided with respect to the increased risk of adverse effects such as infections, osteoporosis, diabetes and hypertension, *etc*. (Hoes et al., 2007, Morrison et al., 2006). Nevertheless, many rheumatologists use low-dose GCs as a routine regime both in early and established RA, and bone protection therapy for osteopenia is recommended in accordance with guidelines (Singh et al., 2012, Smolen et al., 2010, Svensk reumatologisk förening, 2012).

Treatment with Disease modifying anti rheumatic drugs (DMARDs)

The most common DMARD used in clinical practice is methotrexate (MTX), it being well tolerated and effective. Methotrexate should be regarded as the anchor DMARD and can be used in mono or combination therapy with almost all available DMARDs and biological agents (Combe, 2007, Scott et al., 2010). Folic acid substitution reduces the risk of known side effects of MTX (Prey et al., 2009). The use of DMARDs gives relief from symptoms and prevents joint damage. Other DMARDs options for treatment of RA include sulfasalazine (SSZ), hydroxychloroquine (HCQ), leflunomide, cyclosporine, sodium aurothiomalate, auronfine, azathioprine, mykofenolatmofetil and cyclophosphamide, the latter as a treatment of extra-articular RA. A commonly used combination therapy in patients with sustained disease activity is the double or triple-DMARD combination of MTX-SSZ-HAQ, shown to be more effective than MTX alone (O'Dell et al., 2002). There are also many other possible combination strategies available today.

Biological agents

Several biological agents are available for treatment of RA when synthetic DMARD(s) have failed. An improved understanding of the pathogenesis in RA has led to more specific treatment strategies that intervene directly with the disease process. Currently there are five tumor necrosis factor (TNF) blockers; (adalimumab, certolizumab, etanercept, golimumab and infliximab) and three other biologics available, *i.e.*, abatacept with costimulation blockade, rituximab that modifies B-cell activity, and tocilizumab a monoclonal antibody against the interleukin 6 (IL-6) receptor. In the case of inadequate efficacy of MTX treatment, the addition of a TNF inhibitor is recommended and has shown good clinical efficacy (Gartlehner et al., 2006). TNF blocking agents slow the development of joint damage, especially if use of the TNF inhibitor is combined with methotrexate (Klareskog et al., 2004). A meta-analysis of X-ray progression in clinical trials comprising various DMARDs, steroids and biological reagents reported less X-ray detectable destructive progression in patients treated with DMARD combinations, DMARDs in combination with GCs or DMARD in combination with biological agents compared with treatment with a DMARDs as a monotherapy (Graudal et al., 2010).

Aim of this thesis

RA is a systemic inflammatory disease and the inflammation *per se* contributes to joint destruction which in turn leads to loss of function and disability. The disease is associated with an increased morbidity and mortality compared with the general population. Cardiovascular disease (CVD) is the main cause but also other co-morbid conditions contribute to a shorter life expectancy. Early DMARD therapy can suppress disease activity and improve long term outcomes. Identification of prognostic markers early in the disease course that could guide the clinician to optimal treatment decisions are desirable.

Aspects that I have focused on in patients with early RA are:

- To evaluate the diagnostic and predictive value of different anti-citrullinated peptide/protein antibodies (ACPAs), in relation to genetic markers, as predictors of disease outcomes in a two years prospective study

- To evaluate the importance of disease related and traditional CVD risk factors prospectively for the development of CVD during the first five years

- To evaluate prospectively the impact of age at disease onset on prognostic risk factors and pharmacological treatment in patients followed for five years

- To evaluate the prevalence of other co-morbid conditions and the impact of inflammation on new co-morbidity during the first five years of disease

Materials and methods

In this thesis (Papers I-IV) patients diagnosed with early RA (*i.e.*, symptomatic for ≤12 months), and fulfilling the American Rheumatism Association classification criteria for RA (Arnett et al., 1988) were, since December 1995, consecutively included in a large survey into the progress of RA and the development of co-morbidity. All studies are prospective and observational. The Regional Ethics Committee approved the studies and the participants gave their written informed consent.

In the first study (Paper I, Table 1) 210 patients with early RA (145 female and 65 male) attending the Department of Rheumatology, University Hospital, Umeå were consecutively included and followed regularly for two years. Control subjects were randomly selected self- stated healthy individuals from the same geographical area and with the same ethnic background as the patients (figure 1, Table 4).

In Papers II-IV, (Table 4) the studies included patients with early RA from all the four northern-most counties of Sweden.

Figure 1. Geographic distribution of the study cohort

Table 4. Demographic data in early RA patients included in the different papers. N (%) or mean (SD).

	Paper I	Paper II	Paper III	Paper IV
All, RA patients (n)	210	700	YORA 475, LORA 475	950
Sex / female (%)	69	68.7	YORA (75.2), LORA (61.5)	68.3
Mean age at disease onset (years; range)	54 (18-84)	55.2 (18-89)	YORA <58, LORA ≥58 (18-89)	55.6 (18-89)
Mean duration of symptoms at diagnosis (months)	6.4 (3.2)	6.6 (3.3)	YORA 6.9 (3.4), LORA 6.5 (3.2)	6.7 (3.3)
Inclusion period	December 1995- June 2005	December 1995 -April 2008	December 1995 - February 2011	December 1995- February 2011

YORA (young onset RA), LORA (late onset RA)

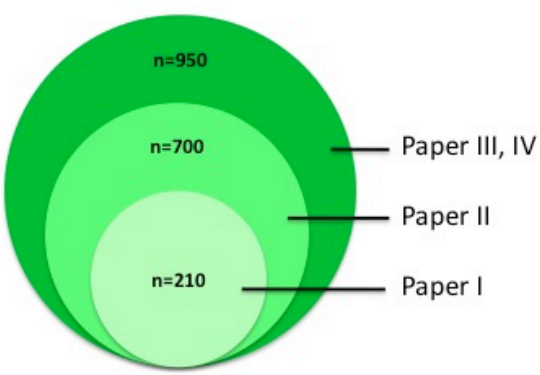


Figure 2. Number of patients included in Paper I-IV

Study populations

The presented studies comprised, by reference to the nation-wide Swedish Early RA Register, (Register, 1995) all eligible patients (Figure 1, Table 4) from Västerbotten county (Paper I) and from the four northern-most counties (Norrbotten, Västerbotten, Västernorrland and Jämtland) of Sweden (Papers II-IV) diagnosed with early RA (i.e., symptomatic for ≤12 months), and fulfilling the American Rheumatism Association classification criteria (Arnett et al., 1988). These patients have, since December 1995, been consecutively included in this large observational study on the progression of RA including complications and co-morbidities (Figure 2, Table 4).

In Paper II, by April 2008, 700 patients (481 women, 219 men) were registered with early RA. Of these, 442 patients had reached a five year follow-up time point, i.e., they had suffered their disease for more than five years.

By February 2011, 950 patients (649 women, 301 men) were registered with incident early RA (Paper III). Of these, 665 patients had been followed for five years. In Paper III, the original cohort was divided into two groups based on the median age at disease onset, i.e., young onset RA (YORA <58 years) and late onset RA (LORA ≥58 years).

In Paper IV, the same cohort of early RA patients as in Paper III (n=950) were included and of these, 726 patients had been ill for five years or more by November 2012.

Information obtained and registered for all patients

The following parameters were recorded at baseline and after 6, 12, 18, 24, 36, and 60 months: the 28-joint count of tender (TJC) and swollen joints (SJC); a visual analogous scale (VAS) for pain and patient's global assessment; completion of a Health Assessment Questionnaire (HAQ) (Ekdahl et al., 1988) and inflammatory markers (i.e., erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)). Disease activity score (DAS28) (Prevoo et al., 1995) was calculated (Papers I-IV).

Lipid levels (total cholesterol (mmol/L), high-density lipoprotein (HDL, mmol/L) and triglycerides (mmol/L)) were analysed in the majority of cases at baseline, otherwise as soon as possible during the follow-up period (Papers II-IV).

All patient records were carefully read and data collected, according to a study protocol, both at inclusion (T0) and after five years (T5). Additionally, the patients in Papers II-IV completed a self-reported questionnaire on co-morbidity at inclusion and five years after they had been diagnosed, to further increase the validity of the collected data. Recorded variables were: all co-morbidity conditions, i.e., having suffered a previous CVE (myocardial infarction (MI)/coronary artery bypass grafting (CABG), stroke/transient ischemic attack (TIA)/deep vein thrombosis (DVT)/pulmonary embolism (PE), and ruptured aortic aneurysm), and/or the first new CVE occurring during the follow-up period. Myocardial infarction was recorded when the diagnosis had been made according to the World Health Organization (WHO) criteria (World Health Organization task force on standardization of clinical nomenclature, 1979). A cerebrovascular lesion was recorded when an intra-cerebral haemorrhage or cerebral infarction had been diagnosed following either computerized tomography or magnetic resonance imaging, or when a typical clinical profile of neurological deficits persisted for more than 24 h. A TIA was recorded in cases when the focal neurological deficit of presumed ischaemic origin had persisted for less than 24 h. Deep vein thrombosis/pulmonary embolism was recorded when the diagnosis had been verified objectively (by phlebography, sonography, scintigraphy, and/or arteriography), or when the clinical signs combined with pulmonary radiography, electrocardiography, and laboratory changes resulted in full time warfarin treatment. The information regarding fatal cardiovascular events was obtained from the National Board of Health and Welfare.

Traditional CV risk factors (ongoing treatment for hypertension (HT) and current blood pressure, diabetes mellitus (DM), smoking (current and previous), body mass index (BMI)), rheumatoid nodules and extra-articular disease (Ex-RA) were registered (Turesson et al., 2004).

Cumulated pharmacological treatment was registered regarding corticosteroids and disease modifying anti-rheumatic drugs (DMARDs, i.e., methotrexate, sulfasalazine, chloroquine, azathioprine, mycophenolatmophetil, myocrisine, auranofin, cyclosporine, leflunomid, alkylating cytotoxic agents) including biological agents (etanercept, adalimumab, infliximab, anakinra, rituximab) at inclusion and at the defined follow-up time-points. Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), before inclusion and any period during the five year follow-up period was registered as "yes" or "no". Treatment with statins was registered as “yes” or “no”. The time for treatment with selective cyclo-oxygenase-2 (COX-2) inhibitors was registered as exact as possible.

Measures of disease activity and outcome

Erythrocyte sedimentation rate (ESR, mm/h), and blood levels of C-reactive protein (CRP, mg/l) were measured using routine chemical pathology methods. A clinical examination was performed and the Disease Activity Score (DAS28) (Prevoo et al., 1995) was calculated from ESR, tender and swollen joint count and the global assessment on the VAS at baseline and after 6, 12, 18, and 24 months. Pharmacological treatment was registered, at baseline and after 5 years (Papers I-IV).

The therapy response at 6 and 12 months (Paper I) and after 24 months (paper I and III) was calculated according to the European League Against Rheumatism (EULAR) response criteria using DAS28, defining "good", "moderate" or "none" response to therapy (van Gestel et al., 1998).

Table 5 . Response criteria calculated in accordance with EULAR guidelines

DAS28	DAS28 reduction from baseline		
At the last observation	>1.2	>0.6 ≤1.2	≤0.6
≤ 3.2	Good	Moderate	None
>3.2 ≤ 5.1	Moderate	Moderate	None
>5.1	Moderate	None	None

Radiographs of the wrists, hands and feet were performed in an antero-posterior projection and the results used in Papers I, III and IV, at baseline and after 24 months. The radiographs were evaluated according to the Larsen score (Larsen, 1995) by two specially trained rheumatologists. The assessments using the Larsen score included 32 joint areas: metacarpophalangeal (MCP) II-V (n=8), proximal interphalangeal (PIP) II-V (n=8), 4 areas in each wrist (n=8) and metatarsophalangeal (MTP) II-V (n=8). Each area was graded from 0-5 based on the degree of destruction. Thus, using this grading the maximum score is 160. Radiological progression was defined as the increase in Larsen score from baseline until 2 years greater than the median value. In Paper III, the presence of erosions at baseline and after 2 years was also registered.

Analysis of autoantibodies

In Paper I, anti-CCP-2 antibodies (ACPA) were analyzed in patients and controls using the commercially available Diastat kit (Axis-Shield Diagnostics, Dundee, UK) with a cut-off for positivity of 5 U/mL according to the manufacturer's instruction. In Paper I, anti-CCP3 antibodies were analyzed using an ELISA for IgG (3rd generation antigen; Inova Diagnostics, San Diego, CA, USA) and an ELISA for anti-CCP3.1 antibodies (Quanta LiteTM, Inova) detecting both antibodies of IgG and IgA isotype (cutoff value 20 units/ml both tests) was used. In Paper I, Anti-MCV antibodies were measured using the Orgentec 548 Anti-MCV ELISA (Orgentec Diagnostika GmbH, Mainz, Germany; cutoff value 20 units/ml) as recommended by the manufacturer.

In Papers II-IV, ACPA were measured using the anti-CCP2 enzyme-linked immunoassays from Euro-Diagnostica kit with the cut-off value of 25 units/ml defining sero-positivity.

In Paper I, rheumatoid factor (RF) of the IgM isotype was measured in serum samples using the Waaler-Rose hemagglutination test with sensitized sheep red blood cells.

In Papers II-IV, RF and anti-nuclear antibodies (ANA), were detected at baseline using the routine chemical pathology methods in current use at each of the participating hospitals.

Genotyping

In Papers I-IV, the detection of Shared epitope (SE) alleles, *i.e.*, HLA-DRB1 genotyping, was performed using polymerase chain reaction sequence-specific primers from a DR low-resolution kit and a DRB1*04 sub-typing kit from Dynal, (Oslo, Norway), and Olerup SSP AB (Saltsjöbaden, Sweden), respectively.The HLA shared epitope (SE) was defined as HLA-DRB1*0401/0404/0405/0408.

PTPN22 1858C/T polymorphisms were determined using the 5'-nuclease assay. Detection of the different genotypes was made using an ABI 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA, USA) using primers and probes designed by Applied Biosystems. Data was processed using SDS 2.1 software.

Co-morbidity data

In Paper IV, co-morbidity data were registered according to Charlson's classification index. The co-morbidity groups that were of interest in Paper IV are presented in Table 6. Emphasis was put on pulmonary and endocrine disorders, and in particular thyroid disease.

Table 6. Registered main co-morbidity groups according to Charlson et al., 1987.

Myocardial	Renal
Vascular	Liver
Pulmonary	Gastrointestinal
Neurologic	Cancer/immune
Endocrine	Miscellaneous

Statistics

Descriptive data collected at baseline and over time are presented as mean (standard deviation: SD) and proportions are presented as a percentage of available data. Chi-square tests were used for testing categorical data between groups. Differences in continuous data between two groups were analyzed using the independent Student´s t-test and data from two different time points for the same individual with a paired Student´s t-test. In all papers, p-values were 2-sided, and p-values ≤ 0.05 were considered statistically significant in the final models.

In Paper I, variations over time between groups were assessed by analysis of variance for repeated measurements. Multiple regression analyses were performed using the ANOVA general linear model. Backward logistic regression analyses were used to estimate the odds ratio for radiological progression at two years. Variables were chosen with respect to results of simple regression analyses and/or clinical assumptions. The degree of explanation of variations in the dependent variable given by the independent variables was expressed as Nagelkerke R2 or R-square. Area under the curve (AUC) at the 24-month time-point was calculated for all clinical and laboratory variables. Receiver-operating characteristic (ROC) curves were

constructed for each ACPA and kappa values were calculated for concordance between tests.

In order to evaluate the total burden of disease activity over time in Papers I-IV, cumulated disease activity measured by DAS28, was calculated according to the trapezoid model (Matthews et al., 1990) referred to as the area under the curve (AUC) of DAS28, at 6, 12 and 24 months after inclusion. When RA register data were missing, the last value was used to impute data once for each parameter assessed up to 24 months.

In Paper II, Cox proportional hazards simple regression models with fixed (time-independent) covariates were used to identify covariates associated with the first new CVE in the group of patients that were followed-up for five years following diagnosis (n = 442). For time dependent covariates, Cox extended models were also used with time-varying variables in simple regression models and in combined regression models with both fixed and time-dependent variables. Covariates reflecting disease activity, traditional risk factors for CVD and pharmacological treatment were considered in multiple regression modeling, based on clinical experience, previous studies or statistical significance ($P < 0.2$) in simple Cox models, and tested in a few appropriate combined models.

To evaluate the association between different prognostic risk factors and the outcomes, in Papers III-IV, simple regression analysis was used and furthermore the variables chosen for multiple regression models were based on clinical assumptions and with respect to results of simple regression analyses.

In Paper III, data analyses were based on stratification of the patients according to the median age (58 years) at disease onset as young onset RA (YORA, *i.e*, <58 years) and late onset RA (LORA ≥58 years). Multiple logistic regression analyses were first used to evaluate the impact of known prognostic factors at baseline, on a chosen treatment, *i.e.*, early DMARD treatment and corticosteroid treatment, as dependent variables. The impact of a chosen treatment, adjusted for prognostic risk factors, on disease outcome, *i.e.*, radiological progression as the dependent variable was tested.

In Paper I, calculations were performed using SPSS for Windows (v. 11.5; SPSS, Chicago, IL, USA) and StatView v. 4.51; Abacus Concepts, Berkeley, CA, USA. In Papers II-IV, all calculations were performed using the IBM SPSS Statistics 18.0-21.0 program (SPSS, Chicago, IL, USA).

Results and discussion

Paper I

Antibodies against mutated citrullinated vimentin are a better predictor of disease activity in patients with early rheumatoid arthritis at 24 months than antibodies against cyclic citrullinated peptides

In this prospective 2 year study the diagnostic and predictive value of four different ACPAs, anti-mutated citrullinated vimentin (anti-MCV), anti-CCP2, anti-CCP3 and anti-CCP3.1 were evaluated, the latter test being a combined test for antibodies of the IgG and IgA isotype. Furthermore, the relation of the various ACPAs with genetic markers for disease progression, in this context PTPN22 1858C/T polymorphism and HLA-DRB1 shared epitope (HLA-SE), was evaluated.

The results of the ROC curve analyses for the various ACPAs are presented in Table 7, the. Anti-CCP3.1 and anti-CCP2 had the highest sensitivities, i.e., 80.5% and 80.4% with specificities of 95.1% and 98.0%, respectively. The 95% CIs for all ACPAs were overlapping. The likelihood ratios (LR) were highest for anti-CCP2 and anti-CCP3, being 41.0 and 40.3 respectively, when calculations at a fixed specificity of 98% for the various ACPAs were performed. The sensitivity was lowest for anti-MCV antibodies at both specificity levels (Table 7).

Table 7. Sensitivity, specificity and positive likelihood ratios (LR) calculated using ROC curves at a given specificity of 98 % for all tests.

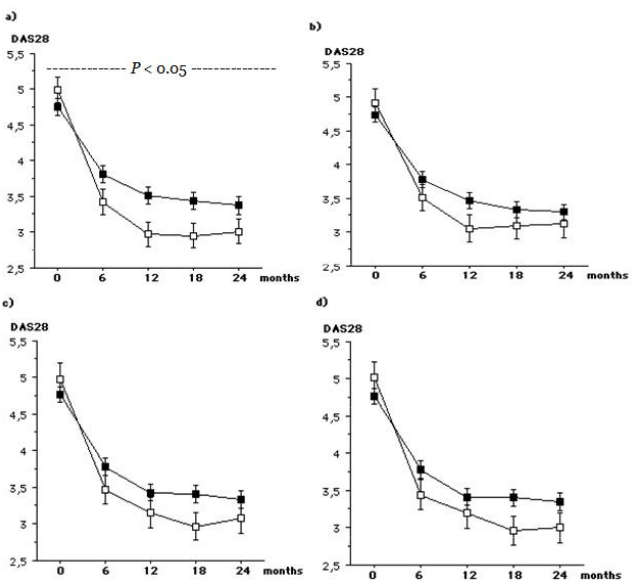
ACPA	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity at 98% specificity (95% CI)	LR*
Anti-CCP2	80.4 (74.3-85.6)	98.0 (93.7-99.7)	80.4 (74.3-85.6)	41.0 (10.3-162.1)
Anti-MCV	74.0 (67.5-79.8)	96.1 (90.8-98.7)	69.0 (62.3-75.1)	35.2 (8.9-139.2)
Anti-CCP3	79.0 (72.9-84.2)	98.0 (93.6-99.7)	79.0 (72.4-84.2)	40.3 (10.2-159.2)
Anti-CCP3.1	80.5 (74.5-85.6)	95.1 (84.4-98.2)	78.5 (72.4-83.8)	40.0 (10.1-158.2)

*n=192, calculated with a specificity of 98%

There was almost 90% correlation between the various ACPAs. All ACPAs except anti-CCP3.1 were significantly ($p<0.05$) associated with HLA-SE. However, there was no relationship between PTPN22 1858T and the various ACPAs in this study.

Regarding disease activity associated with the various ACPAs, there was a significant reduction in DAS28 from baseline, *i.e.*, at the time of a diagnosis of RA, up to 24 months from a mean (SD) of 4.8 (0.9) to 3.3 (1.0) ($p<0.0001$). Patients with anti-MCV antibodies had significantly less reduction in their DAS28 score compared with those without anti-MCV antibodies (ANOVA; F-value=3.391, $p<0.01$), and also the AUC for DAS28 was significantly higher for anti-MCV antibody sero-positive patients compared with sero-negative patients ($p<0.05$) (Figure 4). There were no significant differences among the other ACPAs, or for RF, compared with their sero-negative counterparts. Furthermore, anti-MCV antibody positive patients had a significantly less reduction in the number of swollen joint counts over time compared with patients sero-negative for anti-MCV antibodies ($p<0.05$) and AUC for ESR and CRP were also significantly higher. These differences were not detected for any other antibody in the present study.

Figure 4. DAS28 score in patients sero-positive, compared with sero-negative, for the different ACPA, anti-MCV (a), anti-CCP3 (b), anti-CCP2 (c), and anti-CCP3.1 (d), from baseline to 24 months. Closed squares represent ACPA sero-positive patients and open squares ACPA sero-negative patients



Patients positive for any of the different ACPAs were associated with a greater radiographic damage measured as the Larsen score after 24 months, whilst this outcome was not observed for RF sero-positive patients when compared with those without RF. However, in a multiple regression analysis factors that were significant for radiological progression at 24 months were baseline values for the Larsen score, the swollen joint count, and positivity for all four ACPAs and RF. Patients showing a radiological progression after 24 months had significantly higher concentrations of anti-MCV, anti-CCP3, and anti-CCP3.1 at baseline compared with those without such disease progression (median levels 205 and 66 U/ml, 305 and 222 U/ml, 326 and 219 U/ml, respectively). Those patients sero-positive for any of the 4 ACPAs more often had a significant radiological progression in comparison to anti-body negative patients. Using a backward stepwise logistic regression analysis including all the various ACPAs, RF, swollen joint count, ESR, Larsen score, HLA-SE, carriage of PTPN22 1858 T variant at baseline, and a positive therapeutic response at 6, 12, and 24 months, showed that a positive test for ACPAs, and RF and ESR at baseline were significantly associated with radiological progression after 24 months ($p<0.05$). A therapeutic response alone at 6, 12 and 24 month was associated with less radiological progression ($p<0.05$).

The level of any of the ACPAs at baseline was almost identical in patients already receiving DMARDs (n=43) or prednisolone (n=58) before baseline, compared with that in DMARD-naïve patients. When the data was stratified for response to therapy as “non-responders” versus “good-moderate responders”, it was found that anti-MCV positive patients with a therapeutic response at 6 months ($p<0.05$) had, at 24 months, a significant reduction in their antibody level. There were no significant differences in levels among the other ACPAs, regardless of any therapeutic response.

The main results of this aspect of the study were: that patients with early RA and sero-positive for MCV antibodies had, compared with anti-MCV seronegative patients, more persistent inflammatory activity as measured by the DAS28, ESR, CRP, and swollen joint count (SJC) up to 24 months after diagnosis compared with the results of other ACPAs and RF. This is consistent with previous findings showing that the presence of anti-MCV antibodies at disease onset was associated with persistent disease activity as shown by less reduction of DAS28 score and SJC over time (Bang et al., 2007). In contrast, other groups reported no association between anti-MCV positivity and disease activity, measured as the DAS28, compared with other ACPAs (Ursum et al., 2008).

In line with several other studies all four ACPAs had basically the same accuracy when assessed as being diagnostic markers (Coenen et al., 2007, Dejaco et al., 2006, Raza et al., 2010). Thus, it can be concluded that ACPA positivity predicts a radiological progression better than RF sero-positivity this conclusion is consistent with several previous studies (Nishimura et al., 2007). The results of another study by Mathsson and co-workers study suggested anti-MCV antibodies to be a better prognostic marker for future radiographic changes (Mathsson et al., 2008). This is consistent with a study showing anti-Sa antibodies to be associated with a more severe disease course, as measured by HAQ and/or X-ray compared with anti-CCP antibodies and RF (Boire et al., 2005).

The genetic markers studied, *i.e.*, PTPN22 1858T and HLA-SE, did not reveal any significant differences between the various ACPAs concerning an increased risk for radiological progression or greater disease activity. Published data has resulted in conflicting results in the matter of associations with genetic markers and disease activity/severity. In two large studies, neither PTPN22 1858T nor HLA-SE were associated with disease severity in accord with the results of the present study (Harrison et al., 2006, van der Helm-van Mil et al., 2006). In contrast, others have reported an association between carriage of HLA-SE and a more severe disease course (Gorman et al., 2002, Wagner et al., 1997, van Zeben et al., 1991) and another study reported PTPN22 1858T to be associated with radiological damage (Lie et al., 2007). Overall, in the present cohort of patients, the carriage of HLA-SE was approximately 58% and of PTPN22 1858T 33% with no gender difference. Conversely a study from Germany showed that male patients with RA carried the PTPN22 1858T variant more often than females, additionally there was an association with HLA-SE, suggesting a more genetically homogenous disease in men (Pierer et al., 2006). The discrepancy between studies may be due to clinical heterogeneity, different ethnicity and sample sizes.

In paper III, data analyses were based on stratification of the patients according to the median age at the onset of disease as “young onset RA” (YORA, *i.e.*, <58 years) and “late onset RA” (LORA ≥58 years). The YORA patients were more frequently carriers of PTPN22 T-variant compared with LORA patients; this is consistent with another study which also found a younger age at disease onset to be associated with the presence of PTPN22 (Steer et al., 2005). Furthermore, these results confirm a previous finding, that the combination of PTPN22 1858T variant and the presence of ACPA are independently associated with the onset of RA at an earlier age (Kokkonen et al., 2007).

In papers II and IV, the presence of autoantibodies and genetic markers had no impact on the incidence of CVE nor on comorbidity overall. However, a recent study reported anti-MCV antibody to be associated with severe Ex-RA (Turesson et al., 2013).

Paper II

Cardiovascular events in early RA are a result of the inflammatory burden and traditional risk factors: a five year prospective study

This five year observational study from disease onset aimed to investigate the presence of traditional and disease related risk factors CVD and their predictive effect on development of CVD, as measured by the first cardiovascular event (CVE) during the five year follow-up period, and finally, to assess the potential modulating effect(s) of the prescribed pharmacological treatment

By April 2008, inclusion data for 700 patients with RA (481 women and 219 men) had been compiled and follow-up data for the 442 patients who had been symptomatic for more than five years. The mean age (SD) of the cohort at disease onset was 53.7 (14.8) years for women and 58.6 (12.6) years for men. The mean duration of disease at inclusion, *i.e.*, from the first reported symptom, was 6.6 (3.3) months. In all 76.4% were positive for RF and 67.8% for ACPA, 56.9% had HLA-SE (defined as 0101/0401/0404/0405/0408) and 34.0% carried the PTPN22 T variant. Among those patients followed for five years, 21 (3.0%) had experienced an extra-articular manifestation and 78 (20.7%) had acquired rheumatoid nodules. Forty-eight patients (27 male, 21 female; *i.e.*, 10.9%) experienced a new CVE; 15 MI, 4 coronary artery bypass grafting (CABG), 23 stroke/ transient ischemic attack (TIA), 5 deep vein thrombosis (DVT)/ pulmonary embolism (PE) and 1 ruptured aortic aneurysm. Of these 48 patients, 15 had suffered a CVE prior to inclusion in the present study. For 12 of the 48 cases the CVE proved fatal. Overall 23 of the 442 patients died from various reasons during the follow-up period.

The presence of traditional CV risk factors, inflammatory variables and data on pharmacological treatment at baseline and after 5 years are presented in (Table 8). The number of patients treated for hypertension increased significantly during that period, as did the number of patients acquiring diabetes, compared with baseline. Systolic and diastolic blood pressure decreased, fewer patients were smokers, and BMI decreased over the 5 years. All variables reflecting disease activity (ESR, CRP and DAS28), and the SJC, HAQ, VAS pain and VAS global decreased significantly compared with

baseline values (Table 8). Regarding pharmacological treatment, 393 patients (88.9%) were prescribed DMARDs within the first 3 months following inclusion into the study. The mean time (SD) between the first symptoms of disease and DMARD treatment was initiated was 7.0 (0.3) months, and the mean duration of DMARDs treatment during the five-year period was 51 (16.4) months. Among the patients reaching the 5 year follow-up point, 429 (96.8%) had been treated with DMARDs ; 361 (81.5%) had received methotrexate, and 62 (14.2%) had been treated with biologics. Furthermore, 357 (82.4%) had been treated with NSAIDs during the follow-up period, 112 (25.7%) with COX-2 inhibitors and 367 (72,7%) had had corticosteroids.

Table 8. Variables reflecting traditional cardiovascular risk factors and disease activity in patients with early RA at baseline (T0) and after 5 years of disease (T5). Data is presented as mean (SD) or n(%).

Variables	T0 (N=700)	T5 (n=442)	
Hypertension, n(%)	170(24.5)	164(37,4)***	
BP syst, mm Hg	144.1 (22.6)	141.2(21.8)**	
BP diast, mm Hg	82.7 (10.3)	81.0 (9.6)**	
Diabetes mellitus n(%)	48(7.1)	41 (9.5)**	
BMI	26.3 (4.5)	25.8 (4.3)*	
Smoking, present, n(%)	196 (29.8)	92 (22.4)***	
Smoking, ever, n(%)		451 (69.5)	-
S-Cholesterol, mmolL^{-1}	5.6(1.1)	na	
S-HDL, mmolL^{-1}	1.5 (0.7)	na	
S-Triglycerides, mmolL^{-1}	1.5(1.7)	na	
Statin treatment, n (%)	54 (8.1)	71 (16.4)***	
Previous CVE, n (%)		72 (10.4)	-
ESR (mm)	31.5(23.7)	20.0(19.9)***	
CRP (mgL^{-1})	2.0(24.6)	11.1 (14.3)***	
DAS28[1]	4.8(1.4)	3.2(1.3)***	
HAQ[1]	0.88(0.61)	0.55(0.52)***	
Tender joints[1]	6.7(5.8)	2.6(3.7)***	
Swollen joint count[1]	7.4 (5.2)	3.2(4.1)***	
VAS pain (mm)[1]	44.5(25.2)	28.7(20.7)***	
VAS global (mm)[1]	45.3(24.9)	29.8(20.6)***	
AUC DAS28 (6 mo)[1,2]	-	25.8(7.1)	
AUC DAS28 (12 mo)[1,2]	-	47.2(13.5)	
AUC DAS29 (24 mo)[1,2]	-	87.5 (26.0)	

***p<0.001, **p<0.01; paired t-test, T5 vs T0; na=not analysed
[1] Regularly collected data from the RA registry for 314 patients.
[2]AUC for DAS28 6, 12 and 24 months after inclusion.
[3]Criteria according to (Turesson et al., 2004)

Cox proportional hazard regression models were used to evaluate potential predictors for a new CVE (Table 9). A new CVE was associated with a higher cumulative disease activity, progression of extra-articular disease, a greater age at disease onset, being male, having suffered a previous CVE and with

traditional cardiovascular risk factors such as diabetes mellitus, treated hypertension and higher levels of triglycerides. Furthermore, a shorter duration of treatment with DMARDs during the five year period, treatment with GCs before inclusion and during follow-up, and treatment with COX-2 inhibitors before CVE predicted a new CVE. Treatment with DMARDs within 3 months from inclusion was protective, as was treatment with DMARDs before a CVE.

Table 9. Co-variates for the first new cardiovascular event during the first 5 years after onset of RA in 442 patients. The results of Simple Cox proportional hazards regression models with fixed and time-dependent co-variates.

Covariates	HR	CI 95%	p-value
Sex f/m	0.314/f	0.177, 0.557	<0.001
Age at onset of RA	1.060/year	1.035, 1.086	<0.001
Diabetes mellitus	2.893/+	1.297, 6.452	<0.01
Hypertension, treated	4.066/+	2.308, 7.162	<0.001
BP, syst	1.015/mmHg	1.003, 1.026	<0.05
BP, diast	1.033/mmHg	1.003, 1.063	<0.05
S-HDL-chol	0.318/mmolL^{-1}	0.090, 1.123	=0.075
S-Triglycerides	1.919/mmolL^{-1}	1.461, 2.521	<0.001
Statin treatment	2.237/+	0.950, 5.270	0.065
Previous CVE	5.912/+	3.210, 10.891	<0.001
AUC DAS28 (6 mo)	1.063	1.021, 1.106	<0.01
AUC DAS28[1]	1.025	1.010, 1.040	<0.01
Extra-articular disease[1]	3.343/+	1.421, 7.867	<0.01
Corticosteroids at/before inclusion	1.030/mo	1.004, 1.056	<0.05
Corticosteroids[1]	2.243/+	1.208, 4.164	<0.05
DMARDs within 3 (mo)[2]	0.402/+	0.200, 0.808	<0.05
DMARDs[1]	0.885/mo	0.856, 0.916	<0.001
COX-2-inhibitors[1]	2.392/+	1.206, 4.744	<0.05

[1]=Time-dependent co-variate [2] DMARD treatment started within 3 months from baseline (T0)[2]

When evaluating the impact of disease activity on the CV incidence in Cox regression models adjusting for gender, hypertension and triglyceride level, a higher ESR at baseline independently increased the hazard rate of a new CVE and treatment with DMARDs was protective (Table 10). The same model illustrated graphically showed the synergistic effect of inflammatory activity (increasing ESR at baseline) and a traditional cardiovascular risk factor (higher triglycerides) and how the incidence (HR) was more than doubled when another CV risk factor (hypertension) was added (Figure 5). A similar model, comprising cumulative disease activity (AUC DAS28) after six months showed equal results.

Table 10. Importance of disease related and traditional risk factors for cardiovascular disease and pharmacological treatment for the hazard rate of a new CVE in 442 patients with early RA followed for 5 years. Extended Cox multiple regression model, with fixed and time-dependent co-variates.

Co-variates	HR	CI 95%	P-value
ESR	1.018/+	1.005, 1,030	<0.01
Triglycerides	1.853/mmolL^{-1}	1.376, 2.496	<0.001
Hypertension	2.809/+	1.575,5.008	<0.001
Female sex	0.449	0.249, 0.808	<0.01
DMARDs[1]	0.887/month	0.856, 0.918	<0.001

[1]=Time-dependent co-variate
Global Chi square(LR)=131.45 on 5df (p<0.001)

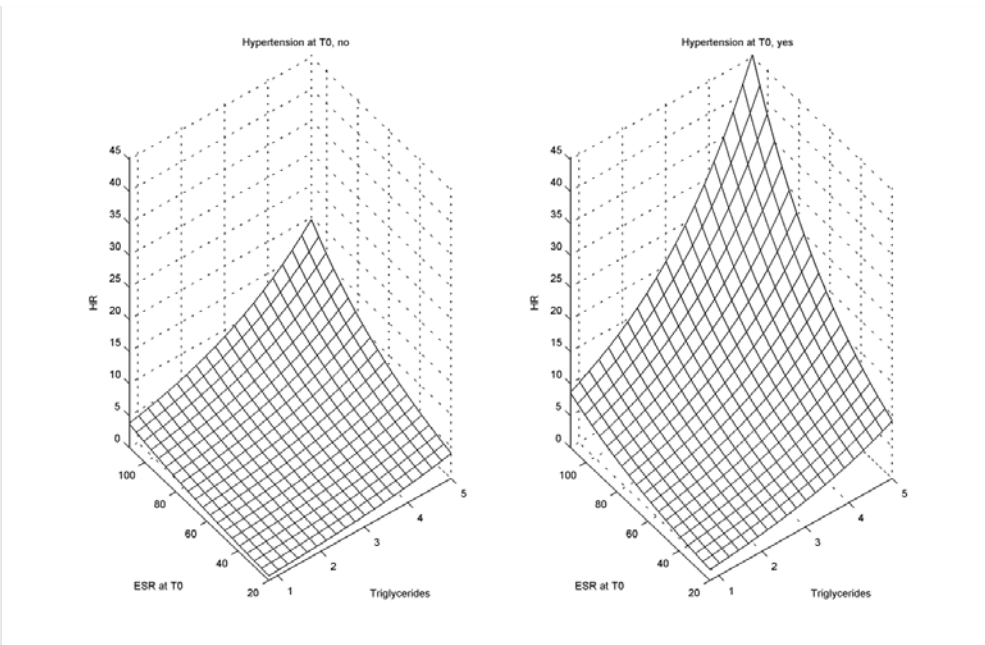


Figure 5. A new CVE is potentiated by the inflammatory activity and CV risk factors. Estimated hazard ratio (HR) of a new CVE, at a given time *t* during five years follow-up in patients with early RA, taking inflammatory activity (ESR) at baseline T0 and the level of triglycerides in consideration. **A** without and **B** with HT at baseline. The model also adjusts for gender and DMARD treatment.

Taken together, the main results of this study were that a new CVE was associated with high disease activity over time, extra-articular disease and most of the traditional risk factors. The presence of DM and/or HT at inclusion and the level of triglycerides could all predict the possibility of a new CVE. Furthermore, traditional risk factors and disease activity appeared to potentiate each other and treatment with DMARDs decreased the risk whilst COX 2-inhibitors seemed to be harmful.

It is now established that patients with RA have an increased morbidity and mortality due to cardiovascular disease compared with the general population (Bjornadal et al., 2002, del Rincon et al., 2001, Goodson et al., 2005a, Kremers et al., 2008, Maradit-Kremers et al., 2005a, Myllykangas-Luosujarvi et al., 1995, Sodergren et al., 2007, Solomon et al., 2003, Wallberg-Jonsson et al., 1997, Wolfe et al., 1994, Young et al., 2007b). The underlying reason for this is not obvious and traditional risk factors have not been regarded to fully explain the increase (del Rincon et al., 2001, Gonzalez et al., 2008, Solomon et al., 2003, Wallberg-Jonsson et al., 1999, Wallberg-Jonsson et al., 1997). However, in the present study, most of the traditional risk factors were identified as being important for the development of a new CVE. These results conflict with several previous reports in which traditional risk factors were not found to be a major reason for the development of CVD in patients with RA (Solomon et al., 2003, del Rincon et al., 2001, Gonzales et al., 2008, Wallberg-Jonsson et al., 1999).

Most importantly, a cumulative inflammatory response over time (*e.g.*, the AUC DAS28) and ESR at baseline were found as potential predictors of a new CVE. Additionally, a more serious disease, for example, in the form of extra-articular disease during follow-up, was associated with a new CVE in line with a previous report of patients with RA associated vasculitis (Turesson et al., 2007b). Moreover, inflammation and traditional CV risk factors appeared to potentiate each other regarding the incidence of a new CVE. It has previously been shown that inflammatory activity is deleterious for the progression of CVD (del Rincon et al., 2005, Gonzalez et al., 2007, Maradit-Kremers et al., 2005b, Wallberg-Jonsson et al., 1999). In one study involving patients with a new onset inflammatory polyarthritis, a high CRP level was directly related to the risk of death as a consequence of CVD (Goodson et al., 2005b). The results of the present study also underline previous findings regarding a synergistic effect of inflammation and traditional CV risk factors (del Rincon et al., 2005). However, some controversy does exist regarding the impact of inflammation on the atherosclerotic process in RA (Radovits et al., 2009b).

In previous studies from Northern Sweden of patients with established RA it was found that hypertension is the only traditional risk factor that clearly predicts a new CVE (Wallberg-Jonsson et al., 1997, Wallberg-Jonsson et al., 1999, Sodergren et al., 2007). Similar results have been presented by others (del Rincon et al., 2005, Gonzalez et al., 2008, Maradit-Kremers et al., 2005b, van Halm et al., 2006). There are some reports on an increased insulin resistance (Dessein et al., 2002, Svenson et al., 1988) and prevalence of diabetes mellitus in patients with RA (Chung et al., 2008) but in the QUEST-RA study, a negative impact of diabetes mellitus was only found in terms of a stroke (Naranjo et al., 2008). Only occasional studies have reported a negative impact of smoking on CVD in RA patients (Gonzalez et al., 2008). In patients with active disease and high inflammatory activity, the role of lipids as a risk factor is complex and the data are conflicting (Boers et al., 2003, Myasoedova et al., 2011a, Park et al., 2002, Rantapaa-Dahlqvist et al., 1991, Svenson et al., 1987). A low BMI, rather than a high, has been reported to increase the risk for CVD mortality (Escalante et al., 2005, Kremers et al., 2004), probably reflecting the impact of continuous inflammation with a certain degree of rheumatological cachexia (Walsmith et al., 2002). In this study, the presence of hypertension, diabetes mellitus and dyslipidaemia was found to be predictive of a new CVE. Previous reports in this field have mostly been retrospective or cross-sectional. It is possible that a prospective study of the size of the present study could reveal connections regarding the impact of traditional risk factors on a new CVE, whereas such associations have been difficult to show in retrospective and cross-sectional studies. One interpretation of this finding is that the relative importance of traditional risk factors decreases when inflammation is part of the picture but that the traditional risk factors *per se* are equally important as in the general population.

An important finding was that early DMARD treatment and continuous DMARD treatment significantly reduce the hazard ratio of a new CVE. This finding is consistent with the concept of an efficient anti-inflammatory treatment being cardioprotective in addition to its more well-known preventive effects on affected joints. The use of corticosteroids predicted a new CVE in simple but not in multiple regression analyses when the inflammatory activity was included. This indicates that confounding by indication may explain the result in simple analyses. Treatment with corticosteroids may be of doubtful benefit, however it has previously been reported to decrease the risk of a new CVE (Wallberg-Jonsson et al., 1999, Maradit-Kremers et al., 2005). A rapid suppression of inflammation by GCs could have a positive net effect whilst long standing GC treatment has been reported to increase the risk of a CVE (Davis et al., 2005). In a recently published review, treatment of patients with RA with low-doses of GCs

(defined as a daily dose <10 mg/day of prednisone) showed a poor association with CV risk but a trend of increasing major CVE's was identified (Ruyssen-Witrand et al., 2011).

Another finding in this study was that treatment with COX 2- inhibitors is significantly predictive of a new CVE even after adjusting the data for a previous CVE. This is in line with other studies showing that consumption of COX-2 inhibitors is associated with increased risk for CVD (Peters et al., 2009). Treatment with NSAIDs was not found to be a risk factor for a new CVE although several studies have shown NSAIDs to be harmful with an increased risk of CVD (Coxib et al., 2013).

Genetic markers (HLA-SE and PTPN22 T-variant) and autoantibodies (ACPA, RF and ANA) were not associated with risk for a new CVD in the present study. This is consistent with a recent study regarding PTPN 22 polymorphism (Palomino-Morales et al., 2010) but in contrast with another study showing that RA patients positive for ACPA and RF suffered more frequently from ischaemic heart disease (Lopez-Longo et al., 2009).

Paper III

Age at onset determines severity and choice of treatment, in early rheumatoid arthritis: a prospective study

In this prospective study the impact of age at onset of disease on prognostic risk factors and pharmacological treatment during the first five years of disease was evaluated. A total of 950 patients with early RA were included, of whom 665 patients had been affected with for five years at follow up. The median age at the onset of disease was 58 years (range 18-89 years). Descriptive data for all of the patients at baseline (T0) and for appropriate variables at follow-up (T5), were stratified into two groups based on the median age at disease onset, *i.e.*, young onset RA (YORA <58 years) and late onset RA (LORA ≥58 years) are presented in Table 11. At baseline, there were 357 (75.2%) women and 118 (24.8%) men in the YORA patient group and 292 (61.5%) women and 183 (38.5%) men in the LORA group. After five years there were 262 women and 89 men in the YORA patient group and 197 female and 117 male patients in the LORA group. At inclusion, the mean duration (SD) of symptoms was 6.9 (3.5) months for YORA and 6.5 (3.2) months for the patients with LORA (p=0.048) with no gender difference in either group. LORA was significantly associated with a lower frequency of ACPA and with less frequent carriage of the PTPN22 T-variant compared with YORA. ACPA was more common (66%) in the youngest quartile (18-47

years) with a gradual decrease (65% in patients 48-58 years, 62% in patients 59-66 years) to 50% in the oldest quartile (67-89 years). The presence of RF and HLA-SE were not related to age at disease onset. Disease activity, as measured by ESR, CRP, and AUC for DAS 28 and HAQ at baseline was higher in LORA patients. The development of extra-articular manifestations was similar in both groups, however LORA patients more often had newly identified CV-related co-morbidity, such as HT, DM and CVD at follow-up. Radiographic findings at inclusion and after 2 years showed that LORA was significantly more often associated with radiologically detected tissue and joint damage compared with YORA. Both YORA and LORA patients exhibited a radiological progression as measured by an increase in the Larsen score over time (YORA 5.31 (5.8) vs 9.46 (9.04) and LORA 8.14 (5.81) vs 12.45 (8.22), $p < 0.001$ for both). Additionally, the presence of damage due to tissue/bone erosion increased significantly between 0 and 24months within both groups (YORA 40.1% vs 61.8% and LORA 54.7% vs 75.3%, ($p<0.001$).

Table 11. Descriptive data for 950 (YORA n= 475, LORA n=475) patients with early RA at inclusion (T0) and at follow-up after 5 years (T5) n= 665. *N* (%) or mean (±SD).

Variable	YORA	LORA	p-value
Age at onset, yrs	< 58	≥58	
Female, (%)	*357/475* (75.2)	*292/475* (61.5)	
Disease Duration (months)	6.90 (3.42)	6.45 (3.20)	<0.05
RF +, (%)	*354/466* (75.9)	*349/468* (75.6)	Ns
ANA +, (%)	*91/377* (24.2)	*69/352* (19.6)	Ns
ACPA +,(%)	*313/429* (72.9)	*267/411*(64.9)	< 0.05
PTPN22 T variant, (%)	*155/405* (38.3)	*106/381* (27.8)	<0.01
HLA-SE, shared epitope, (%)	*237/405* (58.5)	*219/381*(57.5)	Ns
ESR T0, mm/hg; N=*948*	26.0 (21.5)	34.3 (24.2)	<0,001
CRP T0 mg/L; N=*888*	18.3 (23.7)	23.0 (24.6)	<0.01
DAS28 T0; N=788	4.5 (1.5)	4.8 (1.4)	<0.01
AUC DAS28 (6 months)[1]	24.5 (7.2)	25.9 (7.0)	<0.01
AUC DAS28 (12 months)[1]	44.8 (13.4)	47.7 (13.2)	<0.01
AUC DAS28 (24 months)[1]	84.2 (26.8)	89.0 (23.3)	<0.05
HAQ T0; N=796	0.81 (0.6)	0.92 (0.6)	<0.01
Tender joints T0; N=811	6.3 (5.6)	6.5 (5.7)	Ns
Swollen joints T0; N=812	6.8 (5.2)	7.3 (5.2)	Ns
VAS pain T0 (mm); N=800	43.5 (25.5)	43.4 (25.9)	Ns
VAS global T0 (mm); N=800	44.0 (25.8)	45.0 (25.5)	Ns
Smoking ever T0 (%)	*287/443* (64.8)	*300/447* (67.1)	Ns
Ex-RA ≤ T5 (%)[2,3]	*13/344* (3.8)	*14/313* (4.5)	Ns
Nodules ≤ T5 (%)[2]	*60/312* (19.2)	*38/275* (13.8)	p=0.079
DMARDs within 3 mo after T0(%)[2,4]	*325/346* (93.9)	*267/311*(85.9)	<0.001
DMARD's ever ≤ T5 (%)[2]	*344/348* (98.9)	*299/310* (96.5)	<0.05
Methotrexate ever ≤ T5,(%)[2]	*313/346* (90.5)	*253/311* (81.4)	<0.001
Biologics ever ≤ T5 (%)[2]	*84/342* (24.6)	*23/310* (7.4)	<0.001
NSAID T0 (%)	*317/455* (69.7)	*297/466* (63.7)	p=0.056
NSAID ever≤ T5 (%)[2]	*307/340* (90.3)	*233/307* (75.9)	<0.001
COX2-inh T0 (%)	*48/459* (10.5)	*64/468* (13.7)	Ns
COX2-inh ever ≤ T5 (%)[2]	*95/342* (27.8)	*83/309* (26.8)	Ns
Corticosteroids T0-T5 (months)[2]	20.3 (23.8)	24.7 (23.8)	<0.01
Corticosteroids ever ≤ T5 (%)[2]	*230/346* (66.5)	*233/309* (75.4)	<0.01
Hypertension, T0 (%)	*56/471* (11.9)	*199/474* (41.9)	<0.001
DM, T0 (%)	*22/464* (4.7)	*54/470* (11.5)	<0.001
CVD, before T0 (%)[5]	*17/471* (3.6)	*93/474* (19.6)	<0.001
CVD related co-morbidity, T0 (%)[6]	*86/475* (18.1)	*238/475* (50.1)	<0.001
Larsen score 0 (months); N=437	5.3 (5.8)	8.5 (6.2)	<0.001
Larsen score 24 (months); N=381	9.5 (9.1)	12.5 (8.2)	<0.001
Erosions 0 (months); (%)	*85/212* (40.1)	*105/192* (54.7)	<0.01
Erosions 24 (months); (%)	*107/173* (61.8)	*119/158* (75.3)	<0.01
Delta Larsen score 0-24 months ≥ 3 (%)	*103/207 (*49.8)	*97/173 (*56.1)	p=0.131

[1]AUC for DAS28 6, YORA/LORA, n=367/343, AUCDAS28 12, n=308/281, AUCDAS28 24 ,n=231/199 months after inclusion. [2]patients followed for 5 years, n= 665. [3]Criteria for severe extra-articular manifestations defined according to Turesson et al., 2004. [4]DMARD treatment started within three months from baseline (T0). [5]CVD= cardiovascular co-morbidities as previously described in detail (Innala et al., 2011). [6]CVD related co-morbidity at inclusion (T0) = CVD, hypertension or diabetes mellitus present before T0.

Concerning pharmacological treatment, there was a difference between LORA and YORA. LORA patients were less frequently treated with DMARDs early in the disease course, *i.e.*, within the first 3 months following diagnosis, and also significantly less often with methotrexate and/or biologics, but more often with corticosteroids. When the impact of age at disease onset was tested in relation to the choice of treatment, in baseline models adjusted for sex, ACPA status and disease activity (ESR), YORA was significantly associated with early DMARD treatment and LORA was associated with corticosteroid treatment (Table 12). The results were similar regarding age at the onset of disease when the data was corrected for baseline co-morbidity for early DMARD treatment as the dependent variable (OR= 0.371, 95% CI 0.194-0.709, p<0.01) and for corticosteroids OR= 1.453, 95% CI 0.967-2.183, p=0.072.

A reduction in the DAS28 score during the first 24 months (Δ DAS28) was larger in LORA patients compared with YORA (1.72 (1.57) vs 1.44 (1.71); p=0.058). With regards to EULAR treatment response criteria, 26.0 % of patients with LORA were classified as "non-responders" compared with 36.7 % of YORA patients at 12 months (p<0.01).

Table 12. Age in relation to treatment. Multiple logistic regression.

Outcome	DMARD treatment within 3 months			Corticosteroid treatment		
Covariates	OR	CI95%	p-value	OR	CI95%	p-value
Age at disease onset[1]	0.403	0.217, 0.749	<0.01	1.540	1.045, 2.267	<0.05
Sex /female	1.002	0.535, 1.878	0.99	0.803	0.529, 1.219	0.30
ACPA/positive	1.484	0.812, 2.712	0.20	1.127	0.752, 1.693	0.56
ESR (T0)/ mm/h	1.027	1.009, 1.045	<0.01	1.011	1.002, 1.020	<0.05

[1]LORA/YORA

Greater age at disease onset and ACPA status were significantly associated with a progression of the delta Larsen score (0-24 months) adjusted for age, sex, disease activity (ESR), co-morbidity at baseline and chosen therapy, *i.e.*, early treatment with DMARDs and corticosteroids (Table 13), respectively. In both models, male sex was significantly related to a greater radiological progression at 24 months (Tables 13)

Table 13. Age in relation to radiological progression. Multiple logistic regression

	Model I			Model II		
Covariates	OR	CI95%	p-value	OR	CI95%	p-value
Age at disease onset[1]	2.019	1.084, 3.761	<0.05	1.842	1.014, 3.348	<0.05
Sex /female	0.368	0.188, 0.722	<0.01	0.361	0.189, 0.692	<0.01
ACPA/positive	2.179	1.117, 4.251	<0.05	2.473	1.282, 4.769	<0.01
ESR (T0)/mm/h	1.008	0.995, 1.022	0.23	1.010	0.997, 1.024	0,136
CVD related co-morbidity, T0[2]	0.838	0.420, 1.675	0.67	0.889	0.460,1.718	0.727
DMARD treatment within 3 months after T0/yes	1.400	0.594, 3.298	0.44	-	-	-
Corticosteroid therapy ever ≤T5/yes	-	-	-	0.892	0.488,1.631	0.711

[1]LORA/YORA, [2]CVD, HT, DM

The main findings of this study were that late onset RA (LORA ≥58 years) patients had higher disease activity, more reduced function and more radiological damage at disease onset and over time. Despite this LORA patients were less often treated with DMARDs early in the disease course whereas patients suffering young onset RA (YORA <58 years) were not only treated more often early in the disease course with DMARDs but were also prescribed biologics more frequently. This indicates an unequal treatment that may have prognostic implications over time. However, YORA patients were more often positive for ACPA, a finding known to be associated with radiological progression (Berglin et al., 2006, Nishimura et al., 2007). YORA patients were also more often carriers of PTPN22 T-variant compared with LORA but the frequency of RF and HLA-SE positivity was similar. These findings are in line with previous reports showing a combination of ACPA positivity and carriage of PTPN22 T-variant to be associated with disease onset at an earlier age (Kokkonen et al., 2007, Steer et al., 2005). Furthermore, there was a gradual decrease in the frequency of ACPA with increasing age at onset of disease. LORA was found to be more often associated with a reduced function at baseline and more radiological damage over time consistent with other reports (Bukhari et al., 2007, Camacho et al., 2011, Pease et al., 1999, van der Heijde et al., 1991). This finding may indicate a phenotype more predisposed to a progressive disease in younger patients at disease onset. Nevertheless, functional capacity at baseline and radiological findings was more pronounced in the older patients. The LORA patients in this study had higher disease activity at inclusion compared with YORA and after 24 months they also had a greater reduction in DAS28 score. Furthermore, when stratified for response to therapy, LORA patients also had a better response after 12 months. A possible explanation for this finding

is that LORA patients were more often treated with GCs resulting in a rapid reduction in disease activity. Moreover, LORA patients had more erosions and a higher Larsen score after 2 years possibly as a consequence of their receiving DMARDs too late in the disease course and that treatment with GCs is insufficient to delay X-ray progression. However, no difference between the groups regarding radiological progression could be demonstrated irrespective of the chosen drug treatment. It is conceivable that the impact of treatment could have been demonstrated in a larger cohort of patients and by a longer follow-up period. Nor can the fact that a more aging cartilage could contribute to the worse radiographic status in LORA patients be ignored and inflammation mediated cartilage degradation has actually been suggested in elderly individuals (Pease et al., 1999, Bukhari et al., 2007). However, there was a significant progression of erosions after 2 years in LORA patients and the findings of a higher Larsen score cannot easily be explained by the progression of cartilage damage as a part of the disease course in osteoarthritis. These findings concur with a study by Paulus *et al* who found that monotherapy with low-dose GCs did not prevent radiological progression (Paulus et al., 2000). However, there are several reports showing that low doses of GCs as monotherapy, or in combination with DMARDs, induce a successful outcome with respect to remission rate and radiological progression (Kirwan et al,. 2007, Hafstrom et al., 2009, Gorter et al., 2010). It is clearer that older patients, compared with their younger counterparts, show equal, and sometimes worse, disease activity and severity (Bukhari et al., 2007, Calvo-Alen et al., 2005, Pease et al., 1999, Radovits et al., 2009a, Tutuncu et al., 2006, van der Heijde et al., 1991, Villa-Blanco et al., 2009).

According to the findings in this study, rheumatologists are less likely to treat LORA patients with DMARDs early in the disease course and less so with biologics compared with YORA patients. One reason to this fact may be that aging patients more often have co-morbidities requiring treatment with drugs that may interact with the anti-rheumatic treatment used by rheumatologists. However, when co-morbidity at baseline was taken into account, the choice of treatment was age related. At times, this approach may be the correct clinical decision and a risk/benefit profile must always be taken in to consideration. The results in this study indicate that rheumatologist are more concerned about the potential side effects of DMARDs and choose GCs instead of DMARDs early in the disease course in older patients. Several reports have shown the importance of initiating DMARD treatment as quickly as possible, preferably within 3 months from disease onset to improve disease outcome (Gremese et al., 2013, Leirisalo-Repo, 2013). Furthermore, patients with longer disease duration do not respond to treatment to the same extent as those with shorter disease

duration (Anderson et al., 2000, Lard et al., 2001, O'Dell et al., 2004, Rantalaiho et al., 2010). Several studies have shown that treatment with DMARDs, such as methotrexate, sulfasalazine, anti-malarial drugs, biological agents and other DMARDs are well tolerated by the elderly, however before starting any therapy, adverse effects should be evaluated extra carefully (Fleischmann et al., 2003, Schneeweiss et al., 2007, Villa-Blanco et al., 2009). In line with a recent publication LORA patients were found to be treated less often with biologics compared with YORA patients (Huscher et al., 2013). Treatment with GCs can be precarious in elderly patients due to side-effects in the long run but the current literature still lacks data regarding the net effect of GCs in patients suffering an inflammatory condition such as RA (Tutuncu et al., 2007).

Paper IV

Co-morbidity in patients with early rheumatoid arthritis. Does inflammation matter?

In this five year prospective study we investigated the prevalence of co-morbidities in early RA and the role of inflammation in this context.

By November 2012, 950 patients (649 women, 301 men) registered with newly diagnosed early RA had been included in the study at the time of being given a diagnosis of RA (baseline, T0). Of these, 726 patients had been followed for 5 years (T5). The mean age at disease onset was 55.6 years (range 18-89 years). The mean duration (SD) from the first symptom of rheumatoid disease to inclusion into the register was 6.7 (3.5) months (Table 14).

Table 14. Descriptive data at baseline (T0) in 950 patients and at 5-year follow-up (T5) in 726 of the patients with early RA. Mean (SD) or %

Age at onset of symptoms, years	55.6 (14.4)
Female/Male, T0	649/301 (68.3/31.7)
Duration of symptoms at T0, months	6.7 (3.5)
RF+	75.3
ANA+	22.0
ACPA+	69.0
HLA-SE+	58.0
Disease activity and severity at T0	
ESR, mm/h	30.1 (23.2)
CRP, mg/L	20.7 (24.3)
HAQ	0.86 (0.6)
Tender joint count	6.4 (5.7)
Swollen joint count	7.0 (5.2)
VAS pain, mm	43.4 (25.7)
VAS global, mm	44.4 (25.7)
AUC DAS28 (6 months)	25.1 (7.2)
AUC DAS28 (12 months)	46.2 (13.4)
AUC DAS28 (24 months)	86.5 (25.6)
Ex-RA ≤ T5	5.3
Nodules ≤ T5	16.9
Smoking ever, T0	65.8
Treatments	
DMARDs within 3 months after T0	90.1
DMARDs ever ≤ T5	97.8
Methotrexate ever ≤ T5	86.9
Biologics ever ≤ T5	16.5
Time without DMARDs from onset, months	6.8 (4.9)
Time without DARDs T0-T5, months	8.4 (15.7)
NSAIDs ever ≤T5	84.0
Cox-2 inhibitors ever ≤T5	26.5
Corticosteroids T0-T5, months	22.2 (23.8)
Corticosteroids ever ≤ T5	71.0

RF= rheumatoid factor; ANA= anti nuclear antibody; ACPA=anti-citrullinated protein/peptide antibody (analysed as anti-CCP2); ESR= erythrocyte sedimentation rate; CRP= C-reactive protein; HAQ= health assessment questionnaire; AUC= area under curve; Ex-RA= extra articular manifestations of RA; DMARDs= disease modifying anti-rheumatic drugs; NSAIDs= non-steroid anti-inflammatory drugs; Cox-2= cyclo oxygenase 2

In all, 53 % had one or more co-morbidities at RA onset. The most common co-morbidities at inclusion were hypertension (27.3%), obstructive pulmonary disease (asthma and /or COPD); 13.9%), diabetes (8.0%), hypothyroidism (6.3%) and malignancy (5.0%). After 5 years 40.6 %, had developed a minimum of one new co-morbidity. During the first five years, the most common new co-morbidities were hypertension (15.1%), malignancy (7.6%), stroke/TIA (5.1%), myocardial infarction (4.3%) and osteoporosis (3.7%). Obstructive pulmonary disease at inclusion divided as follows: 39 patients (4.1%) had COPD and 106 (11.2%) had asthma. Of these, 13 patients had both diagnoses. After 5 years, 13 patients (1.4 %) had developed new COPD and 4 (0.6 %) had acquired asthma. During follow-up, 40 patients (4.9%) developed an extra-articular manifestation of which 12 were already present at T0. All of these manifestations were RA associated lung disease. At T5, 28 new Ex-RA had developed including 20 with RA associated lung disease. Thus, of all 40 patients with Ex-RA at follow-up as many as 32 had RA associated lung disease. For analyses of predictors for new lung disease, a composite variable comprising obstructive lung disease COPD/asthma and RA associated lung disease, (n=37 in all) was created. The composite variable new endocrine disease during 5 years comprised 75 patients (10.5%) including thyroid disease, (2.4%), osteoporosis (3.7%), DM (3.3%), and hyperparathyroidism (0.6%) (Table 15).
Figure 6 illustrates the various co-morbidity at T0 and T5 in the patients that been followed for five years.

Figure 6. Co-morbidities (%) at T0 and T5

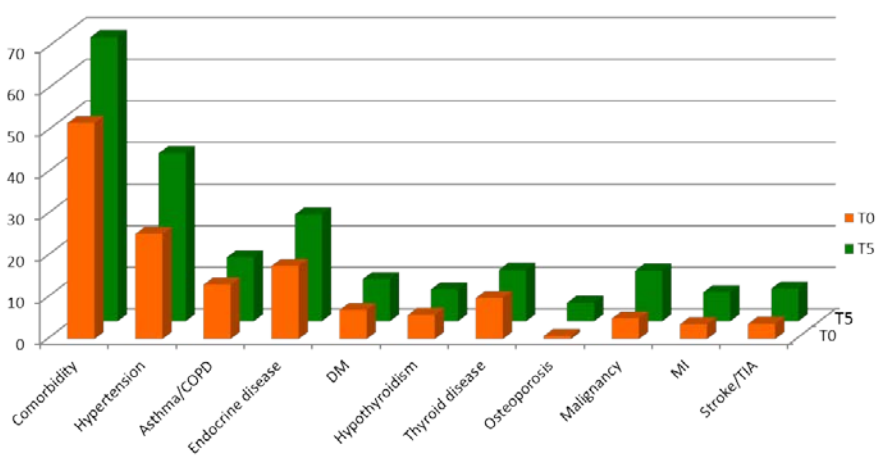


Table 15. Co-morbidity in early RA patients at baseline and after 5 years of disease

	Baseline (N=950) %	New co-morbidity during 5 years of disease (N=726) %
Co-morbidity over all[1]	53.2	40.6
Hypertension	27.3	15.1
Asthma/COPD	13.9 (11.2/4.1)	2.4 (0.6/1.8)
Any endocrine disease	19.2	10.5
Diabetes mellitus	8.0	3.3
Hypothyroidism	6.3	1.9
Thyroid disease[2]	10.4	2.4
Osteoporosis	1.4	3.7
Hyperparathyroidism	0.6	0.6
Malignancy	5.0	7.6
Myocardial infarction	4.5	4.3
Stroke/TIA	3.9	5.1

[1]Co-morbidity defined according to Charlson et al., 1987. [2] Defined as hypothyroidism, hyperthyroid disease and goitre. COPD; chronic obstructive lung disease; TIA; transient ischemic attack.

Predictors of a new co-morbidity after five years; simple logistic regression

In simple regression analyses, co-variates associated with a new co-morbidity over all after five years were, age at inclusion, ESR at baseline, corticosteroid therapy ($p<0.001$ for all), Ex-RA ($p<0.01$), AUC DAS28 (24 months) ($p<0.05$) and smoking ever ($p=0.058$). Co-variates associated with a reduced risk of a new co-morbidity were female gender ($p<0,01$) and treatment with biological agents ($p< 0.05$) (Table 16).

Table 16. Co-variates associated with an increased or reduced risk of a new co-morbidity over all during the first five years. Simple logistic regression

Co- variates	OR	CI 95 %	P-value
ESR (T0), mm/h	1.015	1.008, 1.022	<0.001
AUC DAS28 (24 mo)	1.008	1.000, 1.016	<0.05
Ex-RA, yes/no	2.891	1.483, 5,638	<0.01
Corticosteroids ever ≤ T5	2.087	1.468, 2,960	<0.001
Biologics ever ≤ T5	0.599	0.389, 0.922	<0.01
Smoking ever (T0)	1.373	0.990, 1.905	0.058
Age at disease onset ,years	1.068	1.054, 1.083	<0.001
Sex f/m	0.650	0.472, 0,894	<0.01

AUC: area under the curve; CI; confidence interval; ESR: erythrocyte sedimentation rate; Ex-RA; extra-articular disease; DAS28: disease activity score; OR; odds ratio

Predictors of a new co-morbidity after five years; multiple logistic regression

In multiple regression analyses, ESR at baseline was significantly associated with a new co-morbidity after five years in a model adjusted for age, sex, smoking habits and corticosteroid therapy (Table 17). In a similar model including Ex-RA in place of ESR, Ex-RA ($p<0.05$) was associated with a new co-morbidity (data not shown). In a model evaluating predictors for new lung comorbidity, including also RA associated lung disease, smoking habits, but not ESR at baseline, was significantly associated with the outcome (Table 18). Regarding variables associated with new endocrine co-morbidity as one group, ESR at baseline approached significance ($p=0.10$) in a model adjusted for age, sex, smoking habits and corticosteroid treatment (Table 19).

Table 17. Co-variates associated with a new co-morbidity over all during the first five years. Multiple logistic regression. (N= 623**)**

Co-variates	OR	CI 95 %	P -value
ESR (T0), mm/h	1.008	1.001, 1.016	0.036
Corticosteroids ever ≤ T5	1.760	1.168, 2.654	<0.01
Smoking ever (T0)	1.302	0.877, 1.933	0.190
Age at disease onset, years	1.072	1.055, 1.090	<0.001
Sex f/m	1.058	0.720, 1.554	0.775

CI; confidence interval; ESR: erythrocyte sedimentation rate; OR; odds ratio.

Table 18. Co-variates associated with new lung co-morbidity (obstructive lung disease, RA associated lung disease) during the first five years Multiple logistic regression. (N= 637)

Co-variates	OR	CI 95 %	P –value
ESR (T0), mm/h	1.002	0.988, 1.017	0.760
Smoking ever (T0)	6.479	1.925, 21.800	<0.01
Age at disease onset, yrs	1.043	1.010, 1.078	<0.05
Sex f/m	1.663	0.765, 3.618	0.199

CI; confidence interval; ESR: erythrocyte sedimentation rate; OR; odds ratio.

Table 19. Co-variates associated with new endocrine disease (thyroid diseases, osteoporosis, diabetes, hyperparathyroidism) during the first five-years. Multiple logistic regression. (N= 625)

Co-variates	OR	CI 95 %	P -value
ESR (T0), mmHg	1.009	0.998, 1.020	0.101
Corticosteroids ever ≤ T5	1.542	0.792, 3.002	0.202
Smoking ever (T0)	0.761	0.435, 1.332	0.339
Age at disease onset, yrs	1.052	1.028, 1.077	<0.001
Sex f/m	4.552	2.076, 9.980	<0.001

CI; confidence interval; ESR: erythrocyte sedimentation rate; OR; odds ratio

We found that co-existing disease was common. In all, 53.2 % of the early RA patients had at least one medical condition besides RA and 23% of these had more than one co-existing disease at RA onset. These figures are close to those reported for cohorts of patients with early RA from North America (58% Gabriel et al., 1999), the Netherlands (66% (Kroot et al., 2001), Great Britain (31.6% Norton et al., 2013a) and Southern Sweden (43% (Kapetanovic et al., 2010), respectively. In contrast, a recent study reported a lower rate of co-morbidities with a baseline prevalence of 20% for all co-morbidity in early RA patients (Tiippana-Kinnunen et al., 2013). In the present study the burden of co-existing disease increased during follow up and 40.6% developed at least one new co-morbidity over the first five years of disease. Furthermore, these figures are consistent with other reports of an increasing burden of illness over time (Gabriel et al., 2009, Norton et al., 2013a, Tiippana-Kinnunen et al., 2013, Kapetanovic et al., 2010). However, it is difficult to compare the overall disease burden among the various studies since there is no common definition in terms of co-morbidity. We used the Charlson co-morbidity index to define the co-morbidity groups. This index was developed to predict one-year mortality in a large cohort of patients and comprises, with that purpose, also weighting of diagnosis for

mortality risk. The focus of the present study was, however, the role of inflammation in the context of development of co-morbidity. Thus, we only used this instrument for the purpose of defining co-morbidities.

In this early RA cohort, the age at onset was 56 years and 27.3% were receiving treatment for hypertension at inclusion whilst an additional 15.1% developed hypertension during the first five years of disease. This figure is higher than expected. The prevalence of hypertension requiring treatment in Swedes aged 60-years was calculated to be 10 % in 2003 (www.sbu.se.evidensbaserad.äldrevård). The occurrence of chronic pulmonary disease, in this context COPD and/or asthma at the onset of RA was close to 14% of whom most had been diagnosed with asthma. Furthermore, approximately 85% of the patients with chronic pulmonary disease (COPD and/or asthma) were diagnosed before the onset of RA which is consistent with previous reports (Hyrich et al., 2006, Kroot et al., 2001). Previous studies have shown RA patients to have a higher than expected risk of COPD (Bieber et al., 2013)and an increased risk of asthma (Shen et al., 2014b) compared with the general population but the reported data is conflicting, especially regarding asthma (Rudwaleit et al., 2002). Regarding Ex-RA, almost 5% of the patient cohort developed such a complication during follow-up and RA associated lung disease being the most common Ex-RA manifestation comprising 4 of the 5%. More than a third already had an RA associated lung disease at the onset of RA. This is in keeping with Koduri et al (Koduri et al., 2010). When considering the emerging data suggesting the mucosal surfaces *i.e.*, the lung to be the potential site for an initial immune dysregulation and autoantibody generation in the early development of RA (Demoruelle et al., 2014) these relatively high figures of lung disease involvement at baseline are of great interest. When we evaluated predictors for a new lung disease in a multiple model, disease activity, measured as ESR, had no impact whereas ever being a smoker was, independently associated with the outcome.

Cumulative inflammation over time (*i.e.*, AUC DAS28 24 months) was associated with a new co-morbidity in univariate analysis. Furthermore, ESR at inclusion was independently associated with a new comorbidity during the first five years in a multiple model adjusted for age, sex, use of corticosteroids and smoking. Also, a more serious disease as measured by development of Ex-RA during the follow up period, was associated with a new co-morbidity over time. However, others did not find that co-morbidity was related to diseases activity (Norton et al., 2013a, Kapetanovic et al., 2010)

In all, 6.3% of the patients had hypothyroidism at the time of RA onset, which is consistent with that of Raterman *et al* who reported an increased risk of hypothyroidism in female RA patients compared with controls (Raterman et al., 2008) but in contrast with a recent study reporting no increase of thyroid disease (McCoy et al., 2012). However, both of these studies reported that hypothyroid disease was associated with increased risk for CVD and they suggested that RA patients should be screened for hypothyroidism. In an epidemiological study, thyroxine substitution was suggested to be associated with an increased risk for development of RA (Bengtsson et al., 2013). In the present cohort, there were strong gender differences with an overall four-fold increased risk of endocrine diseases in females. The role of inflammation has been questioned in the context of endocrine disease (McCoy et al., 2012) however, we did find indications of an increased risk; a new endocrine co-morbidity, tended to be associated with higher baseline ESR, and also when adjusted for age, sex, smoking and corticosteroid treatment.

During the five years of follow up period, 7.6 % (n=54) developed a new malignancy, the three most frequent forms being prostate cancer (n=13), gastrointestinal cancers (n=10) and lung cancer (n=10). All of the patients who developed lung cancer had a history of smoking except one patient in for whom we lacked data on smoking status. Smoking is a well-known risk factor for both RA and lung cancer however also systemic inflammation has been reported to be associated with lung cancer (Smitten et al., 2008). Three patients had developed lymphomas at follow-up. Higher inflammatory activity has been shown to be a major risk factor for lymphoma in RA patients and the chronic inflammatory state associated with RA may be the explanation however, treatment with DMARDs did not appear to be a risk factor in that study (Baecklund et al., 2006).

Concerning CV co-morbidity, there were 4.3% of patients with a new MI and 5.1% with a stroke/TIA during the follow-up period. We analysed this co-morbidity thoroughly in a previous prospective study (Innala et al., 2011) and found the inflammatory activity to be harmful in terms of new CV events, a conclusion that is in keeping with several other cross-sectional and retrospective studies (Wallberg-Jonsson et al., 1999, Maradit-Kremers et al., 2005, del Rincon et al., 2005) but at variance with a smaller study from southern Sweden (Kapetanovic et al., 2010). We also found that inflammation potentiated the effect of traditional CV risk factors (Innala et al., 2011).

To summarize, we found that co-morbidity was common in patients with a recent onset RA and considerable new co-morbidity was added during the

first 5 years of disease. We were also able to show that the inflammatory activity, both at disease onset and accumulated over time, was associated with a new co-morbidity during 5 years of follow-up. There was a tendency for inflammation predicting also endocrine diseases, whilst new lung co-morbidity was predicted by smoking. RA patients appear to have several other medical conditions besides the index disease already at onset, adding to the disease burden. In every-day practice, the disease activity should be opposed not only to prevent joint destructions but also with regards to co-morbidity.

Concluding remarks

***** Anti-MCV antibodies are in patients with early RA associated with a more severe disease, as measured by DAS28, ESR, and swollen joint count over time as compared with anti-CCP2, CCP3, and CCP3.1 antibodies.

* All four ACPAs predicted radiological progression equally.

***** The incidence of a new CV event in early RA was predicted by several traditional CV risk factors i.e., the presence of diabetes mellitus and/or hypertension at inclusion, and the level of triglycerides

* A high disease activity, both at baseline and over time, potentiated this risk. On the other hand, treatment with DMARDs decreased the CV risk, confirming the cardio-protective effect of disease modifying anti-rheumatic treatment.

* These results may have implications for cardio-protective strategies in RA: Given the current state of knowledge, it is important to suppress disease activity and great effort should be made to optimize the prevention and treatment of traditional CV risk factors in patients with RA.

***** Patients with young onset of RA were more frequently ACPA positive. A late onset was associated with higher incipient disease activity, reduced function at baseline, and more radiological damage at disease onset and over time.

* Nevertheless, patients with late onset were more often treated with corticosteroids and less with DMARDs early in the disease process compared with patients with young onset.

* This could have implications for development of co-morbidities: We propose that patients with LORA should receive the same treatment as patients with younger onset, however, they may require tighter controls to rapidly detect any potential complicating co-morbidity or other undesirable side effects of the treatment.

* Co-morbidity was common in patients with a recent onset RA and a considerable new co-morbidity developed during the first 5 years of disease.

* Measures of disease activity, both at disease onset and accumulated over time, were associated with occurrence of a new co-morbidity after five years.

These findings indicate that the inflammatory status is of importance for the development of co-morbidities in early RA patients.

* RA patients appear to have several other medical conditions in addition to the index disease already at onset of disease that add to their disease burden. In every-day clinical practice, the patient's disease activity should be treated not only to prevent any destruction of joints but also with regard to their specific co-morbid disease(s).

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References

Alamanos, Y., P. V. Voulgari and A. A. Drosos (2006). Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. *Seminars in Arthritis and Rheumatism* 36(3) 182-188.

Albert, L. J. and R. D. Inman (1999). Molecular mimicry and autoimmunity. *New England Journal of Medicine* 341(27) 2068-2074.

Aletaha, D., et al. (2010). 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis and Rheumatism* 62(9) 2569-2581.

Aletaha, D. and J. S. Smolen (2006). The definition and measurement of disease modification in inflammatory rheumatic diseases. *Rheumatic Diseases Clinics of North America* 32(1) 9-44, vii.

Anderson, J. J., et al. (2000). Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. *Arthritis and Rheumatism* 43(1) 22-29.

Arnett, F. C., et al. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis and Rheumatism* 31(3) 315-324.

Avina-Zubieta, J. A., et al. (2008). Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis and Rheumatism* 59(12) 1690-1697.

Baecklund, E., et al. (2006). Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis and Rheumatism* 54(3) 692-701.

Bang, H., et al. (2007). Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis and Rheumatism* 56(8) 2503-2511.

Bang, S. Y., et al. (2010). Smoking increases rheumatoid arthritis susceptibility in individuals carrying the HLA-DRB1 shared epitope, regardless of rheumatoid factor or anti-cyclic citrullinated peptide antibody status. *Arthritis and Rheumatism* 62(2) 369-377.

Bartels, C. M., et al. (2010). Changing trends in serious extra-articular manifestations of rheumatoid arthritis among United State veterans over 20 years. *Rheumatology* 49(9) 1670-1675.

Begovich, A. B., et al. (2004). A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *American Journal of Human Genetics* 75(2) 330-337.

Bengtsson, C., et al. (2013). Thyroxin substitution and the risk of developing rheumatoid arthritis; results from the Swedish population-based EIRA study. *Annals of the Rheumatic Diseases*.

Berglin, E., et al. (2006). Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. *Annals of the Rheumatic Diseases* 65(4) 453-458.

Berglin, E., et al. (2010). Influence of female hormonal factors, in relation to autoantibodies and genetic markers, on the development of rheumatoid arthritis in northern Sweden: a case-control study. *Scandinavian Journal of Rheumatology* 39(6) 454-460.

Bieber, V., et al. (2013). Autoimmune smoke and fire--coexisting rheumatoid arthritis and chronic obstructive pulmonary disease: a cross-sectional analysis. *Immunologic Research* 56(2-3) 261-266.

Bilgici, A., et al. (2005). Pulmonary involvement in rheumatoid arthritis. *Rheumatology International* 25(6) 429-435.

Bjornadal, L., et al. (2002). Decreasing mortality in patients with rheumatoid arthritis: results from a large population based cohort in Sweden, 1964-95. *Journal of Rheumatology* 29(5) 906-912.

Boers, M. (2003). Understanding the window of opportunity concept in early rheumatoid arthritis. *Arthritis and Rheumatism* 48(7) 1771-1774.

Boers, M., et al. (2003). Influence of glucocorticoids and disease activity on total and high density lipoprotein cholesterol in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases* 62(9) 842-845.

Boire, G., et al. (2005). Anti-Sa antibodies and antibodies against cyclic citrullinated peptide are not equivalent as predictors of severe outcomes in patients with recent-onset polyarthritis. *Arthritis Research & Therapy* 7(3) R592-603.

Bongartz, T., et al. (2010). Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. *Arthritis and Rheumatism* 62(6) 1583-1591.

Bongartz, T., et al. (2006). Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 295(19) 2275-2285.

Boyer, J. F., et al. (2011). Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis. *Joint, Bone, Spine: Revue du Rhumatisme* 78(2) 179-183.

Brink, M., et al. (2013). Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. *Arthritis and Rheumatism* 65(4) 899-910.

Bukhari, M., et al. (2007). Increasing age at symptom onset is associated with worse radiological damage at presentation in patients with early inflammatory polyarthritis. *Annals of the Rheumatic Diseases* 66(3) 389-393.

Burkhardt, H., et al. (2005). Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. *European Journal of Immunology* 35(5) 1643-1652.

Bykerk, V. P., et al. (2013). Achieving remission in clinical practice: lessons from clinical trial data. *Clinical and Experimental Rheumatology* 31(4) 621-632.

Calvo-Alen, J., et al. (2005). Outcome of late-onset rheumatoid arthritis. *Clinical Rheumatology* 24(5) 485-489.

Camacho, E. M., et al. (2011). Influence of age and sex on functional outcome over time in a cohort of patients with recent-onset inflammatory polyarthritis: results from the Norfolk Arthritis Register. *Arthritis Care Res (Hoboken)* 63(12) 1745-1752.

Cannon, G. W. (1997). Methotrexate pulmonary toxicity. *Rheumatic Diseases Clinics of North America* 23(4) 917-937.

Carty, S. M., N. Snowden and A. J. Silman (2003). Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? *Journal of Rheumatology* 30(3) 425-429.

Charlson, M. E., et al. (1987). A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of Chronic Diseases* 40(5) 373-383.

Chatterjee, S. (2004). Severe interstitial pneumonitis associated with infliximab therapy. *Scandinavian Journal of Rheumatology* 33(4) 276-277.

Chung, C. P., et al. (2005). Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors. *Arthritis and Rheumatism* 52(10) 3045-3053.

Chung, C. P., et al. (2008). Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. *Atherosclerosis* 196(2) 756-763.

Coenen, D., et al. (2007). Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. *Clinical Chemistry* 53(3) 498-504.

Combe, B. (2007). Early rheumatoid arthritis: strategies for prevention and management. *Best Practice & Research: Clinical Rheumatology* 21(1) 27-42.

Costenbader, K. H., et al. (2006). Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *American Journal of Medicine* 119(6) 503 e501-509.

Coxib, et al. (2013). Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 382(9894) 769-779.

Crowson, C. S., et al. (2013). Brief report: accelerated aging influences cardiovascular disease risk in rheumatoid arthritis. *Arthritis and Rheumatism* 65(10) 2562-2566.

Davis, J. M., 3rd, H. Maradit-Kremers and S. E. Gabriel (2005). Use of low-dose glucocorticoids and the risk of cardiovascular morbidity and mortality in rheumatoid arthritis: what is the true direction of effect? *Journal of Rheumatology* 32(10) 1856-1862.

Dawson, J. K., et al. (2001). Fibrosing alveolitis in patients with rheumatoid arthritis as assessed by high resolution computed tomography, chest radiography, and pulmonary function tests. *Thorax* 56(8) 622-627.

de Groot, V., et al. (2003). How to measure comorbidity. a critical review of available methods. *Journal of Clinical Epidemiology* 56(3) 221-229.

De Rycke, L., et al. (2004). Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Annals of the Rheumatic Diseases* 63(12) 1587-1593.

Deighton, C. M., et al. (1989). The contribution of HLA to rheumatoid arthritis. *Clinical Genetics* 36(3) 178-182.

Dejaco, C., et al. (2006). Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Arthritis Research & Therapy* 8(4) R119.

del Rincon, I., et al. (2005). Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. *Arthritis and Rheumatism* 52(11) 3413-3423.

del Rincon, I. D., et al. (2001). High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis and Rheumatism* 44(12) 2737-2745.

Demoruelle, M. K., K. D. Deane and V. M. Holers (2014). When and where does inflammation begin in rheumatoid arthritis? *Current Opinion in Rheumatology* 26(1) 64-71.

Dessein, P. H., et al. (2002). The acute phase response does not fully predict the presence of insulin resistance and dyslipidemia in inflammatory arthritis. *Journal of Rheumatology* 29(3) 462-466.

Dessein, P. H., B. I. Joffe and A. E. Stanwix (2003). Inflammation, insulin resistance, and aberrant lipid metabolism as cardiovascular risk factors in rheumatoid arthritis. *Journal of Rheumatology* 30(7) 1403-1405.

Diogo, D., Y. Okada and R. M. Plenge (2014). Genome-wide association studies to advance our understanding of critical cell types and pathways in rheumatoid arthritis: recent findings and challenges. *Current Opinion in Rheumatology* 26(1) 85-92.

Dixon, W. G., et al. (2010). Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). *Annals of the Rheumatic Diseases* 69(3) 522-528.

Doran, M. F., et al. (2002a). Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. *Arthritis and Rheumatism* 46(9) 2287-2293.

Doran, M. F., et al. (2002b). Predictors of infection in rheumatoid arthritis. *Arthritis and Rheumatism* 46(9) 2294-2300.

Doran, M. F., et al. (2002c). Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. *Arthritis and Rheumatism* 46(3) 625-631.

dos Anjos, L. M., et al. (2009). A comparative study of IgG second- and third-generation anti-cyclic citrullinated peptide (CCP) ELISAs and their combination with IgA third-generation CCP ELISA for the diagnosis of rheumatoid arthritis. *Clinical Rheumatology* 28(2) 153-158.

Dougados, M., et al. (2014). Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). *Annals of the Rheumatic Diseases* 73(1) 62-68.

Ebert, E. C. and K. D. Hagspiel (2011). Gastrointestinal and hepatic manifestations of rheumatoid arthritis. *Digestive Diseases and Sciences* 56(2) 295-302.

Ekdahl, C., et al. (1988). Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scandinavian Journal of Rheumatology* 17(4) 263-271.

Emerging Risk Factors, C., et al. (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 375(9709) 132-140.

Eriksson, J. K., et al. (2013). Incidence of rheumatoid arthritis in Sweden: a nationwide population-based assessment of incidence, its determinants, and treatment penetration. *Arthritis Care Res (Hoboken)* 65(6) 870-878.

Escalante, A., R. W. Haas and I. del Rincon (2005). Paradoxical effect of body mass index on survival in rheumatoid arthritis: role of comorbidity and systemic inflammation. *Archives of Internal Medicine* 165(14) 1624-1629.

Finckh, A., et al. (2006). Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. *Arthritis and Rheumatism* 55(6) 864-872.

Fischer, A., et al. (2012). Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease. *Respiratory Medicine* 106(7) 1040-1047.

Fleischmann, R. M., et al. (2003). Response to etanercept (Enbrel) in elderly patients with rheumatoid arthritis: a retrospective analysis of clinical trial results. *Journal of Rheumatology* 30(4) 691-696.

Fleming, A., et al. (1976). Extra-articular features in early rheumatoid disease. *British Medical Journal* 1(6020) 1241-1243.

Frisell, T., et al. (2013). Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number and type of affected relatives, sex, and age. *Arthritis and Rheumatism* 65(11) 2773-2782.

Furst, D. E. (2004). Window of opportunity. *Journal of Rheumatology* 31(9) 1677-1679.

Gabbay, E., et al. (1997). Interstitial lung disease in recent onset rheumatoid arthritis. *American Journal of Respiratory and Critical Care Medicine* 156(2 Pt 1) 528-535.

Gabriel, S. E., C. S. Crowson and W. M. O'Fallon (1999). Comorbidity in arthritis. *Journal of Rheumatology* 26(11) 2475-2479.

Gabriel, S. E. and K. Michaud (2009). Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis Research & Therapy* 11(3) 229.

Garcia Rodriguez, L. A. and S. Hernandez-Diaz (2001). The risk of upper gastrointestinal complications associated with nonsteroidal anti-inflammatory drugs, glucocorticoids, acetaminophen, and combinations of these agents. *Arthritis Res* 3(2) 98-101.

Gartlehner, G., et al. (2006). The comparative efficacy and safety of biologics for the treatment of rheumatoid arthritis: a systematic review and metaanalysis. *Journal of Rheumatology* 33(12) 2398-2408.

Gonzalez, A., et al. (2008). Do cardiovascular risk factors confer the same risk for cardiovascular outcomes in rheumatoid arthritis patients as in non-rheumatoid arthritis patients? *Annals of the Rheumatic Diseases* 67(1) 64-69.

Gonzalez, A., et al. (2007). The widening mortality gap between rheumatoid arthritis patients and the general population. *Arthritis and Rheumatism* 56(11) 3583-3587.

Goodson, N., et al. (2005a). Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. *Annals of the Rheumatic Diseases* 64(11) 1595-1601.

Goodson, N. J., et al. (2005b). Baseline levels of C-reactive protein and prediction of death from cardiovascular disease in patients with inflammatory polyarthritis: a ten-year followup study of a primary care-based inception cohort. *Arthritis and Rheumatism* 52(8) 2293-2299.

Gorman, J. D. and L. A. Criswell (2002). The shared epitope and severity of rheumatoid arthritis. *Rheumatic Diseases Clinics of North America* 28(1) 59-78.

Gorter, S. L., et al. (2010). Current evidence for the management of rheumatoid arthritis with glucocorticoids: a systematic literature review informing the EULAR recommendations for the management of rheumatoid arthritis. *Annals of the Rheumatic Diseases* 69(6) 1010-1014.

Graudal, N. and G. Jurgens (2010). Similar effects of disease-modifying antirheumatic drugs, glucocorticoids, and biologic agents on radiographic progression in rheumatoid arthritis: meta-analysis of 70 randomized placebo-controlled or drug-controlled studies, including 112 comparisons. *Arthritis and Rheumatism* 62(10) 2852-2863.

Greenfield, S., et al. (1993). The importance of co-existent disease in the occurrence of postoperative complications and one-year recovery in patients undergoing total hip replacement. Comorbidity and outcomes after hip replacement. *Medical Care* 31(2) 141-154.

Gregersen, P. K. and F. Batliwalla (2005). PTPN22 and rheumatoid arthritis: gratifying replication. *Arthritis and Rheumatism* 52(7) 1952-1955.

Gregersen, P. K., J. Silver and R. J. Winchester (1987). The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis and Rheumatism* 30(11) 1205-1213.

Gremese, E., et al. (2013). Very early rheumatoid arthritis as a predictor of remission: a multicentre real life prospective study. *Annals of the Rheumatic Diseases* 72(6) 858-862.

Gullick, N. J. and D. L. Scott (2011). Co-morbidities in established rheumatoid arthritis. *Best Practice & Research: Clinical Rheumatology* 25(4) 469-483.

Hafstrom, I., et al. (2009). Remission achieved after 2 years treatment with low-dose prednisolone in addition to disease-modifying anti-rheumatic drugs in early rheumatoid arthritis is associated with reduced joint destruction still present after 4 years: an open 2-year continuation study. *Annals of the Rheumatic Diseases* 68(4) 508-513.

Hakala, M. (1988). Poor prognosis in patients with rheumatoid arthritis hospitalized for interstitial lung fibrosis. *Chest* 93(1) 114-118.

Harris, E. D., Jr. (1990). Rheumatoid arthritis. Pathophysiology and implications for therapy. *New England Journal of Medicine* 322(18) 1277-1289.

Harrison, P., et al. (2006). Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients. *Rheumatology* 45(8) 1009-1011.

Hasegawa, K., et al. (2004). PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 303(5658) 685-689.

Haugeberg, G., et al. (2006). Hand bone loss in early undifferentiated arthritis: evaluating bone mineral density loss before the development of rheumatoid arthritis. *Annals of the Rheumatic Diseases* 65(6) 736-740.

Haugeberg, G., et al. (2000). Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. *Arthritis and Rheumatism* 43(3) 522-530.

Hetland, M. L., et al. (2009). MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Annals of the Rheumatic Diseases* 68(3) 384-390.

Hetland, M. L., et al. (2010). Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Annals of the Rheumatic Diseases* 69(10) 1789-1795.

Hoes, J. N., et al. (2007). EULAR evidence-based recommendations on the management of systemic glucocorticoid therapy in rheumatic diseases. *Annals of the Rheumatic Diseases* 66(12) 1560-1567.

Holmqvist, M. E., et al. (2010). Rapid increase in myocardial infarction risk following diagnosis of rheumatoid arthritis amongst patients diagnosed between 1995 and 2006. *Journal of Internal Medicine* 268(6) 578-585.

Humphreys, J. H. and D. P. Symmons (2013). Postpublication validation of the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis: where do we stand? *Current Opinion in Rheumatology* 25(2) 157-163.

Huscher, D., et al. (2013). Clinical presentation, burden of disease and treatment in young-onset and late-onset rheumatoid arthritis: a matched-pairs analysis taking age and disease duration into account. *Clinical and Experimental Rheumatology* 31(2) 256-262.

Hutchinson, D., et al. (2001). Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. *Annals of the Rheumatic Diseases* 60(3) 223-227.

Hyrich, K., et al. (2006). Baseline comorbidity levels in biologic and standard DMARD treated patients with rheumatoid arthritis: results from a national patient register. *Annals of the Rheumatic Diseases* 65(7) 895-898.

Jonsson, S. W., et al. (2001). Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. *Journal of Rheumatology* 28(12) 2597-2602.

Kapetanovic, M. C., et al. (2010). Prevalence and predictive factors of comorbidity in rheumatoid arthritis patients monitored prospectively from disease onset up to 20 years: lack of association between inflammation and cardiovascular disease. *Scandinavian Journal of Rheumatology* 39(5) 353-359.

Kaplan, M. H. and A. R. Feinstein (1974). The importance of classifying initial co-morbidity in evaluating the outcome of diabetes mellitus. *Journal of Chronic Diseases* 27(7-8) 387-404.

Karlson, E. W., et al. (2004). Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. *Arthritis and Rheumatism* 50(11) 3458-3467.

Kelly, C., et al. (2002). Peripheral bone density in patients with rheumatoid arthritis and factors which influence it. *European Journal of Internal Medicine* 13(7) 423.

Kerola, A. M., et al. (2012). How early in the course of rheumatoid arthritis does the excess cardiovascular risk appear? *Annals of the Rheumatic Diseases* 71(10) 1606-1615.

Kirwan, J. R., et al. (2007). Effects of glucocorticoids on radiological progression in rheumatoid arthritis. *Cochrane Database Syst Rev*(1) CD006356.

Klareskog, L., et al. (2006). A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis and Rheumatism* 54(1) 38-46.

Klareskog, L., et al. (2004). Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet* 363(9410) 675-681.

Kleyer, A. and G. Schett (2014). Arthritis and bone loss: a hen and egg story. *Current Opinion in Rheumatology* 26(1) 80-84.

Koduri, G., et al. (2010). Interstitial lung disease has a poor prognosis in rheumatoid arthritis: results from an inception cohort. *Rheumatology* 49(8) 1483-1489.

Kokkonen, H., et al. (2007). The PTPN22 1858C/T polymorphism is associated with anti-cyclic citrullinated peptide antibody-positive early rheumatoid arthritis in northern Sweden. *Arthritis Research & Therapy* 9(3) R56.

Kokkonen, H., et al. (2011). Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. *Arthritis Research & Therapy* 13(1) R13.

Kremers, H. M., et al. (2008). High ten-year risk of cardiovascular disease in newly diagnosed rheumatoid arthritis patients: a population-based cohort study. *Arthritis and Rheumatism* 58(8) 2268-2274.

Kremers, H. M., et al. (2004). Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. *Arthritis and Rheumatism* 50(11) 3450-3457.

Krishnan, E., T. Sokka and P. Hannonen (2003). Smoking-gender interaction and risk for rheumatoid arthritis. *Arthritis Research & Therapy* 5(3) R158-162.

Kroot, E. J., et al. (2001). Chronic comorbidity in patients with early rheumatoid arthritis: a descriptive study. *Journal of Rheumatology* 28(7) 1511-1517.

Lard, L. R., et al. (2001). Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. *American Journal of Medicine* 111(6) 446-451.

Larsen, A. (1974). *A radiological method for grading the severity of rheumatoid arthritis*. Helsinki.

Larsen, A. (1995). How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long-term studies. *Journal of Rheumatology* 22(10) 1974-1975.

Leirisalo-Repo, M. (2013). What is the best treatment strategy for early RA? *Best Practice & Research: Clinical Rheumatology* 27(4) 523-536.

Libby, P. (2006). Inflammation and cardiovascular disease mechanisms. *American Journal of Clinical Nutrition* 83(2) 456S-460S.

Lie, B. A., et al. (2007). Associations between the PTPN22 1858C->T polymorphism and radiographic joint destruction in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Annals of the Rheumatic Diseases* 66(12) 1604-1609.

Linn, B. S., M. W. Linn and L. Gurel (1968). Cumulative illness rating scale. *Journal of the American Geriatrics Society* 16(5) 622-626.

Listing, J., K. Gerhold and A. Zink (2013). The risk of infections associated with rheumatoid arthritis, with its comorbidity and treatment. *Rheumatology* 52(1) 53-61.

Lopez-Longo, F. J., et al. (2009). Association between anti-cyclic citrullinated peptide antibodies and ischemic heart disease in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 61(4) 419-424.

MacGregor, A. J., et al. (2000). Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis and Rheumatism* 43(1) 30-37.

Magnano, M. D. and M. C. Genovese (2005). Management of co-morbidities and general medical conditions in patients with rheumatoid arthritis. *Curr Rheumatol Rep* 7(5) 407-415.

Makrygiannakis, D., et al. (2008). Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Annals of the Rheumatic Diseases* 67(10) 1488-1492.

Maradit-Kremers, H., et al. (2005a). Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis and Rheumatism* 52(2) 402-411.

Maradit-Kremers, H., et al. (2005b). Cardiovascular death in rheumatoid arthritis: a population-based study. *Arthritis and Rheumatism* 52(3) 722-732.

Masson-Bessiere, C., et al. (2001). The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *Journal of Immunology* 166(6) 4177-4184.

Mathsson, L., et al. (2008). Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides. *Arthritis and Rheumatism* 58(1) 36-45.

Matthews, J. N., et al. (1990). Analysis of serial measurements in medical research. *BMJ* 300(6719) 230-235.

McCoy, S. S., et al. (2012). Hypothyroidism as a risk factor for development of cardiovascular disease in patients with rheumatoid arthritis. *Journal of Rheumatology* 39(5) 954-958.

McGovern, P. G., et al. (2001). Trends in acute coronary heart disease mortality, morbidity, and medical care from 1985 through 1997: the Minnesota heart survey. *Circulation* 104(1) 19-24.

McInnes, I. B. and G. Schett (2007). Cytokines in the pathogenesis of rheumatoid arthritis. *Nature Reviews: Immunology* 7(6) 429-442.

McQueen, F. M. (2013). Imaging in early rheumatoid arthritis. *Best Practice & Research: Clinical Rheumatology* 27(4) 499-522.

Michaud, K. and F. Wolfe (2007). Comorbidities in rheumatoid arthritis. *Best Practice & Research: Clinical Rheumatology* 21(5) 885-906.

Mikuls, T. R., et al. (2012). Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. *Arthritis and Rheumatism* 64(11) 3522-3530.

Miller, M. D., et al. (1992). Rating chronic medical illness burden in geropsychiatric practice and research: application of the Cumulative Illness Rating Scale. *Psychiatry Research* 41(3) 237-248.

Mjaavatten, M. D. and V. P. Bykerk (2013). Early rheumatoid arthritis: the performance of the 2010 ACR/EULAR criteria for diagnosing RA. *Best Practice & Research: Clinical Rheumatology* 27(4) 451-466.

Morrison, E. and H. A. Capell (2006). Corticosteroids in the management of early and established rheumatoid disease. *Rheumatology* 45(9) 1058-1061.

Myasoedova, E., et al. (2011a). Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. *Annals of the Rheumatic Diseases* 70(3) 482-487.

Myasoedova, E., et al. (2011b). Incidence of extraarticular rheumatoid arthritis in Olmsted County, Minnesota, in 1995-2007 versus 1985-1994: a population-based study. *Journal of Rheumatology* 38(6) 983-989.

Myllykangas-Luosujarvi, R., et al. (1995). Cardiovascular mortality in women with rheumatoid arthritis. *Journal of Rheumatology* 22(6) 1065-1067.

Naranjo, A., et al. (2008). Cardiovascular disease in patients with rheumatoid arthritis: results from the QUEST-RA study. *Arthritis Research & Therapy* 10(2) R30.

Neovius, M., J. F. Simard and J. Askling (2011). Nationwide prevalence of rheumatoid arthritis and penetration of disease-modifying drugs in Sweden. *Annals of the Rheumatic Diseases* 70(4) 624-629.

Newkirk, M. M. (2002). Rheumatoid factors: host resistance or autoimmunity? *Clinical Immunology* 104(1) 1-13.

Nishimura, K., et al. (2007). Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Annals of Internal Medicine* 146(11) 797-808.

Norton, S., et al. (2013a). A study of baseline prevalence and cumulative incidence of comorbidity and extra-articular manifestations in RA and their impact on outcome. *Rheumatology* 52(1) 99-110.

Norton, S., et al. (2013b). Trajectories of functional limitation in early rheumatoid arthritis and their association with mortality. *Rheumatology* 52(11) 2016-2024.

O'Dell, J. R. (2004). Therapeutic strategies for rheumatoid arthritis. *New England Journal of Medicine* 350(25) 2591-2602.

O'Dell, J. R., et al. (2002). Treatment of rheumatoid arthritis with methotrexate and hydroxychloroquine, methotrexate and sulfasalazine, or a combination of the three medications: results of a two-year, randomized, double-blind, placebo-controlled trial. *Arthritis and Rheumatism* 46(5) 1164-1170.

Okada, Y., et al. (2014). Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 506(7488) 376-381.

Oliver, J. E. and A. J. Silman (2006). Risk factors for the development of rheumatoid arthritis. *Scandinavian Journal of Rheumatology* 35(3) 169-174.

Ostergaard, M. and M. Szkudlarek (2005). Ultrasonography: a valid method for assessing rheumatoid arthritis? *Arthritis and Rheumatism* 52(3) 681-686.

Padyukov, L., et al. (2004). A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis and Rheumatism* 50(10) 3085-3092.

Palomino-Morales, R., et al. (2010). Lack of association of PTPN22, STAT4 and TRAF1/C5 gene polymorphisms with cardiovascular risk in rheumatoid arthritis. *Clinical and Experimental Rheumatology* 28(5) 695-701.

Palosuo, T., et al. (2003). Filaggrin related antibodies among the aged. *Annals of the Rheumatic Diseases* 62(3) 261-263.

Park, Y. B., et al. (2002). Effects of antirheumatic therapy on serum lipid levels in patients with rheumatoid arthritis: a prospective study. *American Journal of Medicine* 113(3) 188-193.

Paulus, H. E. (2004). Defining remission in rheumatoid arthritis: what is it? Does it matter? *Journal of Rheumatology* 31(1) 1-4.

Paulus, H. E., et al. (2000). Progression of radiographic joint erosion during low dose corticosteroid treatment of rheumatoid arthritis. *Journal of Rheumatology* 27(7) 1632-1637.

Pease, C. T., et al. (1999). Does the age of onset of rheumatoid arthritis influence phenotype?: a prospective study of outcome and prognostic factors. *Rheumatology* 38(3) 228-234.

Pedersen, M., et al. (2007). Strong combined gene-environment effects in anti-cyclic citrullinated peptide-positive rheumatoid arthritis: a nationwide case-control study in Denmark. *Arthritis and Rheumatism* 56(5) 1446-1453.

Peters, M. J., et al. (2009). Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. *Arthritis and Rheumatism* 61(11) 1571-1579.

Pierer, M., et al. (2006). Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients. *Arthritis Research & Therapy* 8(3) R75.

Pikwer, M., et al. (2012). Early menopause and severity of rheumatoid arthritis in women older than 45 years. *Arthritis Research & Therapy* 14(4) R190.

Prevoo, M. L., et al. (1995). Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis and Rheumatism* 38(1) 44-48.

Prey, S. and C. Paul (2009). Effect of folic or folinic acid supplementation on methotrexate-associated safety and efficacy in inflammatory disease: a systematic review. *British Journal of Dermatology* 160(3) 622-628.

Prince, F. H., et al. (2012). Sustained rheumatoid arthritis remission is uncommon in clinical practice. *Arthritis Research & Therapy* 14(2) R68.

Quirke, A. M., et al. (2011). Citrullination of autoantigens: upstream of TNFalpha in the pathogenesis of rheumatoid arthritis. *FEBS Letters* 585(23) 3681-3688.

Radovits, B. J., et al. (2009a). Missed opportunities in the treatment of elderly patients with rheumatoid arthritis. *Rheumatology* 48(8) 906-910.

Radovits, B. J., et al. (2009b). Disease activity as a risk factor for myocardial infarction in rheumatoid arthritis. *Annals of the Rheumatic Diseases* 68(8) 1271-1276.

Rambiharilal Shrivastava, S., P. Saurabh Shrivastava and J. Ramasamy (2013). Coronary heart disease: pandemic in a true sense. *J Cardiovasc Thorac Res* 5(3) 125-126.

Rantalaiho, V., et al. (2010). Early combination disease-modifying antirheumatic drug therapy and tight disease control improve long-term radiologic outcome in patients with early rheumatoid arthritis: the 11-year results of the Finnish Rheumatoid Arthritis Combination Therapy trial. *Arthritis Research & Therapy* 12(3) R122.

Rantapaa-Dahlqvist, S., et al. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis and Rheumatism* 48(10) 2741-2749.

Rantapaa-Dahlqvist, S., S. Wallberg-Jonsson and G. Dahlen (1991). Lipoprotein (a), lipids, and lipoproteins in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases* 50(6) 366-368.

Rasch, E. K., et al. (2003). Prevalence of rheumatoid arthritis in persons 60 years of age and older in the United States: effect of different methods of case classification. *Arthritis and Rheumatism* 48(4) 917-926.

Rashid, T. and A. Ebringer (2007). Rheumatoid arthritis is linked to Proteus--the evidence. *Clinical Rheumatology* 26(7) 1036-1043.

Raterman, H. G., et al. (2008). Rheumatoid arthritis is associated with a high prevalence of hypothyroidism that amplifies its cardiovascular risk. *Annals of the Rheumatic Diseases* 67(2) 229-232.

Raza, K., et al. (2010). Anti-modified citrullinated vimentin (MCV) antibodies in patients with very early synovitis. *Annals of the Rheumatic Diseases* 69(3) 627-628.

Register, S. S. R. (1995). *Välkommen till Svenska Reumatologi Register* [*online*]. Available at: http://www.swerre.se/cms/sv/home.

Ridker, P. M., P. W. Wilson and S. M. Grundy (2004). Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? *Circulation* 109(23) 2818-2825.

Ropes, M. W., et al. (1958). 1958 Revision of diagnostic criteria for rheumatoid arthritis. *Bulletin on the Rheumatic Diseases* 9(4) 175-176.

Rudwaleit, M., et al. (2002). Atopic disorders in ankylosing spondylitis and rheumatoid arthritis. *Annals of the Rheumatic Diseases* 61(11) 968-974.

Ruyssen-Witrand, A., et al. (2011). Cardiovascular risk induced by low-dose corticosteroids in rheumatoid arthritis: a systematic literature review. *Joint, Bone, Spine: Revue du Rhumatisme* 78(1) 23-30.

Sattar, N., et al. (2003). Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 108(24) 2957-2963.

Saulot, V., et al. (2002). Presence of autoantibodies to the glycolytic enzyme alpha-enolase in sera from patients with early rheumatoid arthritis. *Arthritis and Rheumatism* 46(5) 1196-1201.

www.sbu.se.evidensbaserad äldrevård.

Schellekens, G. A., et al. (1998). Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *Journal of Clinical Investigation* 101(1) 273-281.

Schellekens, G. A., et al. (2000). The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis and Rheumatism* 43(1) 155-163.

Schneeweiss, S., et al. (2007). Anti-tumor necrosis factor alpha therapy and the risk of serious bacterial infections in elderly patients with rheumatoid arthritis. *Arthritis and Rheumatism* 56(6) 1754-1764.

Scott, D. L. (2004). Interstitial lung disease and disease modifying anti-rheumatic drugs. *Lancet* 363(9416) 1239-1240.

Scott, D. L., F. Wolfe and T. W. Huizinga (2010). Rheumatoid arthritis. *Lancet* 376(9746) 1094-1108.

Seegobin, S. D., et al. (2014). ACPA-positive and ACPA-negative rheumatoid arthritis differ in their requirements for combination DMARDs and corticosteroids: secondary analysis of a randomized controlled trial. *Arthritis Research & Therapy* 16(1) R13.

Shen, T. C., et al. (2014a). Increased risk of chronic obstructive pulmonary disease in patients with rheumatoid arthritis: a population-based cohort study. *QJM*.

Shen, T. C., et al. (2014b). The risk of asthma in rheumatoid arthritis: a population-based cohort study. *QJM*.

Shi, J., et al. (2013). Anti-carbamylated protein antibodies are present in arthralgia patients and predict the development of rheumatoid arthritis. *Arthritis and Rheumatism* 65(4) 911-915.

Silman, A. J., et al. (1993). Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *British Journal of Rheumatology* 32(10) 903-907.

Silman, A. J., J. Newman and A. J. MacGregor (1996). Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. *Arthritis and Rheumatism* 39(5) 732-735.

Silman, A. J. and J. E. Pearson (2002). Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res* 4 Suppl 3 S265-272.

Singh, J. A., D. Furst and K. G. Saag (2012). 2012 update of the 2008 ACR recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of RA: Reply to letter by Graudal et al. *Arthritis Care Res (Hoboken)*.

Smit, M. D., et al. (2012). Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. *Arthritis Research & Therapy* 14(5) R222.

Smitten, A. L., et al. (2008). A meta-analysis of the incidence of malignancy in adult patients with rheumatoid arthritis. *Arthritis Research & Therapy* 10(2) R45.

Smolen, J. S., et al. (2010). EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Annals of the Rheumatic Diseases* 69(6) 964-975.

Sodergren, A., et al. (2007). Increased incidence of and impaired prognosis after acute myocardial infarction among patients with seropositive rheumatoid arthritis. *Annals of the Rheumatic Diseases* 66(2) 263-266.

Sokka, T., B. Abelson and T. Pincus (2008). Mortality in rheumatoid arthritis: 2008 update. *Clinical and Experimental Rheumatology* 26(5 Suppl 51) S35-61.

Sokka, T. and H. Makinen (2009). Drug management of early rheumatoid arthritis - 2008. *Best Practice & Research: Clinical Rheumatology* 23(1) 93-102.

Solomon, D. H., et al. (2004). Cardiovascular risk factors in women with and without rheumatoid arthritis. *Arthritis and Rheumatism* 50(11) 3444-3449.

Solomon, D. H., et al. (2003). Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. *Circulation* 107(9) 1303-1307.

Stahl, H. D., et al. (2000). Detection of multiple viral DNA species in synovial tissue and fluid of patients with early arthritis. *Annals of the Rheumatic Diseases* 59(5) 342-346.

Steer, S., et al. (2005). Association of R602W in a protein tyrosine phosphatase gene with a high risk of rheumatoid arthritis in a British population: evidence for an early onset/disease severity effect. *Arthritis and Rheumatism* 52(1) 358-360.

Stolt, P., et al. (2003). Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Annals of the Rheumatic Diseases* 62(9) 835-841.

Svensk reumatologisk förening (2012). *Riktlinjer för läkemedelsbehandling vid reumatoid artrit*: Svensk reumatologisk förening.

Svenson, K. L., et al. (1987). Serum lipoprotein in active rheumatoid arthritis and other chronic inflammatory arthritides. II. Effects of anti-inflammatory and disease-modifying drug treatment. *Archives of Internal Medicine* 147(11) 1917-1920.

Svenson, K. L., et al. (1988). Impaired glucose handling in active rheumatoid arthritis: relationship to peripheral insulin resistance. *Metabolism: Clinical and Experimental* 37(2) 125-130.

Symmons, D. P. (2002). Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. *Best Practice & Research: Clinical Rheumatology* 16(5) 707-722.

Symmons, D. P. and S. E. Gabriel (2011). Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. *Nat Rev Rheumatol* 7(7) 399-408.

Symmons, D. P., et al. (1998). Longterm mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well. *Journal of Rheumatology* 25(6) 1072-1077.

Tiippana-Kinnunen, T., et al. (2013). Co-morbidities in Finnish patients with rheumatoid arthritis: 15-year follow-up. *Scandinavian Journal of Rheumatology* 42(6) 451-456.

Toms, T. E., D. P. Symmons and G. D. Kitas (2010). Dyslipidaemia in rheumatoid arthritis: the role of inflammation, drugs, lifestyle and genetic factors. *Current Vascular Pharmacology* 8(3) 301-326.

Turesson, C. (2013). Extra-articular rheumatoid arthritis. *Current Opinion in Rheumatology* 25(3) 360-366.

Turesson, C., L. Jacobsson and U. Bergstrom (1999). Extra-articular rheumatoid arthritis: prevalence and mortality. *Rheumatology* 38(7) 668-674.

Turesson, C. and L. T. Jacobsson (2004). Epidemiology of extra-articular manifestations in rheumatoid arthritis. *Scandinavian Journal of Rheumatology* 33(2) 65-72.

Turesson, C., et al. (2007a). Rheumatoid factor and antibodies to cyclic citrullinated peptides are associated with severe extra-articular manifestations in rheumatoid arthritis. *Annals of the Rheumatic Diseases* 66(1) 59-64.

Turesson, C., et al. (2013). Antibodies to modified citrullinated vimentin are associated with severe extra-articular manifestations in rheumatoid arthritis. *Annals of the Rheumatic Diseases* 72(12) 2047-2048.

Turesson, C., et al. (2007b). Severe extra-articular disease manifestations are associated with an increased risk of first ever cardiovascular events in patients with rheumatoid arthritis. *Annals of the Rheumatic Diseases* 66(1) 70-75.

Tutuncu, Z. and A. Kavanaugh (2007). Rheumatic disease in the elderly: rheumatoid arthritis. *Rheumatic Diseases Clinics of North America* 33(1) 57-70.

Tutuncu, Z., et al. (2006). Do patients with older-onset rheumatoid arthritis receive less aggressive treatment? *Annals of the Rheumatic Diseases* 65(9) 1226-1229.

Ulubas, B., et al. (2004). Bronchiolitis obliterans organizing pneumonia associated with sulfasalazine in a patient with rheumatoid arthritis. *Clinical Rheumatology* 23(3) 249-251.

Ursum, J., et al. (2008). Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. *Arthritis Research & Therapy* 10(1) R12.

Wagner, U., et al. (1997). HLA markers and prediction of clinical course and outcome in rheumatoid arthritis. *Arthritis and Rheumatism* 40(2) 341-351.

Wallberg-Jonsson, S., et al. (1999). Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. *Journal of Rheumatology* 26(12) 2562-2571.

Wallberg-Jonsson, S., M. L. Ohman and S. R. Dahlqvist (1997). Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *Journal of Rheumatology* 24(3) 445-451.

Walsmith, J. and R. Roubenoff (2002). Cachexia in rheumatoid arthritis. *International Journal of Cardiology* 85(1) 89-99.

van den Broek, M., et al. (2011). Drug-free remission: is it already possible? *Current Opinion in Rheumatology* 23(3) 266-272.

van der Heijde, D. M. (1996). Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability. *Bailliere's Clinical Rheumatology* 10(3) 435-453.

van der Heijde, D. M., et al. (1991). Older versus younger onset rheumatoid arthritis: results at onset and after 2 years of a prospective followup study of early rheumatoid arthritis. *Journal of Rheumatology* 18(9) 1285-1289.

van der Helm-van Mil, A. H., et al. (2006). The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis and Rheumatism* 54(4) 1117-1121.

van Gestel, A. M., C. J. Haagsma and P. L. van Riel (1998). Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. *Arthritis and Rheumatism* 41(10) 1845-1850.

van Halm, V. P., et al. (2006). Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study. *Arthritis Research & Therapy* 8(5) R151.

van Staa, T. P., et al. (2006). Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis and Rheumatism* 54(10) 3104-3112.

van Venrooij, W. J. and A. J. Zendman (2008). Anti-CCP2 antibodies: an overview and perspective of the diagnostic abilities of this serological marker for early rheumatoid arthritis. *Clinical Reviews in Allergy and Immunology* 34(1) 36-39.

van Zeben, D., et al. (1991). Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis. Results of a followup study. *Arthritis and Rheumatism* 34(7) 822-830.

Watts, R. A., et al. (2004). Rheumatoid vasculitis: becoming extinct? *Rheumatology* 43(7) 920-923.

Wegner, N., et al. (2010). Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis and Rheumatism* 62(9) 2662-2672.

Villa-Blanco, J. I. and J. Calvo-Alen (2009). Elderly onset rheumatoid arthritis: differential diagnosis and choice of first-line and subsequent therapy. *Drugs and Aging* 26(9) 739-750.

Wilson, P. W., et al. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation* 97(18) 1837-1847.

Visser, H., et al. (2002). How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. *Arthritis and Rheumatism* 46(2) 357-365.

Wolfe, F., et al. (1994). The mortality of rheumatoid arthritis. *Arthritis and Rheumatism* 37(4) 481-494.

World Health Organization task force on standardization of clinical nomenclature (1979). Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. *Circulation* 59(3) 607-609.

Vossenaar, E. R., et al. (2004a). Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Research & Therapy* 6(2) R142-150.

Vossenaar, E. R., et al. (2004b). The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis and Rheumatism* 50(11) 3485-3494.

Vossenaar, E. R., et al. (2003). PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays* 25(11) 1106-1118.

Young, A. and G. Koduri (2007a). Extra-articular manifestations and complications of rheumatoid arthritis. *Best Practice & Research: Clinical Rheumatology* 21(5) 907-927.

Young, A., et al. (2007b). Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. *Rheumatology* 46(2) 350-357.

Zeng, Q. Y., et al. (2008). Rheumatic diseases in China. *Arthritis Research & Therapy* 10(1) R17.