Clinical and epidemiologic aspects of Rheumatoid Arthritis. Special emphasis on cardiovascular outcome and risk factors

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Cover page photo of painting ”Two Sisters (On the Terrace)”
by Pierre-Auguste Renoir (1841-1919)

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Dedicated to my beloved and most missing Parents Berit and Greger.

The pain passes, but the beauty remains

Pierre-Auguste Renoir

Every day you may make progress. Every step may be fruitful. Yet there will stretch out before you an ever-lengthening, ever-ascending, ever-improving path. You know you will never get to the end of the journey. But this, so far from discouraging, only adds to the joy and glory of the climb.

Sir Winston Churchill

Contents

Abstract

Rheumatoid arthritis (RA) is a systemic disease, with an increased risk of co-morbidity from cardiovascular disease (CVD), in particular among those with severe disease. Environmental risk factors are of potential interest for both prevention and treatment of RA.

Our aim was to examine changes in the occurrence of CVD over time and, using immunohistochemistry, study markers of inflammation in vascular endothelial cells during treatment with a TNF-inhibitor. We also investigated predictors of RA.

Two community based of RA cohorts were established in 1978 and 1995 and compared to the corresponding background population regarding CVD. Patients were followed for 8 years, and fatal and non-fatal cardiovascular first events were identified.

To investigate markers of endothelial activation, we used fourteen patients with active RA who started anti-TNF treatment. Muscle biopsies were taken at baseline and 3 months after start of treatment.

To identify incident cases of RA for evaluation of predictors of RA, we used two large surveys, the Malmö Preventive Medicine Program and the Malmö Diet Cancer Study.

Cardiovascular morbidity and mortality in the two community based RA cohorts was increased compared to the background population. Treatment with adalimumab was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA.

Smoking and a low level of formal education were independent risk factors for RA. Moderate alcohol consumption was associated with a reduced risk of RA. Reduced pulmonary function was not associated with future RA, but smoking and low socioeconomic status were independent predictors of RA.

Abbreviations

aCL	Anti-cardiolipin
ACPA	Anti-citrullinated protein antibody
ACR	American College of Rheumatology
ACS	Acute coronary syndrome
AIRDs	Autoimmune rheumatic diseases
AMI	Acute myocardial infarction
Anti-CCP	Anti-cyclic citrullinated protein antibodies
anti-TNF	TNF-alpha inhibitors
APO	Apo lipo proteins A1 and B
BAL	Broncho-alveolar lavage
BFT	Bentonite flocculation test
BMI	Body mass index
CAD	Coronary Artery Disease
CD	Cluster Diffrentiation
CEVD	Cerebrovascular disease
CHD	Coronary Heart Disease
CI	Confidence Interval
CIC	Circulating immune complexes
COMP	Cartilage oligomeric matrix protein
COPD	Chronic obstructive pulmonary disease/dysfunction
COX	Cyclo-oxygenase
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease activity score 28 swollen and tender joints
DIP	Distal interphalangeal joints
DMARDs	Disease-modifying anti-rheumatic drugs
E	Expected
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
ExRA	Extra-articular Rheumatoid Arthritis
FEV1	Forced expiratory volume within 1 second
FEV1 %	Forced expiratory volume within 1 second; % of predicted
FVC	Forced vital capacity
GOLD	Global initiative for obstructive lung disease
HAQ	Health assessment questionnaire

HDL	High density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfon (buffering agent)
HLA	Human leukocyte antigen
HR	Hazards ratio
ICAM	Intracellular adhesion molecule
ICD	International Classification of Diseases
IFN-γ	Interferon gamma
Ig	Immunglobulin
IGT	Impaired glucose tolerance
IHD	Ischemic heart disease
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
ILD	Interstitial lung disease
IMT	Intima-media thickness
IP	Interstitial pneumonitis
IQR	Interquartile range
KIR	Killer immunoglobulin-like receptors
LDL	Low density lipoprotein
MCP	Metacarpophalangeal joints
MDCS	Malmö Diet and Cancer Study
MHC	Major histocompatibility complex
MPMP	Malmö Preventive Medicine Program
MTP	Metatarsophalangeal joints.
N	Number of
NA	Not applicable
NAn	Not Analyzed
ND	Not Done
NSAIDs	Non-steroidal anti-inflammatory drugs
O	Observed
OCT	Optimal cutting temperature
OR	Odds Ratio
oxLDL	Oxidized low-density lipoprotein
PAD	Peripheral artery disease
PEG	Polyethylene glycol
PFT	Pulmonary Function Test
PIP	Proximal interphalangeal joints
PTPN22	Protein tyrosine phosphatase, non-receptor type 22

Pyr	Person-years
RA	Rheumatoid Arthritis
RAI	Ritchie's articular index
RF	Rheumatoid Factor
RPD	Restrictive pulmonary disease/dysfunction
RR	Relative Risk
SCAT	Sheep cell agglutination test
SD	Standard deviation
SE	Shared Epitope
SEI	Socioeconomic index
SIR	Standard Incidence Ratio
SLE	Systemic lupus erythematosus
SMoR	Standardized Morbidity Ratio
SMR	Standardized Mortality Ratio
ß2GPI	ß2-glycoprotein-I
TNF	Tumor necrosis factor
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
Yrs	Years

List of papers

The studies upon which this thesis is based have been reported in the following papers, referred to in the text by their respective Roman numerals. The papers have been reprinted with permission of the publishers.

I

Cardiovascular morbidity and mortality remain similar in two cohorts of patients with long-standing rheumatoid arthritis seen in 1978 and 1995 in Malmö, Sweden. *Ulf Bergström, Lennart TH Jacobsson and Carl Turesson.* ***Rheumatology (Oxford) 2009;48:1600–1605.***

II

Smoking, Low Formal Level of Education, Alcohol Consumption and the Risk of Rheumatoid Arthritis. *Ulf Bergström, Lennart TH Jacobsson, Jan-Åke Nilsson, Elisabet Wirfält, Göran Berglund and Carl Turesson.* ***Manuscript.***

III

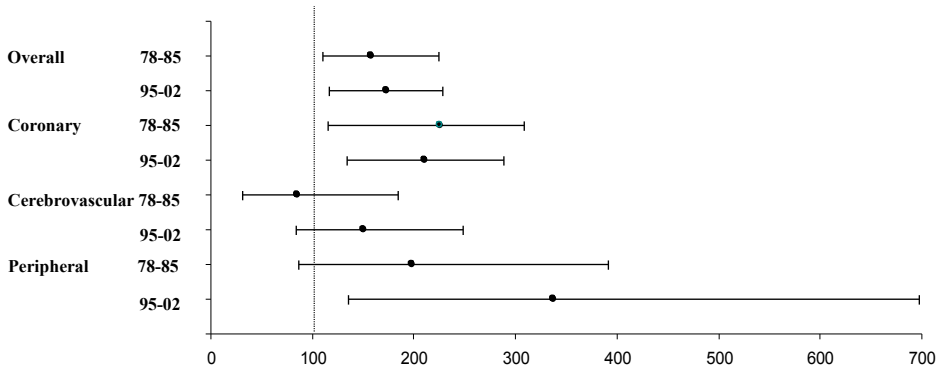
Effects of Adalimumab Treatment on Endothelial Cell Activation markers in Skeletal Muscle of Patients with Rheumatoid Arthritis. *Ulf Bergström, Cecilia Grundtman, Ingrid E. Lundberg, Lennart TH Jacobsson, Käth Nilsson and Carl Turesson.* ***Manuscript.***

IV

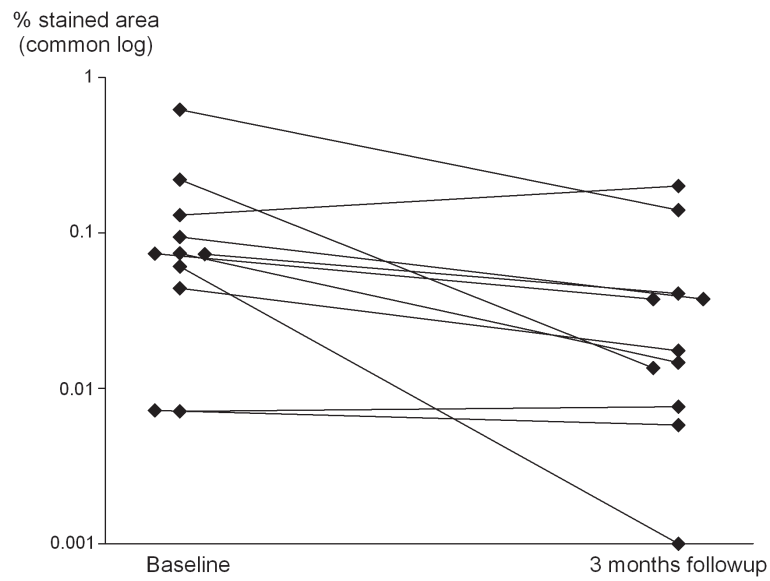
Pulmonary Dysfunction, Smoking, Socioeconomic Status and the Risk of Developing Rheumatoid Arthritis. *Ulf Bergström, Lennart TH Jacobsson, , Jan-Åke Nilsson, Göran Berglund and Carl Turesson.* ***Accepted by Rheumatology (Oxford) in May 2011 for publication.***

Thesis at a glance

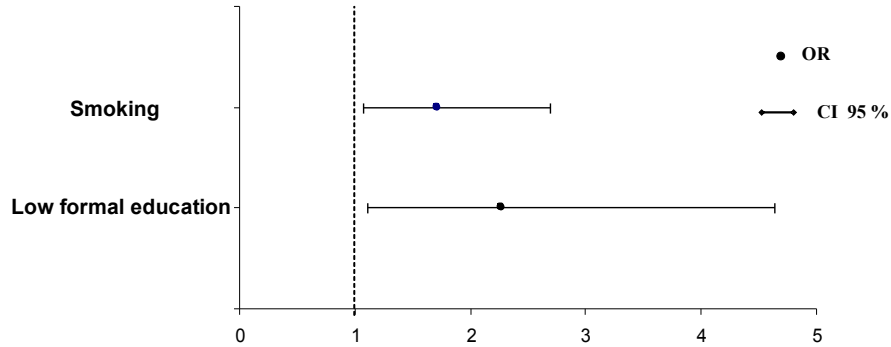
Article I. Cardiovascular morbidity over a period of 8 year follow-up for the two cohorts of RA patients identified 1978 and 1995 respectively for overall CVD and its subsets (CAD, CEVD and PAD). SMoR with 95 % CI.



Article III. Treatment with adalimumab was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA. Our findings could indicate reduced endothelial activation in patients treated with anti-TNF drugs. Endothelial HLA-DQ expression by computer assisted image analysis, at baseline and after 3 months of treatment



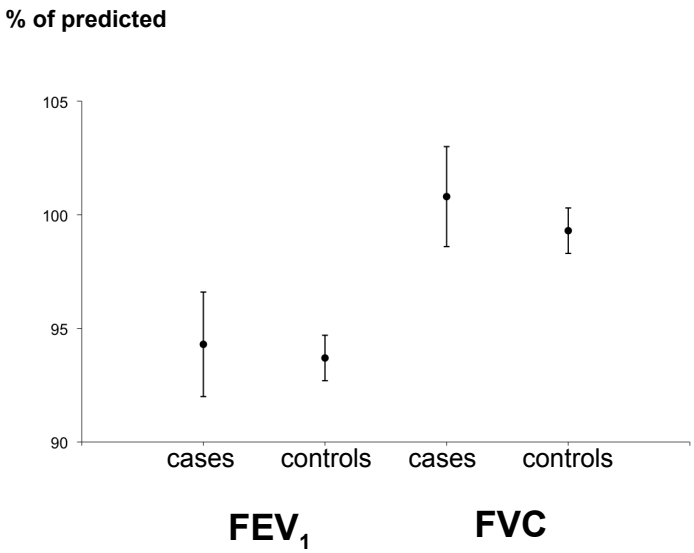
Article II. Smoking and formal education as predictors of RA in multivariate analysis, adjusted for both factors



In multivariate analyses, adjusted for current smoking and formal level of education, individuals with
- reported moderate alcohol intake, measured during one week, had a decreased risk of RA (OR 0.47; 95 % CI 0.22 to 0.99 vs. low intake)

- reported infrequent alcohol intake (within the last year, but not the last month) had an increased risk of RA (OR 3.52; 95 % CI 1.92 to 6.45 vs recent intake (within the last month)).

Article IV. Our results do not support a role for smoking induced pulmonary dysfunction in the pathogenesis of RA. Other effects of smoking may be important for RA susceptibility. Pulmonary function tests FEV1 and FVC in pre RA cases and controls. Values are given as % of predicted; Means, 95 % confidence intervals.



Background

Rheumatoid Arthritis

The patient had previously worked as a cold-buffet manageress, but had retired prematurely because of back and hip problems. She had given birth to one child, and gone into menopause at 50 years of age. She had been hospitalized once for pneumonia a few years ago, and took painkillers occasionally because of back problems – otherwise her medical history was unremarkable. She reported a normal Swedish diet. Since her early youth she had been a smoker, currently consuming about 10 cigarettes per day.

Last year at the age of 61 she had noted onset of swelling in both knee joints, which had been treated with traditional analgetics via the general practitioners, however with no major effect. Barely a year later there was worsened swelling of several joints in both hands with marked morning stiffness as well as discomfort in the shoulders, elbows, hips and knees. She had not been able to use her hands properly for many months which affected her ability to perform daily tasks satisfactorily. She also reported weight loss and feeling feverish at times. The first physical examination by a rheumatologist revealed symmetric synovitis in several metacarpophalangeal joints in both hands with a tendency to ulnar deviation, increased pulp to palm distance and also reduced strength in both hands. There was muscle atrophy of interosseus and thenar muscles in both hands, and tenderness in both ankles and toe bases, knees and hips with synovitis in both knees.

This is an authentic disease history from a patient's first presentation to a rheumatologist for consultation. Three months later she was diagnosed with Rheumatoid Arthritis (RA) and treatment was started. In most cases like hers, this will be a lifelong treatment of varying degrees and intensity depending on the disease severity.

The typical case of RA begins insidiously, with the slow development of signs and symptoms over weeks to months. Often the patient first notices stiffness in one or more joints, usually accompanied by pain on movement and by tenderness in the joint. The number of joints involved is highly variable, but almost always the process is eventually polyarticular, involving five or more joints. The joints most often involved are the proximal interphalangeal (PIP) and metacarpophalangeal (MCP) joints of the hands, the wrists (particularly at the ulnar-styloid articulation), shoulders, elbows, knees, ankles, and metatarsophalangeal (MTP) joints.

The distal interphalangeal (DIP) joints are generally spared. The spine except the atlanto-axial articulation in late disease is never affected.

Morning stiffness, persisting more than one hour but often lasting several hours, may be a feature of any inflammatory arthritis but is especially characteristic of RA. Its duration is a useful gauge of the inflammatory activity of the disease. Similar stiffness can occur after long periods of sitting or inactivity.

Non-specific systemic symptoms, primarily fatigue, malaise, and depression, may commonly precede other symptoms of the disease by weeks to months. Some patients complain of severe fatigue for hours after wakening. Fever occasionally occurs and is almost always low grade 37° to 38°C. It is typical of patients with RA that their symptoms wax and wane, often making diagnosis and treatment decisions difficult.

Historical notes

The first known traces of arthritis (symmetrical erosive arthritis, in the absence of periostal reaction or ankylosis, suggesting a diagnosis of RA) date back at least as far as 4500 BC in late archaic Indian groups from the Green River region in west central Kentucky and the west branch of the Tennessee River in northwest Alabama and Tennessee [1,2]. The first written evidence of RA may be a report on chronic symmetric polyarthritis in the Caraka Samhita, a medical text from India written between 500 BC and AD100. Subcutaneous nodules, contractures and atrophy of the limbs were described [3,4,5]. In the Old World the disease appears to have been very rare before the 1600's and on this basis some investigators believe it spread across the Atlantic during the Age of Exploration. The truth of this has been questioned and debated in many contexts [6].

The first recognized description of RA in 1800 by French physician Dr. Augustin Jacob Landra-Beauvais who was based at the famous Salpêtrière hospital in Paris. The name "rheumatoid arthritis" itself was coined in 1859 by British rheumatologist Dr. Alfred Baring Garrod.

A number of artists and other prominent persons have been affected with the disease. It is well documented that the famous impressionist painter Pierre-Auguste Renoir suffered from RA. Although no medical records remain, it is possible, thanks to photographs (Picture 1), his personal letters, and biographical notes by people who knew him well to get a reasonable idea about the course of his disease. The arthritis started around the age of 50, took on an aggressive form from 1903 onwards, when he was about 60, and made him quite disabled from the age of 70 for the last seven years of his life. Compared to current standards, the available therapeutic opportunities at that time were extremely limited.

Picture 1. Shows how Renoir had to adapt his painting technique. The brushes had to be fixed in his hands by his wife or model and he couldn't hold his palette, so he let it balance on his knees and the edge of the easel.

Picture taken from article Boonen A, van de Rest J, Dequeker J, van der Linden S. How Renoir coped with rheumatoid arthritis. BMJ. 1997 Dec 20-27;315(7123):1704-8 [7]. With permission from the author.

Epidemiology

RA is a disease that is described in all populations, but with varying prevalence (the total number of cases of the disease in the population at a given time). In Western Europe and North America, the prevalence of RA has been estimated to be between 0.5 - 1.0% [8]. In a recent study from southern Sweden, using a large patient administrative database, the overall prevalence was approximately 0.6% [9]. The highest prevalence has been observed in some North American Indian groups: the Chipewa Indians [10] and the Pima Indians [11] (up to 5%), while lower prevalence is noted in China and other parts of the Far East [12]. The lowest reported prevalence is among blacks in Africa [13]. This is probably partly due to variable frequency of the known disease risk alleles in the HLA-DRB1 region. Among the Pima Indians in Arizona, U.S., for example, 95% of the population have at least one gene copy of the risk alleles while the corresponding figure for western Europe is 35% [14] and lower for other groups with lower prevalence of RA. Other genetic and environmental factors, and also methodological issues, may explain differences in prevalence estimates between studies of various ethnic groups.

Incidence, i.e. the number of new cases in the population over a certain period of time, according to a study from southern Sweden in 2002 [15], figures are 24/100 000 (29/100 000 for women and 18/100 000 for men) inhabitants becoming ill per year. Data from both Finland [16] and England [17] as well as from North American Indians [8] suggested that the incidence has declined in recent decades. However, in a recent study from Olmsted County, Minnesota, there was an increased incidence after 1995 [18]. Overall, RA is 2-3 times more common in women, but the sex ratio is different depending on age at onset. In individuals 20-30 years of age, the incidence is much higher in women, whereas rates are higher in men aged > 50 and closer to those seen in postmenopausal women [9]. The median age of onset is 55-60 years, but RA can occur at any age. The incidence appears to be rising with increasing age up to the age of 80 [9].

Diagnosis, treatment and outcome

Diagnosis

RA is a clinical diagnosis, which is supported by laboratory tests and imaging. For the purpose of research studies a consistent and valid classification of patients is necessary [19].

Over the years, a number of different sets of classification criteria have been used.

In the main part of our papers in this thesis the 1987 criteria for classification of RA, established by the American College of Rheumatology (ACR) (formerly the American Rheumatism Association) has been used (Table 1) [20].

Table 1

1987 ACR Criteria for the Classification of RA.

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

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

At least four criteria must be met for classification as RA. As previously mentioned, the criteria were primarily intended to categorize patients in research studies. For example: one of the criteria is the presence of bone erosion on X-ray. Prevention of bone erosion is one of the main goals of treatment, since such joint damage is a sign

of progression of the disease and usually irreversible. To wait until all of the ACR criteria, including the presence of erosions, are fulfilled before making the diagnosis and initiating treatment would lead to a worse outcome in many patients. Most patients and rheumatologists agree that it would be better to treat the disease as early as possible and prevent the occurrence of bone erosion, even if it means treating people who do not meet the criteria. Because of criticism against the old criteria, mainly because of lack of sensitivity in early disease, a new classification has been proposed (Table 2). This is not surprising, because the 1987 criteria were developed in order to define homogeneous patient groups for research purposes and were based on patients in whom the average disease duration was 7 years [21].

Table 2.

Modified from the 2010 ACR-EULAR classification criteria for RA

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

Aletaha D, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum 2010; 62: 2569–81.* [317]

**** The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of RA with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.***

‡ Although patients with a score of <6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. DIP joints , first carpometacarpal joints, and first MTP joint are excluded from assessment. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

¶ "Large joints" refers to shoulders, elbows, hips, knees, and ankles.

 # "Small joints" refers to the MCP joints, PIP joints, second through fifth MTP joints, thumb PIP or DIP joints, and wrists.

*** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).*

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where RF information is only available as positive or negative, a positive result should be scored as low-positive for RF.

§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

The new ACR/EULAR classification criteria for RA identify many patients with positive test for the rheumatoid factor (RF) or antibodies to cyclic citrullinated peptides (anti-CCP) at an earlier time point, and most likely reflect current management in many early arthritis clinics better than the 1987 ACR criteria. A recent study of an early arthritis cohort from Holland [21] reported that, compared with the 1987 criteria, the 2010 ACR/EULAR criteria classify more patients with RA and at an earlier phase of the disease. They also found that the discriminative ability of the 2010 criteria is acceptable. On the other hand, in epidemiological studies of established RA, it is possible that some seronegative patients who responded quickly to treatment and did not develop erosive disease, but were classified as having RA bases on criteria I to IV during early phase, may never meet the 2010 criteria.

Autoantibodies for the diagnosis

Autoantibodies can be detected in a spectrum of rheumatic diseases where they may be highly associated with distinct clinical syndromes. These are often helpful for diagnosis, and to some extent, prognosis. In RA, RF is detected in 70-80% of patients with established disease, and is an integral part of the definition of this disorder [22].

Anti-citrullinated protein antibodies (ACPA) are directed against one or more of an individual's own, post translationally modified proteins, and frequently detected in the blood of RA patients. Anti-CCP assays are the most widely used methods to study ACPA. Anti-CCP have been evaluated in patients with early synovitis, and were found to be more specific than RF for early RA, while having comparable sensitivity [23]. Interestingly, RF and anti-CCP have both been found in blood samples taken several years before disease onset in a subset of patients [24-26].

Treatment

The aim of RA treatment is to reduce inflammation in the joints, relieve pain, prevent or slow joint damage, reduce disability and provide support to help the patient live as active a life as possible. There is good evidence that early treatment and support can reduce joint damage and limit the impact of RA. Drug treatment, lifestyle changes and other non-pharmacological interventions and surgery can all help reduce the negative effects of the disease.

Many different drugs are used to treat RA. Some aim to relieve symptoms and others help slow the progression of the condition. Everyone experiences RA differently, so it may take time to find the best treatment strategy for the individual patient. Different medicine groups are outlined below.

Disease-modifying anti-rheumatic drugs (DMARDs) help to ease symptoms and slow down the progression of RA. The immune reaction and the chronic inflammatory process leads to local release of pro-inflammatory cytokines and metalloproteinases in the joints, which can cause further damage to the bones, tendons, ligaments and cartilage. DMARDs work by blocking various stages of this process. In general, the earlier you start taking a DMARD, the more effective it will be. The ultimate goal of treatment is remission, with no or very limited clinically detected disease activity and no long-term progression of the disease. There are many different conventional DMARDs including methotrexate, gold, leflunomide, hydroxychloroquine and sulfasalazine. Methotrexate is recommended as first-line treatment for most patients [27-29], and it is often the first drug given for RA. It can be combined with other DMARDs in an initial combination or in a step-up strategy in the case of insufficient response. Based on current evidence, methotrexate is usually used in combination with biologics when such treatment is initiated.

Biologics is a newer form of treatment for RA (the first were introduced in 1999 and since used mainly in patients with severe disease). They include TNF-alpha inhibitors (anti-TNF) (adalimumab, certolizumab, etanercept, golimumab and infliximab) but also some more recently developed compounds with different mechanisms, such as abatacept, rituximab and tocilizumab,. They are usually taken in

combination with methotrexate or sometimes with another DMARD. They work by stopping particular chemicals in the blood from activating your immune system to attack the lining of your joints. Anti-TNF are mainly used in patients who have already tried methotrexate and/or another DMARD at standard doses and still have a quite active RA, although recently issued guidelines recommend their use as first-line agents together with methotrexate in patients with high disease activity at presentation and a poor prognosis [28,29].

Corticosteroids have potent anti-inflammatory effects and help reduce pain, stiffness and swelling. They can be used as systemic (mainly oral) treatment or using local injection in a single or several joints. Relief is often rapid, and this treatment may be useful as bridging therapy while the effect of other interventions is awaited. High dose corticosteroids are usually only used on a short-term basis, as long-term use could have serious side effects. Long-term treatment with low-dose corticosteroids is sometimes used in addition to traditional DMARDs in patients with early RA, and has been shown to reduce joint damage [30] and disability [31]. Non-steroidal anti-inflammatory drugs (NSAIDs) is a group of drugs that is mainly used for relief of symptoms. NSAIDs work by blocking production of pro-inflammatory mediators by cyclo-oxygenase (COX) and work in slightly different ways depending on their selectivity for different subtypes of the enzyme. There are traditional NSAIDs, such as ibuprofen, naproxen or diclofenac, and selective COX-2 inhibitors, such as celecoxib or etoricoxib. NSAIDs help relieve pain and stiffness while also reducing inflammation. However, they will not slow down the progression of RA. It is important to consider pros and cons using NSAIDs, including co-morbid conditions. NSAID tablets may not be suitable in patients with asthma, a peptic ulcer, renal failure, heart failure or a history of coronary artery disease (CAD) or stroke.

Analgetics reduce pain rather than inflammation and are used to control the symptoms of RA. The most commonly prescribed painkiller is paracetamol. In patients with severe pain, codeine in combination with paracetamol, or weak opioids such as Tramadol, are sometimes prescribed.

Finally, physiotherapy and physical exercise is very important in many patients with RA. Supervised training may be helpful to exercise the muscles and joints in a gentle way without unnecessarily tear on the joints. Physiotherapy, occupational therapy and rehabilitation play important roles in the management of RA, in particular in patients with extensive disease-related disability and pain.

Outcome

The course of RA is variable. Approximately 15 to 20 percent of patients have intermittent disease with periods of exacerbation and a relatively good prognosis.

The disease course may follow one of several patterns, such as a spontaneous remission, particularly in patients who are seronegative for RF and anti-CCP, within the first 6 months of symptoms or recurrent explosive attacks followed by periods of quiescence most commonly in the early phases, though the most common pattern is of persistent and progressive disease activity that waxes and wanes in intensity. Prognostic indicators for progressive joint destruction are the presence of RF and/or anti-CCP antibodies, high ESR (erythrocyte sedimentation rate) or CRP (C-reactive protein), early presence of radiographic erosions, and a high number of swollen joints [27]. The more numerous the unfavourable prognostic factors presented by the patient, the worse the prognosis, but the difficulty in judging prognosis in an individual patient is still considerable.

Inflammation and joint damage lead to major disability in many patients with RA [32], and this is associated with a reduced quality of life. RA is a systemic disease, which may also feature diffuse inflammation in many other organs and tissues such as lungs, heart and eyes, leading to extra-articular organ involvement RA [33]. Such extra-articular RA (ExRA) manifestations include interstitial lung disease, pleuritis, pericarditis, scleritis and severe vasculitis complications. The median life expectancy is lower in RA patients compared to the general population, mainly because of a higher occurrence of cardiovascular disease (CVD) among patients with RA [34].

Risk factors - environmental and genetic

There is major interest in attempting to understand what the factors are that lead to the development of RA and potentially even reduce the risk of developing RA. Twin studies have proved a genetic component but also quantified the non-genetic risk. These studies suggest that over 40% of the etiological fraction is due to environmental factors [35].

HLA-DRB1 alleles featuring the shared epitope are suggested to be involved in the etiology of RA and can be found in most patients with RA [36]. Among other genes, the main RA susceptibility factor is the tyrosine-phosphatase gene PTPN22 on chromosome 1 [37]. Genetic factors have also an effect on disease progression [38,39].

Changes in the female hormonal environment related to pregnancy, menopause and breast feeding have been suggested to play an important role in the development of RA. Some such factors may be associated with an increased risk, whereas others may be protective [40,41]. A number of pieces of evidence, including the fact that autoantibodies, such as RF [24] and anti-CCP [24], can develop several years before the onset of clinical disease, suggest that environmental exposure occurring long before disease onset may influence disease susceptibility. Several recent studies lend weight to the hypothesis that early life events play a role, with a reported increased risk of RA in the offspring of mothers who smoked during pregnancy [42] and in individuals with higher birth weight [42]. This may indicate that early modulation of the immune system, e.g. by higher levels of estrogen in utero leading to increased birth weight, may have long-term effects on the risk of RA. The association between high birth weight and RA is supported by several studies [43], including a case-control study from Malmö [44], but not by a case-control study based on a Swedish national survey [45]. Cigarette smoking is a risk factor for a number of other chronic diseases, and in many studies has also been associated with the development of RA [46-54]. This association is particularly strong for seropositive RA, and has been a consistent finding especially in men [49,50,54-57]. Most studies have also shown an association between smoking and RA in women [50,54], although there are exceptions [47]. Smoking individuals who carry the shared epitope have a multifold increased risk of developing RA [38]. A positive interaction between PTPN22 and smoking has also been described [58]. These studies establish that both genetic and environmental factors are important to the development and clinical outcome of RA.

The interaction between smoking and the shared epitope of HLA-DRB1 may be restricted to RA patients with anti-CCP [59]. This is of particular interest, since anti-CCP may be detected years before RA onset [24,-26]. It is unknown to what extent passive inhalation of smoke may also be associated with development of RA. Smoking individuals who carry the shared epitope have a multifold increased risk of developing RA, suggesting a gene-environment interaction [59]. This has led to the hypothesis that the autoimmune response in RA may be induced in the lungs [59]. Tissue studies have proposed smoking as an environmental factor that might lead to citrullination, potentially contributing to anti-citrulline autoimmunity in genetically susceptible individuals [60].

Most chronic diseases are reported more frequently by individuals with less than 12 years of formal education [61]. Obviously, the level of formal education may be a marker for socio-economic status or occupation. Certain occupations have previously been associated with an increased risk of developing RA [62,63]. This may be a marker for specific occupational exposures [64,65] or other factors. Infections may be related to the socio-economic background, and could trigger immune events leading to the development of RA and other disorders. In particular, RA with onset at young age may be associated with early-life infections [45].

Diet may play a role in the etiology of RA. Some prospective data indicate that red meat consumption may be associated with an increased risk of developing RA [66], although conflicting results have been published [67]. Coffee consumption was a significant predictor for RF positive RA in a prospective study from Finland [68], but not in the US Nurses' Health Survey [69].

Several factors have been reported to reduce the risk of developing RA, such as vitamin-D intake [70] in prospective studies, and regular alcohol intake in case control studies [47,71]. Issues such as confounding, recall bias and systematic misreporting may be relevant in all retrospective studies of life-style factors, in particular those on diet history.

RA pathogenesis

At present, the prevailing hypothesis is that RA involves a deleterious synergy among several processes. Thus, RA is an autoimmune disease characterized by the production of autoantibodies – e.g. RF and anti-CCP – against common autoantigens that are widely expressed outside the joints. RA is also a chronic inflammatory disease involving many cytokines that act both in series and in parallel, a phenomenon known as cytokine network redundancy. The clinical expression of RA is both systemic and local, the most devastating manifestation being joint destruction, which is ascribable in part to the inflammatory process. Experimental models have provided valuable insights into these various successive or coexisting processes [72].

Immunology

Apart from the presence of autoantibodies, RA is associated with a number of abnormalities of the immune system, including the presence of clonally expanded, autoreactive T-cells [73].

The function of the immune system is to protect the body from invasion. Immune cells recognise and remove foreign antigens (mainly proteins and carbohydrates) derived from other organisms, while tolerating very similar substances specified by the host genome. The distinction is normally precise: the body can often detect a single amino-acid substitution, but the system is not perfect and mistakes can give rise to serious disease. The immune response normally includes both cell-mediated immunity and humoral immunity. Cell-mediated immunity involves the direct killing and / or swallowing of infected host cells and pathogens by immune cells, whereas humoral immunity involves the production of soluble antibody proteins which circulate in the blood, and are secreted into other body fluids.

The mechanisms underlying the breaking of tolerance and the development of autoreactive T cells and B cells are incompletely defined for most autoimmune disorders, including RA. These are key issues for the understanding of the early disease process.

Synovitis

Rheumatoid synovitis is a complex process in which systemic and local homeostatic dysregulation is expressed in the joint. Rheumatoid synovitis consists of resident cells and invading immune cells that together arrange the inflammatory process in RA. There are 3 major types of synovitis that RA can comprise: germinal-center synovitis, aggregate synovitis, and diffuse synovitis [74]. Germinal centers are highly organized complex structures that are functionally competent. Aggregates are B cells and T cells arranged in defined follicles, yet they lack germinal-center reactions. Diffuse synovitis is the least organized type but can still cause significant damage. Differences in lymphoid microorganizations draw attention to the process of lymphoid organogenesis as a fundamental pathway of rheumatoid synovitis, a process that lends stability and sustainability to dysfunctional immune responses [74]. These subtypes are associated with distinct clinical phenotypes of RA. Patients with diffuse synovitis are more likely to be RF negative, and those with germinal center synovitis are at greater risk of ExRA manifestations [75].

In RA, disruptions in self-tolerance lead to abnormalities such as recognition of citrullinated antigens by B and T cells. The balance of lymphocyte differentiation in RA is skewed toward the Th1 phenotype, to the detriment of the Th2, and T regulator Treg phenotypes. Among the cytokines produced by T cells, interleukin (IL)-17A (previously known as IL-17) and IL-17F constitute the signature cytokines of the recently described Th17 T helper cell subset. In the presence of TNF, IL-17A and IL-17F increase the migration, chemokine gene expression and invasiveness of synoviocytes. They also inhibit synoviocyte apoptosis and enhance metalloprotease secretion, leading to cartilage damage. These properties support the combined inhibition of IL-17A and -F to control RA inflammation and joint destruction [76].

Other imbalances in the main cytokine systems including IL-1, TNF, IL-6, IL-18, IL-15, IL-33, IL-22, and IL-13 contribute to the joint destruction seen in RA [72].

In addition, specific effects of the Wnt system and osteoprotegerin on osteoclasts and matrix production dysregulation are involved in cartilage damage [72].

Extra-articular manifestations

Severe ExRA manifestations occur mainly in RF seropositive patients [77], and very high levels of RF and antibodies to anti-CCP have been found in patients with RA-associated vasculitis and Felty's syndrome [78]. Circulating immune complexes and complement activation have been implicated in vasculitis [79] and other severe ExRA manifestations [80]. Microvascular abnormalities may play a role in a number of exRA organ manifestations and in vascular co-morbidity through effects on cell migration, tissue oxygenation and hemostasis. The concept of ExRA as a systemic vascular disorder is supported by findings of perivascular cell infiltrates in rheumatoid nodules [81] and pericarditis specimen [82].

There is extensive evidence for a role for genetics in ExRA [83]. A double dose of RA-associated *HLA-DRB1*04* alleles is seen more frequently in patients with severe disease and ExRA manifestations [84,85]. This may be due to the effects of HLA-DR on selection and activation of T cells predisposing to RA and extra-articular organ involvement. T cells have also been implicated in localized extra-articular lesions. In patients with RA-associated interstitial pneumonitis (IP), immunohistochemistry studies of lung biopsies with computer-assisted image analysis have revealed increased numbers of CD4+ T cells [86] and CD20+ B cells [87] compared to patients with idiopathic IP.

Systemic inflammation in severe RA is associated with the emergence of clonally expanded, circulating T cells with features of immunosenescence, including down-regulation of the co-stimulatory molecule CD28 [73].Circulating CD4+ CD28null T cells are particularly frequent in patients with severe exRA [88]. $CD28^{null}$ T cells isolated from patients with severe RA express markers of natural killer cells, including killer immunoglobulin-like receptors (KIR) and CD56. In a recent study, the mean frequency of CD56+ $CD28^{null}$ cells in the circulating CD4+ T cell compartment in patients with ex-RA was 22.7% compared to 6.9% in age matched RA controls without extra-articular manifestations [89]. Such cells may be pathogenic through cell-cell interaction with cytotoxic effects on endothelium and other cell types [90] and increased production of interferon-γ. Similar mechanisms have been implicated in other chronic inflammatory disorders and in CAD [91].

Vascular abnormalities

Notably, patients with severe ExRA (vasculitis, pericarditis, pleuritis, or Felty's syndrome) have an increased expression of Interleukin-1α (IL-1α) and Human Leukocyte Antigen DQ (HLA-DQ) in the endothelium of small vessels in skeletal muscle compared to RA patients without extra-articular manifestations, matched for age, sex and disease duration [92]. Such endothelial activation has been suggested to contribute to systemic inflammation and vascular damage [93], leading to premature atherosclerosis. Several vascular abnormalities have been reported to be more common among patients with RA compared to healthy controls, such as increased thickness of the intima and media of the carotid artery [94,95], increased vascular stiffness [96] and endothelial dysfunction [97]. These findings fit well with the concept of an inflammatory process that leads to activation of vascular endothelium and

damage to the vessel wall, which may lead to cardiovascular events. The expression of cytokines and HLA molecules in muscle biopsy findings may indicate that this is driven by the inflammatory reaction, and effective anti-inflammatory treatment could therefore have an effect on these processes.

Artherosclerosis and inflammation

Atherosclerosis is a multifactorial process that commences in childhood but manifests clinically later in life. Atherosclerosis is increasingly considered an immune-system–mediated process of the vascular system. The presence of macrophages and activated lymphocytes within atherosclerotic plaques supports the concept of atherosclerosis as an immune system–mediated inflammatory disorder [98,99]. Inflammation can aggravate atherosclerosis via different mechanisms secondary to autoimmunity, infectious diseases, and other proatherogenic changes that occur during the inflammatory state.

Autoimmune rheumatic diseases (AIRDs), including RA, are associated with higher rates of cardiovascular morbidity and mortality, primarily secondary to accelerated atherosclerosis. This phenomenon can be partly attributed to traditional risk factors for atherosclerosis and use of specific drugs, such as corticosteroids, but might also be the result of other autoimmune and inflammatory mechanisms that are aggravated in AIRDs. Several AIRDs are associated with an increased risk of cardiovascular events, as well as signs of advanced subclinical atherosclerosis, which may precede the appearance of a clinical CVD and thus be a target of early diagnosis and preventive therapy.

Cells of the immune system can be found within atherosclerotic plaques, which suggests that they have a role in the atherogenic process. Their migration and activation within the plaques can be secondary to various stimuli, including infectious agents [100]. These cells probably aggravate atherosclerosis, because CD4+ and CD8+ T-cell depletion reduced fatty-streak formation in the C57BL/6 atherosclerosis mouse model [101]. In addition, after crossing of apolipoprotein E (ApoE)-knockout mice with immunodeficient scid/scid mice, the offspring had a 73% reduction in aortic fatty-streak lesions compared with the immunocompetent apoE mice. Moreover, when CD4+ T cells were transferred from the immunocompetent to the immunodeficient mice, the increased lesion area was increased in the latter by 164% [102]. It is therefore not surprising that like in primary lesions of AIRDs, the cellular components within atherosclerotic plaques secrete various pro-inflammatory cytokines, mainly those reflecting a Th1 type cellular immune response [103].

A cellular immune response specifically directed against heat-shock proteins, oxidized low-density lipoprotein (oxLDL), and ß2-glycoprotein-I (ß2GPI) has been reported, suggesting a direct involvement of these molecules in atherosclerosis [98]. ß2GPI can be found in human atherosclerotic lesions obtained from carotid endarterectomies, it is abundantly expressed within the subendothelial regions and the intimal-medial border of human atherosclerotic plaques, and it co-localizes with CD4+ lymphocytes [104]. Animal studies indicate that T cells specific for ß2GPI are capable of increasing atherosclerosis [105], suggesting that ß2GPI is a target autoantigen in atherosclerosis. There are probably many more such specific cell lines reacting with specific antigens that can modulate atherosclerosis by either aggravating or decreasing its extent (pro-atherogenic or anti-atherogenic).

Several autoantibodies are associated with atherosclerosis and its manifestations in humans. Animals provide good models for studying the effect of these autoantibodies on atherosclerosis. Active immunization of LDL-receptor–deficient mice

with anti-cardiolipin (aCL) antibodies resulted in development of high titers of mouse aCL and increased atherosclerosis compared with control subjects [106]. Immunization of mice with ß2GPI resulted in pronounced cellular and humoral responses to ß2GPI, with high titers of anti-ß2GPI antibodies concomitant with larger atherosclerotic lesions that contained abundant CD4+ cells. The presence of anti-ß2GPI antibodies is a marker of the antiphospholipid syndrome, which includes arterial and venous thromboembolic events.

OxLDL is the type of LDL that is more likely to undergo uptake by macrophages, which turn into the foam cells characterizing atherosclerotic lesions. Anti-oxLDL antibodies are present in patients with atherosclerosis, those with AIRDs, and healthy individuals [107]. In multivariate analyses, anti-oxLDL autoantibodies discriminated better between patients with peripheral vascular disease and control subjects than did any of the different lipoprotein analyses. There was also a tendency for higher autoantibody levels in patients with more extensive atherosclerosis [108]. Autoantibodies to oxLDL were investigated in several AIRD groups, including patients with systemic sclerosis [109], systemic vasculitides [107], and systemic lupus erythematosus (SLE) [109]. The antibody levels were higher in those patient groups than in control subjects. There was a correlation between the total level of immunoglobulins and the level of antibodies against oxLDL, whereas no correlation was demonstrated between the total immunoglobulin levels and the levels of antibodies to unrelated antigens (Epstein-Barr virus and purified protein derivative of Myocobacterium tuberculosis). This finding suggests that elevated total immunoglobulin levels in SLE patients are selective for some specific antibodies, including autoantibodies against oxLDL [109].

Atherosclerosis and RA

RA by itself seems to represent a significant risk factor for early atherosclerosis and CVD development [110]. In this setting, a number of epidemiological, clinical, and laboratory investigations suggested that chronic inflammation and immune dysregulation characterizing RA have a key role in accelerating atherosclerosis [111-113]. Like the RA synovium, atherosclerotic plaques are characterized by enhanced expression of adhesion molecules and by an abundance of proinflammatory cytokine-secreting cells attracted by locally activated endothelium and chemokines. The release of a number of collagen-breaking mediators is likely to exert a fundamental role in the destabilization of atherosclerotic plaques as well as erosion of cartilage and bone into the RA joint. According to these observations, it is conceivable that the chronic systemic inflammation characterizing RA may trigger early events, accelerating diffuse atherosclerosis development. Excess cardiovascular mortality has been demonstrated in particular in RA patients with severe extra-articular organ manifestations, such as those with lung involvement and vasculitis, and those who have elevated markers of systemic inflammation [114].

Although this may support a role for rheumatoid vasculitis in promoting atherosclerosis, there are several lines of evidence suggesting that a dysfunction, rather than a full-blown “vasculitic phenotype”, is the leading event to early endothelial damage

in RA. Functional abnormalities of the endothelium have been found in distinct cohorts of RA patients, independently of patients' age, duration of the disease, degree of disease activity, or seropositivity [94,115]. Despite the fact that different factors could alter endothelium homeostasis, prevalent data support the view that abnormal endothelial function in RA is essentially linked to inflammation. In a recent evaluation of young RA patients with low disease activity, endothelial dysfunction, assessed by brachial flow–mediated vasodilatation, was predicted independently by LDL cholesterol and by the mean levels of CRP, as evaluated at different time points. Persistent endothelial dysfunction predisposes to damage to the vascular wall that, in a preclinical stage, before overt disease, can be detectable by ultrasound measurement of carotid intimal-medial thickness (IMT). Many investigations have provided evidence of increased carotid IMT in RA [116-118].

Cardiovascular disease in the general population

Common CVD complications to atherosclerosis include those related to CAD, cerebrovascular disease (CEVD) , and peripheral artery disease (PAD). In Sweden, as in many other parts of the world, CAD is a dominant cause of death, especially at older ages. CAD also affects people in productive age, sometimes with fatal outcome. In Sweden, more than 50 000 persons are hospitalized each year for CAD, about half due to acute myocardial infarctions (AMI) [119]. There are major differences in the occurrence of CAD in different population groups, including large geographical differences within Sweden. The highest incidence of CAD in Sweden has been reported from the mainly rural county of Dalarna, in northern Sweden, and in the city of Malmö [119] . There are also significant differences between sexes, with women appearing to be relatively better protected from CAD before menopause. After menopause, this difference is quickly reduced, and it is worth pointing out that CVD is the overall leading cause of death also for women [120].

ng ACS, together with the simultaneously reduced incidence of smoking in the general population, are certainly two very important factors for decreased morbidity and mortality from CAD in recent decades [120]. It remains to be seen whether this positive trend will be continued or interrupted. For the latter alternative speaks the major increase in obesity in most populations, mainly due to less physical exercise and higher intake of carbohydrate-rich diet, and the consequent increased incidence of type-2 diabetes, in itself an important risk factor forCAD.

A large number of risk factors associated with CAD have been identified (Table 3). Such risk factors apply to both men and women and are common to most geographical and ethnic areas. The two most important risk factors for CAD are smoking and dyslipidemia. These two together togheter with diabetes, hypertension, abdominal obesity, psychosocial factors such as depression, and lack of protective factors such as daily consumption of fruits and vegetables, regular and moderate alcohol consumption and regular physical activity together accounted for 90% of the population attributable risk in men and 94% in women in the international INTERHEART study [121].

Daily intake of fruit and vegetables, regular physical activity and a moderate alcohol intake are associated with a reduced risk of AMI.

A positive impact of alcohol in this context has been reported, but this is a controversial issue. A protective effect against CAD with a J-shaped correlation has been described, indicating a reduced risk at moderate consumption and a highly increased risk among individuals with a high consumption [122]. One reason for the controversy is concern for the cost-benefit relation, due to the risks associated with an increased general alcohol intake in the population. This can lead to an increase in alcohol-related diseases, accidents and violence, in particular in younger individuals. On the other hand, intervention against CVD-associated life-style factors is likely to be even more important for long-term outcomes in younger subjects, and early onset of CAD may be preventable with lifestyle interventions.

The risk factors for CVD include factors that can not be modified, e.g. advanced age, male gender and family history of CVD. Diabetes or impaired glucose tolerance (IGT) are more important for the development of ACS than has previously been recognized [123]. This indicates that in patients with diabetes or IGT, one should be alert for symptoms suggestive of CAD and be generous with investigations which may lead to an early diagnosis. In addition, repeat checks of fasting blood glucose and, if possible, also oral glucose tests, are recommended.

This paragraph was partly based on the Medicine guide in Swedish [124].

Table 3

Predictors and protective factors for a first cardiovascular event.

Risk Factors:

Smoking
Lipid abnormalities
- High total cholesterol or LDL
- High LDL/HDL ratio
- High ApoB/ApoA1 ratio

Hypertension
Diabetes
Abdominal obesity
Psychosocial stress

Protective Factors:

High intake of fruit and vegetables
High levels of physical activity
Moderate alcohol intake

This table was modified from a corresponding table in the Medicine guide in Swedish [124].

Cardiovascular disease in Rheumatoid Arthritis patients

Increased mortality from CVD in patients with RA has been reported in a number of epidemiological surveys [125-130]. Several studies have shown increased mortality specifically from CAD [126,127,129,131], while reports on mortality and morbidity in CEVD are more contradictory [127,129,132]. The relative impact of RA on the risk of death from CVD appears to be greater in younger women, a group which in the general population has a low baseline risk. For example, in a community-based study from Finland [125], the largest reported increase of cardiovascular death in a group of RA patients was found among women aged 15-49 years, with a Standardized Mortality ratio (SMR) of 3.64.

Studies of cardiovascular morbidity in patients with rheumatic disease have been more frugal, but several recent epidemiological studies have provided support for the increased incidence of cardiovascular events in RA compared with normal population [109,111,113,131,133-136]. The risk of AMI seems to be increased already within the first year after RA diagnosis [137] as well as in patients with early inflammatory polyarthritis, especially in those who are RF positive [138].

This contrasts with the pattern for overall mortality, which has been found to be significantly increased only after more than 10 years of disease [139,140]. When studied, most traditional risk factors for developing cardiovascular disease appeared to be of importance also in patients with RA, but there is clearly an independent role for RA and a further increase of the risk in patients with severe disease and signs of systemic vascular inflammation [113]. RA per se has been suggested to be an independent cardiovascular risk factor comparable to diabetes mellitus [141].

Confirmed risk factors for developing CVD in RA patients are listed in Table 4.

Table 4

Risk factors for CVD in patients with RA.

Risk factor	Predicted outcome	References
Severe extraarticular manifestations	Total mortality and CVD mortality First-ever CVD events New onset PAD or venous thromboembolic events	Gabriel et al 2003,Maradit-Kremers et al 2005; Turesson et al 2007; Liang et al 2006
Disability (HAQ score)	Total mortality and CVD mortality First-ever CVD events	Wolfe et al 2003,Young et al 2007,Jacobsson et al 2005
ESR (≥60 mm/h at least 3 times)	Death from CVD	Maradit-Kremers et al 2005;
	CVD events	Wållberg-Jonsson et al 1999;
Last ESR	Death from CVD	Goodson et al 2005;
CRP	Death from CVD	Maradit-Kremers et al 2005;
Smoking	New onset PAD	Liang et al 2006
Hypertension	Death from CVD	Wållberg-Jonsson et al 1999; Maradit-Kremers et al 2005
Low BMI	Death from CVD	Kremers et al 2004,Escalante et al 2005

Modified from *'Cardiovascular co-morbidity in rheumatic diseases' by Turesson C, Jacobsson LTH and Matteson EL. Published Vasc Health Risk Manag. 2008;4(3):605-14 [142].*

Some studies also indicate an increased incidence of atypical myocardial infarction onset and increased incidence of silent CAD and sudden death associated with RA [109,143-145]. Case fatality after AMI is increased in patients with RA [136,146,147]. This may be due to differences in the severity of CAD between RA patients and others, or to differences in management with a tendency towards less aggressive interventions among patients with RA.

Rheumatoid Arthritis and NSAIDs

Treatment with COX inhibitors is associated with known risks in patients with established heart disease or renal impairment. A growing body of evidence indicates that traditional NSAIDs as well as coxibs have some impact on the development of CVD in patients with RA and patients with osteoarthritis [148]. On the other hand, in a survey of elderly individuals in the Pennsylvania Medicare database, except for the confirmed association with rofecoxib, no significantly increased risk of AMI in patients with RA using COX inhibitors compared to non-users was detected [149], and RA remained a significant predictor of CVD mortality in an analysis adjusted for traditional risk factors and NSAID treatment [150]. Furthermore, in a recent study of an inception cohort of patients with early inflammatory polyarthritis from Norfolk, UK, surprisingly, regular NSAID use at baseline was associated with a significantly reduced risk of death from CVD [151]. The latter finding may be due to unmeasured confounders, but, taken together, these data indicate that it is unlikely that the use of COX inhibitors is the main reason for the observed increased risk of CVD in patients with RA.

Guidelines for the prevention of cardiovascular morbidity and mortality among the general population and RA patients

Prevention of CAD involves measures designed to prevent or delay the onset in healthy individuals (primary prevention), early detection of risk factors for CAD or measures to prevent recurrence or complications of CAD (secondary prevention). According to some studies, 90 percent of AMI in the general population can be explained by known risk factors, such as lifestyle habits (smoking, diet, physical inactivity and alcohol consumption), psychosocial factors and markers related to the metabolic syndrome (obesity, hypertension, dyslipidemia and diabetes) [121].

Interventions with drug therapy to prevent CAD should be based on a comprehensive risk assessment such as the SCORE system or similar algorithms [152] and not on single risk factors such as blood pressure or lipids.

Lifestyle factors related to CVD (smoking habits, physical activity, dietary habits, alcohol habits) should increasingly be identified and documented in connection with health-care contact. It has been advocated that advice on healthy lifestyles to prevent illness and death from CAD should be considered even in the absence of hypertension, obesity or dyslipidemia. According to current guidelines, advice and support for a healthier lifestyle should in general be implemented for at least 12 months before pharmacologic treatment for hypertension or dyslipidemia is considered [153] In cases with severe hypertension (systolic blood pressure > 180 mm Hg, diastolic blood pressure > 110 mm Hg) or major elevation of S-cholesterol> 8.0 mmol / L, found on repeated occasions, drug therapy should be initiated immediately, in parallel with advice on better ways of life [153].

The Swedish Society of Rheumatology [154] has recently presented guidelines for primary prevention of CVD in patients with RA and other rheumatic disorders. They recommend that RA patients with persistent elevation of ESR or CRP and/or severe extra-articular manifestations should be considered as a group with particularly increased risk. As a risk score model, they recommend using the SCORE system [152]. Patients with rheumatic disease should be screened regularly for modifiable risk factors as mentioned before. A modified algorithm for RA patients based on the general algorithm provided by the Swedish Medical Products Agency is recommended [154]. In the modified algorithm, patients with persistent elevation of CRP/ESR or severe extra-articular manifestations are considered to be at a risk level comparable to those with CVD-related organ damage [152]. For such individuals, the recommended target levels are: blood pressure < 140/90 mm Hg, total serum cholesterol < 5.0 mmol/L, and LDL cholesterol < 3.0 mmol/L.

The guidelines issued by the European League Against Rheumatism, based on work by an international panel of experts [155] also use SCORE [152] to calculate the 10-year risk of death from CVD, but differ in some important ways from the Swedish guidelines. They recommend using a multiplication factor of 1.5 when estimating the risk based on the SCORE value in patients who fulfill two of the following three criteria: Disease duration of more than 10 years, RF or anti-CCP positivity and presence of severe extra-articular manifestations. Interventions should then be based on national guidelines for the general population.

Rheumatoid Arthritis and the lung

RA is described as a systemic disease, which may involve the lungs. The nature of the relationship between inflammation in the lungs and in the joints is incompletely understood. In 1948, Ellman and Ball described three cases with classic manifestations of RA and extensive pulmonary involvement [156]. Since then a number of studies have focused on interstitial lung disease (ILD) among patients with established RA, with widely varying estimates of incidence and prevalence depending on the methods used [157-163]. There is limited data on obstructive pulmonary disease in RA. Geddes et al found that 32 out of 100 studied patients with established RA had an obstructive lung disease, based on standard pulmonary function tests [164].

A number of pleuropulmonary manifestations which are quite typical for RA, have been described, including íntrapulmonary rheumatoid nodules (Table 5).

Pulmonary complications are common and have been reported to be directly responsible for 10 to 20% of all mortality [165-167]. Furthermore, while the prevalence of other severe ExRA seems to be declining (168,169), estimates of the incidence of RA-associated lung disease have been increasing, probably due to the improved availability of diagnostic tests [168,170]. Pulmonary infection and drug-induced lung disease are common and important differential diagnoses [171,172]. The prevalence of a particular complication varies based on the characteristics of the population studied, the definition of lung disease used and the sensitivity of the clinical investigations employed. In a clinic-based study, one-third of the subjects described clinically relevant respiratory symptoms [173], but approximately two-thirds had significant radiographic abnormalities on high-resolution computed tomography [173]. The latter is compatible with other studies (174).

Table 5

Pleuropulmonary manifestations of RA.

Interstitial
RA-ILD
• Usual interstitial pneumonitis
• Non-specific interstitial pneumonitis
• Desquamative interstitial pneumonitis
• Lymphocytic interstitial pneumonitis
Cryptogenic organizing pneumonitis (formely Bronchilitis obliterans organizing pneumonia)
Rheumatoid nodule
Apical fibrobullous disease
Rheumatoid pneumoconiosis (Caplan's Syndrome)

Airway
Upper airway obstruction
• Cricoarytenoid arthritis
• Laryngeal obstruction
Bronchiolitis
• Follicular bronchiolitis
• Constrictive bronchiolitis
• Obliterative bronchiolitis
Bronchiectasis

Pleural
Pleuritis with/without pleural effusion
Secondary empyema, pneumothorax or pleural fistula
Chyliform effusion

Vascular
Pulmonary hypertension
Vasculitis

Infection
(increased risk due to pharmacological immunosuppression or disease-related immunodeficiency)

Drug reaction
(i.e, Methotrexate-related pneumonitis etc.)

Modified from *"The lung in rheumatoid arthritis" by Amital A, Shitrit D, Adir Y. Presse Med. 2011 Jan;40(1 Pt 2):e31-48 [175].*

Risk estimate measures

Several measures are commonly used to summarize comparisons of disease rates between populations. Usually, exposed populations are being compared with those unexposed. The exposure might be to risk factors suspected of causing the disease, e.g. smoking for CVD, or of protecting against it, e.g. physical exercise. Parallel definitions can be used to compare disease rates between people with different levels of exposure to a risk factor, e.g. amount of cigarettes smoked daily. Relative risk or risk ratio (RR) is often used in medical epidemiology, and defines the risk of an event occurring (binary, i.e. zero or one, outcomes), or of developing a disease relative to exposure. It is thus often suited to clinical trial data, where it is used to compare the risk of developing a disease, in people not receiving the new medical treatment (or receiving a placebo) versus people who are receiving an established (standard of care) treatment. It is particularly attractive because it can be calculated by hand in the simple case, but is also amenable to regression modelling.

The odds ratio (OR) is a measure of effect size, describing the strength of association or non-independence between two binary data values. It is used as a descriptive statistic, and also plays an important role in logistic regression. The OR is the ratio of the odds of an event occurring in one group to the odds of it occurring in another group. Unlike other measures of association for paired binary data such as the relative risk, the odds ratio treats the two variables being compared symmetrically, and can be estimated using some types of non-random samples.

An OR or RR of 1 implies that the event is equally likely in both groups during the observation period. An OR or RR greater than one implies that the event is more likely in the first group. An OR or RR less than one implies that the event is less likely in the first group.

Logistic regression is used to predict a categorical (usually dichotomous) variable from a set of predictor variables and may be chosen if the predictor variables are a mix of continuous and categorical variables and/or if they are not nicely distributed (logistic regression makes no assumptions about the distributions of the predictor variables). Logistic regression has been especially popular with medical research in which the dependent variable is whether or not a patient has a disease.

Cox regression is comparable to logistic regression though the time factor before an event occurs is also taken into account. Hazard ratios based on cox regression are commonly used when presenting results in clinical trials involving survival data. When hazard ratios are used in survival analysis, this may have nothing to do with dying or prolonging life, but reflects the analysis of time survived to an event. A hazard is the rate at which events happen, so that the probability of an event happening in a short time interval is the length of time multiplied by the hazard. Although the hazard may vary with time, the assumption in proportional hazard models for survival analysis is that the hazard in one group is a constant proportion of the hazard in the other group. This proportion is the hazard ratio.

The SMR is the ratio of observed deaths to expected deaths according to a specific health outcome in a population and serves as an indirect means of adjusting a rate. The figure for observed deaths is usually obtained for a particular sample of a population. The figure for expected deaths reflects the number of deaths for the

larger population from which the study sample has been taken e.g. national level of mortality attributed to a particular health outcome. The calculation used to determine the SMR is simply: number of observed deaths/number of expected deaths (often multiplied by 100). If the SMR is quoted as a ratio and is equal to 1 (or 100) then this means the number of observed deaths equals that of expected cases. If higher, then there is a higher number of deaths than is expected.

A confidence interval (CI) gives an estimated range of values which is likely to include an unknown population parameter, the estimated range being calculated from a given set of sample data. If independent samples are taken repeatedly from the same population, and a confidence interval calculated for each sample, then a certain percentage (confidence level) of the intervals will include the unknown population parameter. CI are usually calculated so that this percentage is 95%, but we can produce 90%, 99%, 99.9% (or whatever) confidence intervals for the unknown parameter. The width of the confidence interval gives us some idea about how uncertain we are about the unknown parameter. A very wide interval may indicate that more data should be collected before anything very definite can be said about the parameter.

Aims

To estimate the relative risk for cardiovascular co-morbidity and mortality in two cohorts of patients with RA initiated in 1978 and 1995 compared to the background population and to investigate changes over time in RA associated co-morbidity. To evaluate traditional risk factors for cardiovascular disease, as well as disease severity markers of RA, and study their effect on the cardiovascular burden in these patients.

To investigate markers of endothelial cell activation in muscle biopsies from patients with RA before and after 3 months of treatment with adalimumab, and to correlate these data to clinical outcome variables.

To examine the effect of smoking, alcohol consumption and level of education on the risk of developing RA in nested case-control studies based on a prospective cohort health survey. To investigate the relation between pulmonary function and the risk of RA, and in the same sample analyze the impact of current and previous smoking habits and socioeconomic status on RA development.

Ethical considerations

All studies were conducted according to the Declaration of Helsinki, and the study protocols were approved by the regional research ethics committee in Lund, Sweden.

Paper III was also approved as a phase IV clinical trial by the Swedish Medical Products Agency. The study was monitored according to a standard protocol by an independent agent (the Region Skåne Competence Centrefor Clinical Research, Lund, Sweden). All participating patients gave their written informed consent to participate.

The patients included in the Malmö RA register gave their informed consent for this. All subjects included in papers II and IV gave their informed consent to be included in the MDCS or/and MPMP database. No informed consent was obtained specifically for the present studies, but the scope of the planned studies of RA was advertised in two local newspapers with good coverage, with a notice indicating that the studied subjects could contact the investigators if they did not want to participate. This procedure was also approved by the regional research ethics committee in Lund, Sweden.

Patients and methods

Paper I

Patients in the 1978 cohort and the 1995 cohort.

The cohort from 1978 consisted of 148 consecutive patients who fulfilled the 1958 American Rheumatism Association criteria for rheumatoid arthritis [176] and were seen during one month at the outpatient rheumatology clinic at Malmö University Hospital [177]. This is the only hospital serving the population of Malmö (230000 inhabitants in 1978 and 240000 inhabitants in 1995). In 1978, this was the only outpatient clinic for rheumatology in the city.

The patient cohort from 1995 consisted of 161 consecutive patients (ten patients were included in both cohorts) who fulfilled the 1987 ACR criteria for RA [20] and who during one month visited either the outpatient rheumatology clinic at Malmö University Hospital or the outpatients clinics of two board certified rheumatologists working outside the hospital. Previous surveys indicate that > 90 % of all patients with RA in the city were seen at these clinics [135,178].

All patients were systematically evaluated at baseline, using a structured protocol, including joint tenderness, disability, CRP and RF analysis as part of a previous study evaluating differences in disease severity and treatment between 1978 and 1995 [179].

Disease activity was clinically evaluated using Ritchie's articular index (RAI), which is a score for joint tenderness based on the examination of 53 joints with scoring of 26 joint areas (score range 0-78) [180]. Functional disability was evaluated according to the Steinbrocker functional class index, which categorises the patients into four classes based on the patient's ability to perform everyday activities [181].

The serum levels of CRP were analysed by the routine method at the Department of Clinical Chemistry, Malmö University Hospital, which had the same reference values for normal/abnormal values in both periods. RF was analysed at the Department of Microbiology using the bentonite flocculation test (BFT) and the sheep agglutination test (SCAT) on both occasions [182]. In this study, the patients were considered as RF seropositive when they had a SCAT titer of ≥1/16 or BFT ≥1/40. In both patient groups information on present and previous treatment with DMARDs and glucocorticosteroids, was obtained through a structured review of all the clinical records.

Through a second structured review in 2007 of both cohorts we registered information on diabetes (diagnosed before inclusion or on treatment at inclusion), blood pressure (at inclusion or one year before or after inclusion registered in subsequent case records) and current pharmacologic treatment focusing on heart and anti-hypertensive agents, anti-thrombotic drugs and on NSAIDs at inclusion. Hypertension was defined as blood pressure over 140/80 at inclusion, treatment with antihypertensive agents or diagnosis of hypertension predating inclusion. The process of data extraction and the availability of these data were similar for both cohorts.

Smoking habits (mentioned at inclusion, in case records after inclusion, indicating that they smoked at inclusion or mentioned in the case records one year before inclusion), and lipid levels (limited to total cholesterol because of lack of data on other parameters) were analyzed from other visits between 1972 and 1993 in 98 patients (66 %) for the 1978 cohort, and between 1981 and 2007 in 62 patients (39 %) for the 1995 cohort), were also registered. At the time of this review five medical records (two in the 1978 cohort and three in the 1995 cohort respectively) could not be obtained.

Standard population and cardiovascular event definition.

The general population of Malmö, aged 16 years and above, served as the standard population. The Swedish population register [183] was used to obtain the number of residents in Malmö during the studied periods.

Data on cardiovascular events were retrieved from The Swedish National Hospital Discharge Register and the Causes of Death Register [183]. The underlying assumption is that virtually all clinically significant CAD, CEVD or PAD events would lead to either hospitalization or death. These registers are both administered by the Swedish National Board of Health and Welfare. The hospital discharge register is based on reports from local registers, and includes information on age, sex, and place of residence for each individual, as well as the time for hospitalization and discharge diagnoses (primary and secondary), classified according to the International Classification of Diseases (ICD), eight, ninth and tenth revision (ICD-8, ICD-9 and ICD-10) [184-186] for each in-patient episode. ICD-9 uses the same codes as ICD-8 for diagnoses used in these analyses. The proportion of hospital discharges not reported to the register has been estimated to be 1-2 % [183]. In evaluations of the accuracy of registered diagnoses conducted in 1987 and 1995, 86 % of episodes classified as being due to AMI fulfilled predefined criteria for definite AMI, whereas 9 % were considered to have had possible AMI [183]. The Causes of Death Register is based on compulsory reporting of underlying and contributing causes of death, and contains information on age, sex, place of residence and date of death. In 1996, the register was estimated to include data on 99.64 % of all deaths [183]. Previous validation studies of death certification for AMI have reported confirmation rates of 92-96 % [187].

Table 6 Classification of CVD events based on ICD codes.

	ICD-8 (1969-1986) and ICD-9 (1986-1997)	ICD-10 (1997-)
Coronary artery disease		
AMI	410,412	I21-I23
Other acute and subacute forms of ischemic heart disease	411	I24
Angina pectoris	413	I20
Other forms of chronic ischemic heart disease	414	I25
Cerebrovascular disease		
Intracerebral hemorrhage	431,432	I61,I62
Other and unspecified stroke	434	I63,I64
Occlusion and stenosis of cerebral and precerebral arteries	433	I65,I66
Other cerebrovascular disease	435-438	I67-I69
Peripheral vascular disease:		
Atherosclerosis	440	I70
Major arterial aneurysm	441,442	I71,I72
Other peripheral vascular disease	443x	I73.9
Arterial embolism and thrombosis	444	I74

For both cohorts data on all hospital episodes with a registered diagnosis (both underlying and contributing causes) of CAD, CEVD or PAD (Table 6), and all deaths using the same ICD codes were retrieved from the Hospital Discharge Register and Causes of Death Register. For the period Jan 1st, 1974 up to Jan 1st 1997 (for the causes of Death Register) and Jan 1st 1998 (Hospital Discharge Register) ICD-8 and ICD-9 codes were used, and for the period after these dates to the end of the study the corresponding ICD-10 codes were used. To get an estimation of prevalent CVD, events for a period of four years prior to baseline, i.e. 1974-1978 and 1991-1995, were captured in a similar fashion through linkage with the Hospital Discharge Register. The period of four years prior to baseline was used since the starting date for the Hospital Discharge Register in Malmö was Jan 1st 1974. Patients with prevalent CVD were excluded from the analysis of first ever CVD events.

Paper III

Phase IV clinical trial – regulatory issues, inclusion and exclusion criteria

This phase IV clinical trial was approved by the Swedish Medical Products Agency and the study was registered with ClinicalTrials.gov, number NCT01270087.

The study was monitored according to a standard protocol by an independent agent.

Patients who fulfilled the 1987 ACR classification criteria for RA [20], and for whom treatment with adalimumab (Humira) was indicated according to their rheumatologist, were enrolled. Additional inclusion criteria and exclusion criteria are described in Table 7.

Table 7 Inclusion and exclusion criteria

Inclusion Criteria
Non-responders to ≥ 1 DMARD
≥ 6 swollen joints in 28-joint index at inclusion visit
CRP > 8 mg / L within the last 3 months before inclusion
Exclusion criteria
Anti-TNF treatment within the last 3 months prior to inclusion
Corticosteroids intravenous treatment < 15 days before inclusion ongoing high-dose oral treatment (equivalent to ≥ 20 mg of prednisolon daily) or completed such treatment < 15 days before inclusion
Contraindications to muscle biopsy, such as severe bleeding disorder extensive or refractory leg ulcers severe peripheral vascular disease

Tissue samples, clinical evaluation and laboratory parameters.

Muscle biopsies were performed before and after three months of treatment with adalumimab. Biopsies were taken from the tibialis anterior muscle and were obtained under local anesthesia using a semi-open technique [188]. At least three biopsy samples were taken from each patient, snap frozen in isopentane chilled with liquid nitrogen, and stored at -70°C. The second biopsy was taken from the contralateral leg.

All muscle samples were assessed without knowledge of the patient history for histopathological changes by an experienced neuropathologist - Dr I Nennesmo at the Division of Pathology, Karolinska University Hospital Huddinge, Sweden. Muscle biopsies were evaluated using conventional histopathology and immunohistochemistry on serial sections to identify pathological changes. The first and last section of each series of consecutive sections were stained with Mayer's haematoxylin and

eosin [189], to confirm that the histopathology of the biopsies remained unchanged in the consecutive series of sections. These sections were also used for evaluation of the presence of degeneration, regeneration, atrophy, central nuclei and inflammatory cell infiltrates. All slides were coded, and blinding was maintained during this assessment.

Patients were evaluated at baseline and after 3 months of treatment with adalimumab for RA disease activity, using standard measures (number of swollen joints, number of tender joints, RF, CRP, health assessment questionnaire (HAQ) disability index, patient's assessment of pain, patient's global assessment of disease activity and physician's assessment of disease activity). The disease activity score, based on 28-joint counts of swollen and tender joints (DAS28) was calculated [190]. In addition, a standard physical examination was performed, and data on medications and cardiovascular risk factors such as smoking, current hypertension and history of cardiovascular events were recorded using a structured clinical interview.

Immunohistochemistry studies

The skeletal muscle biopsy specimens were frozen in pre-cooled isopenthane, embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek BV, Zoeterwoude, The Netherlands) and stored at -70˚C until sectioning was performed. Cryostat sections from the biopsies (6-8μm) were placed on chrome gelatin-coated slides (Novakemi AB, Enskede, Sweden) and air dried for 30 minutes. The sections were initially fixed for 20 minutes with freshly prepared 2% formaldehyde (SigmaChemicals, St Louis, MO, USA) at +4˚C, washed twice in phosphate buffered saline (PBS) for 5 minutes and then left to air-dry before storage at -70°C. Immunohistochemical staining was performed in the following way.

The fixed sections were washed in buffer (900 ml sterilized water and 100 ml Earl's balanced salt solution [EBSS; Gibco BRL, Paisley, UK]), 10 ml HEPES (Gibco BRL), and 0.1% saponin (Reidel-de Haen AG, Seelze, Germany) at pH 7.4 (referred to as EBSS-Sap) for 10 minutes. After the washing procedure a blocking step was performed with 1% H2O2 and 2% NaN3 in EBSS-Sap for 1 hour in darkness at room temperature. The sections were washed 3 times in EBSS-Sap and blocked for 15 minutes with 1% normal horse serum (Dako, Glostrup, Denmark) for HLA-DQ, IL-1α, IL-1β, TNF, ICAM-1, VCAM-1 and CD31. After washing with EBSS-Sap, the sections were blocked with avidin (with 0.1% saponin) (Vector, Burlingame, CA) and, after a further washing with EBSS-Sap, with biotin (with 0.1% saponin) (Vector) for 15 minutes, respectively.

The primary antibodies used to identify expression of endothelial and negative controls are listed in Table 8. The sections were incubated with the primary antibody overnight at room temperature in a humid chamber. After washing, the secondary antibody (biotinylated horse anti-mouse IgG1, Vector Laboratories, Burlingame, CA, dilution 1/320) , was diluted in EBSS-Sap containing 1% normal horse serum, and the antibody-serum mix was then applied to the sections for 30 minutes. After washing, sections were incubated with peroxidase-conjugated ExtrAvidin (1:2000; Sigma, St. Louis, MO) for 45 minutes. Reactions were developed using a Peroxidase Substrate Kit (Vector) containing 3,3'-diaminobenzidine, with Mayer's hematoxylin counterstaining. Slides were mounted using buffered glycerol. Staining was performed on consecutive sections. Slides were evaluated using a Polyvar II microscope (Reichert-Jung, Vienna, Austria) connected to a 3CCD color camera (DXC-750P; Sony, Tokyo, Japan).

Evaluation of muscle biopsy stainings.

The sections were coded, and the investigator was blinded to the clinical information for each section. Whole tissue sections were assessed using computer assisted image analysis. The method has been described in detail previously [191]. Analysis of an entire tissue section typically involved 10-30 microscopic fields. The area of specific immunostaining was expressed as a percentage of the total tissue area evaluated.

Table 8. Antibodies used for immunohistochemical stainings*

Antigen	Clone	**Dilution or concentration used for tissue staining**	**Isotype**	**Supplier**
HLA-DQ	SK10	1/80	Mouse IgG_1	Becton-Dickinson, San Jose, CA
IL-1α	1277-89-7	1 mg/ml	Mouse IgG_1	Immunokontakt, Bioggo, Switzerland
IL-1β	2D8 combined with 1437-96-5	1 mg/ml	Mouse IgG_1	Immunokontakt, Bioggo, Switzerland
TNF *	2C8	5 mg/ml	Mouse IgG_1	Biodesign, Saco, ME
TNF *	Mab1 combined with Mab11	500 µg/ml	Mouse IgG_1	BD, PharMingen, San Diego, CA
ICAM-1	84H10	1 mg/ml	Mouse IgG_1	AbD Serotec, UK
VCAM-1	51-01C9	0,5 mg/ml	Mouse IgG_1	Becton-Dickinson, San Jose, CA
CD31	6002-1	1/400	Mouse IgG_1	Novakemi, Stockholm, Sweden
Negative control	X 0931	100 µg/ml	Mouse irrelevant IgG_1	Dakopatts A/S, Glostrup, Denmark

** Clone 2C8 is a non-neutralizating antibody and Mab1 combined with Mab11 is neutralizing antibodies.*

Paper II and IV

Malmö Diet and Cancer Study (MDCS)

Paper II used information from the MDCS which is a prospective cohort study performed in Malmö, a city in the south of Sweden (approximately 245,000 inhabitants in 1995). The MDCS source population was, in 1991, defined as all persons living in the city of Malmö and born during 1926–1945. However, in May 1995, the cohort was extended to include all women born during 1923–1950 and all men born during 1923–1945. With this extension, 74,138 persons constituted the source population. Inadequate Swedish language skills and mental incapacity were the only exclusion criteria. During the baseline examinations from March 1991 to October 1996 a total of 30447 persons joined the MDCS (Figure 1). Details of recruitment and the cohort are described elsewhere [192]. Participants visited the MDCS screening centre twice. During the first visit, they were informed about the study and instructed how to register meals in the menu book and how to fill in the diet questionnaire. Participants completed all questionnaires at home. During the second visit, approximately 2 weeks after the first, the socioeconomic questionnaire was checked for completeness and the dietary interview conducted.

Malmö Preventive Medicine Program (MPMP)

Paper IV used information from the MPMP, which was a preventive case-finding program, focusing on cardiovascular risk factors and alcohol abuse and started in 1974 at the Department of Preventive Medicine, Malmö University Hospital in the city of Malmö, Sweden (population 235 000 in 1974). The aim was to screen large strata of the adult population in order to find high risk individuals for preventive interventions [193]. Subjects were invited to participate in health screening, including a physical examination and laboratory tests [193]. In addition, every participant filled out a self-administered questionnaire, including questions on lifestyle. Between 1974 and 1992, a total of 22,444 males and 10,902 females (Figure 1) attended the screening program with an overall attendance rate of 71%, differing somewhat between years (range 64–78%). Males were mostly screened in the first part of the period (1974–82), and females in the latter part (1981–92), thus implying different length of follow-up time. Various interventions (lifestyle modification, drug therapy) engaged nearly 25% of the screened subjects [193].

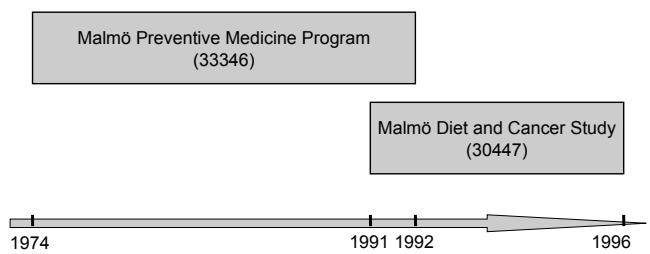


Figure 1. Inclusion of participants over time in MPMP and MDCS

Case and control selection.

From both MDCS and MPMP cohorts we identified persons who developed RA (from inclusion until 2004-12-31) after inclusion by linking both registers to a community based RA register [194], the local outpatient clinic administrative register for Malmö University Hospital, the National Hospital Discharge Register [195] and the National Cause of Death Register [196]. In a structured review of the medical records, patients were classified according to the 1987 ACR criteria for RA [20], and the year of RA diagnosis was noted together with RF status (ever vs never positive; MDCS only). Four controls for every case, matched for sex, year of birth and year of screening, who were alive and free of RA when the index person was diagnosed with RA, were selected from the corresponding register.

Variables in the MDCS

Data on level of formal education, smoking (including cigarettes, cigars, cigarillos and pipe tobacco) and alcohol consumption were collected from the self administered MDCS questionnaire. Formal education was classified into five levels: ≤ 8 years of elementary school, 9-10 years, 11-12 years, >12 years and university degree. University degree was used as the reference. Based on the smoking data from the questionnaire patients were classified into four categories : current regular smoker, occasional smoker, former smoker and never smoker. We created a dichotomous variable, comparing current regular smokers to a reference group containing all other. Three patterns of reported alcohol consumption were registered from one single questionnaire item requesting information during the preceding year (Figure 2), no consumption (abstainers), infrequent consumption and recent consumption. The pattern variable has been used in previous studies, and when used together with follow-up questions on alcohol consumption habits, it is a valid method for assessing alcohol intake [197]. Alcohol exposure in the cohort was also assessed from the menu book, completed during 7 consecutive days following the first visit to the screening centre [198,199], and expressed in grams/day (figure 2).

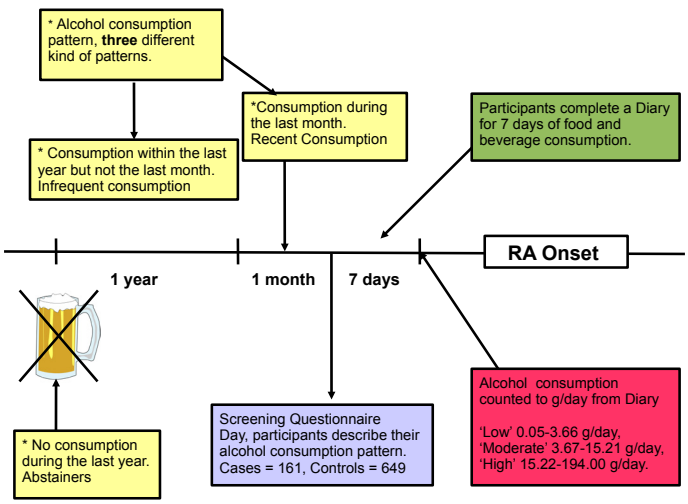


Figure 2. How information on alcohol intake was collected in two separate ways in MDCS.

Among those who reported alcohol intake in the menu book, we used quartiles to define them in three categories, the middle category containing quartiles two and three: 'Low' 0.05-3.66 g/day, 'Moderate' 3.67-15.21 g/day, 'High' 15.22-194.00 g/day (Figure 2). For comparison, one glass containing approximately 150 millilitres of wine with an alcoholic strength of about 12 % corresponds to 14.0 g of alcohol. The two alcohol variables, i.e., alcohol consumption patterns during the last year from the questionnaire and alcohol intake from the menu book, were treated as independent exposures. For example, an individual could report no alcohol within the last year, but some alcohol consumption in the menu book, or individuals reporting no consumption in the menu book may have reported some alcohol intake during the last year. In logistic regression models, recent alcohol consumption was used as reference for the analyses of alcohol consumption pattern. All models of quantified alcohol intake were adjusted for total energy intake.[198,199] In the analyses concerning alcohol consumption per day we excluded persons who were considered to be misreporters of total energy intake. Energy misreporting was defined as having a ratio of energy intake to basal metabolic rate outside the 95% CI limits of the calculated physical activity level [199]. This definition (i.e., of low-, adequate-, and high-energy reporters in the MDCS cohort) was previously described in detail by Mattisson et al. using the approach described by Goldberg et al. [200] and later refined by Black et al. [201]. The exclusions left 97 cases and 432 controls to be included in these analyses. Low alcohol intake was used as the reference.

Variables in the MPMP

Pulmonary function tests were assessed by a screening spirometry performed by a spirotron apparatus according to a standard protocol as part of the health survey [202]. Obstructive pulmonary dysfunction was defined using the Global initiative for obstructive lung disease (GOLD) criteria for chronic obstructive pulmonary disease (COPD) diagnosis using measured spirometry values of the forced vital capacity (FVC) and the forced expiratory volume within 1 second (FEV1), in this case without previous bronchial dilatation. COPD stages are defined in Table 9 [203].

Table 9. Stages of COPD according to the GOLD standard:

		FEV_1/FVC	FEV_1 - % of predicted
Stage I	Mild COPD	<0.70	≥ 80%
Stage II	Moderate COPD	<0.70	50-79%
Stage III	Severe COPD	<0.70	30-49%
Stage IV	Very Severe COPD	<0.70	FEV_1 <30%, or <50% with chronic respiratory failure present*

Restrictive pulmonary dysfunction (RPD) was defined as reduced FVC without major reduction of the FEV1/FVC ratio [204], with cut-offs of ≤ 80 % of predicted FVC and ≥ 70 % for the FEV1/FVC ratio. Predicted FEV1 and FVC were calculated using the European Respiratory Society standard [205]. Based on the smoking data from the survey questionnaire, subjects were classified using three dichotomous variables: current smoker (yes vs no), current smoker with a history of smoking for more than 10 years (yes vs no), and current smoker with reported smoking of more than 20 cigarettes per day (yes vs no). Data on socioeconomic status was derived from self-reported job titles in the Swedish national censuses carried out in the years 1960, 1970, 1980, 1985, and 1990 [206]. In the 1975 census no job title was asked for. Occupation was coded using national adaptations of the Nordic Occupational Classification. Three-digit codes were combined into 53 occupational groups and 1 group of people who were economically active but whose occupations were unknown [207], and converted into standardized social class categories expressed as a socioeconomic index (SEI) [208]. Both men and women were classified according to their reported occupation in the census immediately before or following inclusion in the MPMP. Retired individuals were classified according to the last reported occupation in a previous census. Housewives, students, and unemployed without any classification during the study period were excluded [209]. Subjects were classified as blue-collar workers, white-collar workers and "other". Blue-collar workers included manual workers, both skilled and unskilled. White-collar workers included non-manual employees of high-level, medium-level and low-level, as well as self-employed professionals such as lawyers and architects. Finally, the "other" category covered other self-employed individuals, including farmers.

Statistical analyses

In ***paper I***, the groups were compared using the students t-test for normally distributed variables, the Mann-Whitney U-test for variables without a normal distribution, and the chi-square test for categorical variables.

Cardiovascular event rates in ***paper I*** were calculated for the time periods 1978-1985 for the 1978 cohort and 1995-2002 for the 1995 cohort. To enable age- and sex-specific cardiovascular event rates, the male and female subgroup of patients and controls were stratified into 15 age groups (i.e.14 5-year periods; 15-19 yr olds, 20-24 yr olds etc., and a ≥85 yr old group). We also stratified the events into time intervals of four years, for the 1978 cohort (1978-1981 and 1982-1985) and the 1995 cohort (1995-1998 and 1999-2002). Based on indirect standardization to the Malmö general population, the expected number of events, based on information from the same national registers, i.e. the Hospital Discharge Register [195] and the Causes of Death Regíster [196], for each group in the RA cohorts was estimated. Age adjusted SMR (see risk estimate measures in background) and standardized morbidity ratios (SMoR) with 95 % CI, were calculated for each sex separately for CAD, CEVD and PAD and also for CVD overall. Both fatal and non-fatal events were included when estimating the morbidity rates and the SMoR. In these analyses, a single patient can contribute to all three subgroups (CAD, CEVD and PAD) if the patient has been discharged (or died) with several diagnoses during the follow up period. In each of the RA cohorts separately, the impact of disease severity markers and traditional CVD risk factors on the risk of new onset cardiovascular morbidity during the study period was examined using Cox regression (see **risk estimate measures** in background), adjusted for age at baseline and sex.

In the comparison of baseline findings and after three months treatment in ***paper III***, the paired T test was used for parameters with a normal distribution (i.e. IL-1α and CD31 expression by computer assisted image analysis); and the results were presented as mean pairwise differences with 95 % CI. For parameters without a normal distribution (i.e. HLADQ expression by computer assisted image analysis), the Wilcoxon sign rank test was used, and the results were presented as median pairwise differences and p-values for between group differences. These comparisons were stratified by EULAR good/moderate responder [210] status at 3 months after start of adalimumab. In an additional post-hoc exploratory analysis, the analyses were stratified by current smoking status at baseline.

Also in ***paper III*** we analyzed correlations between changes (from inclusion to after three months of treatment) in clinical parameters (i.e. DAS28 [190]) and endothelial markers (i.e. IL-1α and HLA-DQ expression by computer assisted image analysis). For parameters with a normal distribution, Pearson's correlation test was used, and for parameters without a normal distribution we used Spearman's correlation test.

In ***paper II and IV*** potential predictors were examined in conditional logistic regression models, taking into account the matched design of the study. Each case and the corresponding controls were assigned a group number, and this was entered into the logistic regression models (see risk estimate measures in background) as a categorical variable. Analyses were performed bivariately and also adjusted for the

other risk factors evaluated in multivariate models . The analyses in ***paper II*** were also stratified by RF status (positive vs negative) at diagnosis or later in the cases. The analyses in ***paper IV*** were also stratified by gender (men vs women) and we also stratified by time from inclusion to RA diagnosis in ***paper IV***. FVC, FEV1 and FVC/FEV1 values in cases and controls in ***paper IV*** were compared using Student's T test.

Results and Discussion

Brief summary of Results

- In papers I and III we have studied different aspects of cardiovascular disease, clinical events e.g. myocardial infarction and markers of inflammation in vascular endothelial cells.

In a longitudinal study (paper I), which mainly covers the period before anti-TNF drugs were in regular use, we evaluated changes over time (1978-2002) in cardiovascular morbidity and mortality in patients with established RA. We found that there was an increased incidence of cardiovascular events and deaths from CVD in patients with RA compared to the general population. There was no change over time in excess CVD morbidity, in spite of more extensive treatment with DMARDs and reduced disease severity in the later of the two cohorts.

In a phase IV clinical trial (paper III), we showed that treatment with the anti-TNF drug adalimumab was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA. Our findings could indicate reduced systemic endothelial activation in patients treated with anti-TNF drugs, which might reduce the risk of cardiovascular co-morbidity.

- In papers II and IV, we have studied risk factors for developing RA in two studies with similar design.

In these nested case-control studies, based on two separate prospective health surveys, we found that smoking and low level of formal education were independent risk factors for RA (paper II). Smoking and low SEI, defined as blue-collar worker status based on occupation, were also independent risk factors for developing RA (paper IV).

Our results from analysis of spirometry data do not support a role for smoking induced pulmonary dysfunction in the pathogenesis of RA (paper IV). Moderate alcohol consumption tended to be protective for RA development in multivariate analyses (adjusting for smoking and formal level of education) and infrequent alcohol intake pattern was associated with an increased risk of developing RA (paper II).

Results *Paper I*

Lower disease activity was found in the 1995 cohort compared to the 1978 cohort, measured by CRP ($p < 0.001$) as well as by RAI for joint tenderness ($p < 0.001$). Disability classified by Steinbrocker's functional class index showed a pattern with twice as many patients in class I and only half as many in class III-IV in the 1995 cohort (Table 10).

Table 10.

Baseline data for the two RA cohorts.

	1978 Cohort (N =148)	**1995 Cohort (N=161)**
Age at disease onset (yrs)	46.3 (14.2)	48.3 (16.4)
Female (N; %)	117 (79.1)	125 (77.6)
Disease duration (yrs)	14.0 (10.3)	13.4 (11.5)
RF seropositive (%)	88**	73
CRP (mg/l)†		
: 0-9 (N, (%))	41 (28)	85 (53)
: 10-49 (N, (%))	46 (31)	39 (24)
: ≥50 (N, %)	61 (41)	37 (23)
Steinbrockers Functional index†		
: Class 1 (N, (%))	26 (18)	59 (37)
: Class 2 (N, (%))	82 (55)	81 (50)
: Class 3 (N, (%))	31 (21)	19 (12)
: Class 4 (N, (%))	9 (6)	2 (1)
RAI (Median;IQR)	7 (3-15) ***	4 (2-8)
Number of DMARDs ever taken	1.46 (0.98) ***	2.66 (2.00)
Current NSAID (N,%)	133 (90)	123 (76)
Current DMARD (N,%)	75 (51)	108 (67)
Current methotrexate (N,%)	0	33 (20.4)
Current anti-malarial (N,%)	36 (24)	17 (10)
Current biologics (N,%)	0	0
Current Glucocorticosteroids (N,%)	18* (12)	35 (22)

Values are given as means (standard deviation) where not otherwise indicated. P = 0.03. ** P = 0.001; *** P < 0.001 vs 1995 cohort.*

† P < 0.001 for the distribution of CRP levels and Steinbrocker's

Functional Index in the 1978 cohort vs 1995 cohort (Chi square test).

Blood pressure measured at inclusion was slightly lower in the 1995 cohort (Table 11). Overall, the proportions treated for hypertension were similar in the two groups (24 % in 1978 vs 23 % in 1995). Lipid lowering drugs were not used at all in either of the cohorts. Anti-platelet drugs were only used by nine patients (4.5 %) in the 1995 cohort, and by none in the 1978 cohort. Cholesterol levels were higher in the 1995 cohort compared to the 1978 cohort (mean 5.68 vs 4.75 mmol/l; p=0.001).

Data on smoking were available for 104 patients (70 %) in the 1978 cohort and 82 (51 %) patients in the 1995 cohort. There was a trend towards fewer current smokers in the 1995 cohort (24 % vs 31 %).

Table 11

Cardiovascular risk factors at inclusion.

	1978 Cohort (N =148)	**1995 Cohort (N=161)**
Hypertension (N; %)	81 (55)	90 (56)
Systolic blood pressure (mmHg)	145 (23.6)	144 (18.2)
Diastolic blood pressure (mmHg)	84 (9.4)	81 (9.9)
Antihypertensive treatment (N; %)	35 (24)	37 (23)
Diabetes (N; %)	5 (3.4)	4 (2.5)
Smoking: Current (N; %)	33 (31)	20 (24)
Former (N; %)	8 (8)	15 (18)
Non smoker (N; %)	63 (61)	48 (58)
Cholesterol (mmol/l) **	4.75 (1.13) *	5.68 (1.22)

**p=0.001 vs the 1995 cohort. Values are given as means (standard deviation) where not otherwise indicated. Hypertension was defined as blood pressure over 140/80 at inclusion, treatment with antihypertensive agents or diagnosis predating inclusion.*

Diabetes was defined as a documented clinical diagnosis at the time of assessment.

*** Based on 98 subjects in the 1978 and 62 subjects in the 1995 cohorts, respectively.*

Cardiovascular Morbidity

Sixty-eight patients had a first ever cardiovascular event during the study period (31 in the 1978 cohort, 37 in the 1995 cohort). In the 1978 cohort 24 first ever events of CAD, 6 of CEVD and 8 of PAD occurred, where as in the 1995 cohort, the corresponding figures were 23 for CAD , 15 for CEVD and 7 for PAD. Overall CVD morbidity was elevated compared to the background population in the 1978 cohort (SMoR 158 (CI 111-225)) as well as in the 1995 cohort (SMoR 173 (CI 117-228)). This was mainly due to a significantly increased risk of CAD in both RA cohorts. Hospitalization due to PAD occurred more frequently than expected in both cohorts, although fewer events result in less precise estimates for PAD compared to CAD (Figure 3).

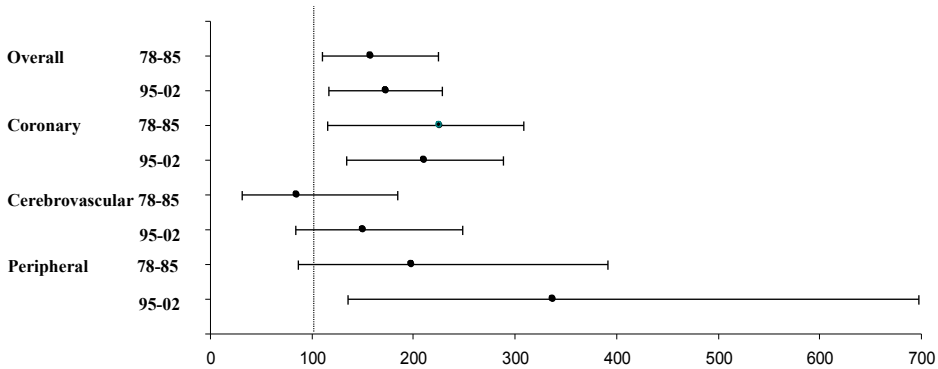


Figure 3. Cardiovascular morbidity over a period of 8 year follow-up for the two cohorts of RA patients identified 1978 and 1995 respectively for overall CVD and its subsets (CAD, CEVD and PAD). SMoR with 95 % CI.

Stratification by sex revealed a significantly increased morbidity from CVD overall and CAD in women with RA compared to the general population (Tables 12 and 13). The estimated SMoRs were slightly higher in the 1995 cohort than in the 1978 cohort, suggesting that RA associated excess CVD morbidity did not decrease over time in women.

Table 12

Cardiovascular morbidity (fatal and non-fatal) in the 1978 cohort during the 8-year follow-up in patients with RA compared to the background population stratified by sex

	1978 Cohort (N =148)		
	O	E	SMoR (95 % CI)
CVD Overall Women	23	13.9	165 (105-248)
CVD Overall Men	8	5.7	141 (61-277)
CAD Women	16	7.3	219 (125-355)
CAD Men	8	3.3	240 (104-473)
CEVD Women	4	5.3	75 (20-193)
CEVD Men	2	1.7	115 (14-414)
PAD Women	6	2.9	205 (75-446)
PAD Men	2	1.1	180 (22-652)

O=Observed. E=Expected.

Table 13

Cardiovascular morbidity (fatal and non-fatal) in the 1995 cohort during the 8-year follow-up in patients with RA compared to the background population stratified by sex

	1995 Cohort (N=161)		
	O	E	SMoR (95 % CI)
CVD Overall Women	28	15.0	186 (124-269)
CVD Overall Men	9	6.4	140 (64-266)
CAD Women	17	7.6	224 (130-359)
CAD Men	6	3.3	181 (66-393)
CEVD Women	11	7.2	153 (76-274)
CEVD Men	4	2.8	145 (39-371)
PAD Women	5	1.0	485 (157-1133)
PAD Men	2	1.0	193 (23-697)

O=Observed. E=Expected.

In analysis restricted to RF positive patients, fifty-five patients had a first ever cardiovascular event during the study period (27 in the 1978 cohort, 28 in the 1995 cohort). Overall CVD morbidity in RF positive patients was increased compared to the background population in the 1978 cohort as well as in the 1995 cohort, with similar pattern in women and men when studied separately (Table 14).

Table 14

Cardiovascular morbidity (fatal and non-fatal) in the 1978 and 1995 cohort during the 8-year follow-up in patients with RF positive RA compared to the background population.

	1978 Cohort (N =148)			1995 Cohort (N=161)		
	O	E	SMoR (95 % CI)	O	E	SMoR (95 % CI)
CVD overall	27	17.3	156 (103-227)	28	16.9	166 (110-239)
CVD overall women	19	11.8	161 (97-251)	22	12.2	180 (113-273)
CVD overall men	8	5.5	145 (63-287)	6	4.7	128 (47-278)

O=Observed. E=Expected.

Predictors for CVD events

Substantial RA related disability (Steinbrocker's Functional Index class III-IV) was associated with an increased age-sex adjusted risk of CVD in the 1995 cohort, as well as in the 1978 cohort (Table 15). A high baseline CRP (³ 50 mg/l) level also tended to predict CVD in both cohorts, although the association did not reach statistical significance. Hypertension at inclusion was a predictor for CVD events, in particular in the 1995 cohort.

Table 15

Impact of baseline factors on the risk of cardiovascular events (fatal and non-fatal) for RA patients during the 8-year follow-up, adjusted for age at inclusion and sex

	1978 Cohort (N =148) HR (95 % CI)	**1995 Cohort (N=161) HR (95 % CI)**
RF		
: Negative	1.00	1.00
: Positive	1.80 (0.42-7.60)	1.03 (0.50-2.14)
CRP (mg/l)		
: 0-9	1.00	1.00
: 10-49	1.88 (0.64-5.51)	1.03 (0.44-2.43)
: ≥50	1.86 (0.67-5.15)	1.43 (0.68-3.02)
Steinbrocker's Functional index		
: Class I-II	1.00	1.00
: Class III-IV	1.60 (1.01-2.53)	3.72 (1.76-7.86)
Hypertension *		
: No	1.00	1.00
: Yes	1.65 (0.63-4.32)	3.36 (1.27-8.85)

*HR= Hazards ratio. * Hypertension was defined as a documented clinical diagnosis at the time of assessment or as blood pressure over 140/80 at inclusion or finally using antihypertensive treatment.*

Mortality

Excess CVD mortality over time was similar in the two cohorts [SMR 175 (CI 100-284) and SMR 172 (CI 100-276) for the 1978 and 1995 cohorts respectively] (Figure 4). On the other hand, the overall mortality during the eight-year follow up period was significantly increased in the 1978 cohort, but not in the 1995 cohort (Figure 4). However, the 95 % confidence intervals overlap, indicating that the difference in mortality between the cohorts was not statistically significant. This difference was mainly due to a lower number of deaths attributed to other causes than CVD. In the 1978 cohort, there were 20 deaths due to other causes (two malignances, three infections, five arthritis and ten miscellaneous) compared to 13.2 expected. In the 1995 cohort there were 17 deaths due to other causes (three malignances, three infections, six arthritis and five miscellaneous) compared to 19.8 expected.

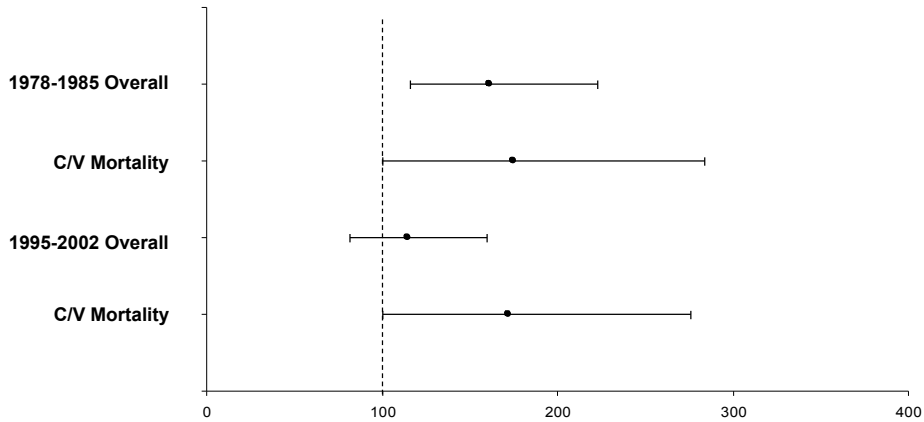


Figure 4. *Cardiovascular and overall mortality over a period of 8 year follow-up for the two cohorts of RA patients identified 1978 and 1995 respectively. SMR with 95 % CI.*

Results *Paper III*

Fourteen patients with active RA were started on treatment with adalimumab 40 mg subcutaneously every two weeks. Muscle biopsies for evaluation were available from eleven patients, who were enrolled in this study (Table 16).

None of the patients had clinical signs of myositis or myopathy at baseline or after three months. Three of the patients had extra-articular involvement in the form of rheumatoid nodules, but no other ex-RA manifestations were recorded.

Table 16

Baseline characteristics for patients in paper III.

Sex	9 female/ 2 male
Age (mean yrs; SD)	54.2 (10.9)
Disease duration (median yrs; IQR)	6.5 (1.5 to 15)
RF positive	9/11 (82 %)
Anti-CCP positive	10/11 (91 %)
Methotrexate treated at inclusion* Previously on Methotrexate Previously on anti-TNF drugs	7/11 (64 %) 4/11 (36 %) 2/11 (18 %)
Current smoker Former smoker Never smoked	6/11 (54 %) 2/11 (18 %) 3/11 (27 %)
Diagnosis of hypertension Use of anti-hypertensive drugs Systolic blood pressure – mmHg (mean; SD) Diastolic blood pressure – mmHg (mean; SD) Diabetes or Hyperlipidemia diagnosis	2/11 (18 %) 3/11 (27 %) 135 (15) 78 (13) 0/11
DAS28 (mean; SD)	5.5 (1.4)
HAQ (mean; SD)	1.44 (0.76)
CRP (mg/L) (median; IQR)	20 (13 to 43)

IQR = Interquartile range. SD = Standard deviation.
** Median dose 20 mg/week, range 10-25 mg/week*

RA clinical and laboratory outcomes, paper III.

Six out of the eleven showed a good/moderate EULAR response. A clinically significant decrease in DAS28 was seen after the three-month period. Disability measured by HAQ and CRP was also reduced, but the differences did not reach statistical significance (Table 17).

Table 17

Clinical and laboratory RA outcomes, paper III

Good/moderate EULAR response *	6/11 (54 %)
DAS28	Mean change -1.40; p = 0.018
HAQ	Mean change -0.27; p=0.11
CRP (mg/L)	Median change -11 mg/L; p=0.29

** Improvement of DAS28 [190] > 1.2 from last examination or improvement of DAS28 ≤1.2 and > 0.6 from last examination and DAS28 ≤ 5.1 at current examination [210].*

Results of endothelial marker expression in muscle tissue at baseline compared to after three months of treatment with adalimumab

HLA-DQ was mainly expressed in endothelial cells in capillaries, whereas IL-1α was mainly seen in larger vessels. Staining for HLA-DQ decreased significantly after treatment (median (M) 0.073 %, IQR 0.027-0.121 vs M vs 0.023%, IQR 0.009-0.040; p=0.041) (Figure 5 and Table 18). There was a similar trend for IL-1α measured from baseline to three months follow up (mean difference 0.049 %; 95 % confidence interval(-0.069-0.168). Capillairy density, measured as the percentage of CD31 positive area, also tended to be reduced after adalimumab treatment (Table 18).

Decreased expression of IL-1α was seen in EULAR good/moderate responders, but not in non-responders (mean difference -0.114% vs 0.028%) (Table 18). HLA-DQ expression decreased in both groups (median difference -0.046 % vs -0.036 %). There were no significant correlations between changes in DAS28 and changes in HLA-DQ or IL-1α.

There was no major difference in baseline HLA-DQ expression between current smokers and non-smokers (M 0.084% vs 0.073%). There was a significant reduction of HLA-DQ expression in non-smokers (median difference 0.059 %; p=0.043), but not in current smokers (median difference 0.019%, p=0.345).

Table 18.

Endothelial markers; % of total tissue area.

		Baseline	Change from baseline to 3 months follow up	P
IL-1α	Total n=11	0.122 (0.161)	-0.049 (0.176)	0.38
(mean;SD)	Responder n= 6	0.184 (0.200)	-0.114 (0.206)	0.23
	Non responders n=5	0.047 (0.042)	+0.028 (0.105)	0.58

HLA-DQ	Total n= 11	0.073 (0.044-0.130)	-0.036 (-0.061 to -0.001)	0.04
(median;IQR)	Responder n=6	0.073 (0.047-0.152)	-0.046 (-0.097 to 0.018)	0.34
	Non responders n=5	0.073 (0.026-0.357)	-0.036 (-0.268 to -0.014)	0.04

CD31	Total n=11	1.16 (1.36)	-0.51 (1.41)	0.26
(mean;SD)	Responder n=6	1.09 (0.91)	-0.44 (1.36)	0.46
	Non responders n=5	1.25 (1.89)	-0.58 (1.35)	0.47

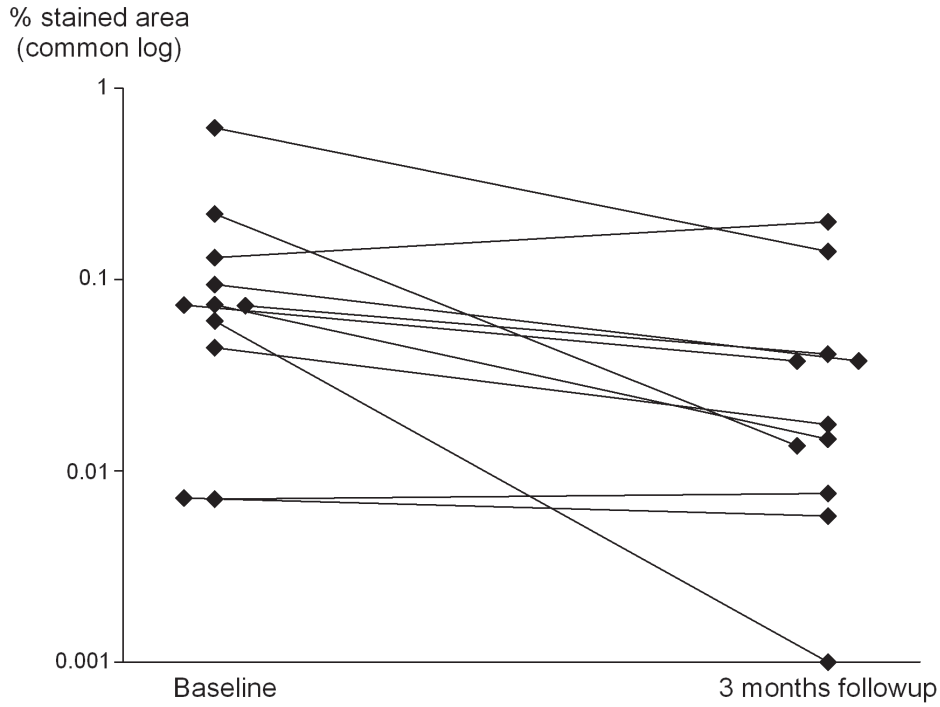


Figure 5. Endothelial HLA-DQ expression by computer assisted image analysis; at baseline and after three months of treatment in the 11 studied patients.

Increased cardiovascular morbidity and mortality in patients with RA recruited in 1978 as well as in 1995 (Paper I) – Discussion.

The pattern of increased CVD morbidity and mortality was similar in both cohorts in spite of more intensive treatment and more favourable markers of inflammation and disability in the 1995 cohort. Substantial RA related disability (Steinbrocker's Functional Index class III-IV) was associated with an increased age-sex adjusted risk of CVD in both cohorts, whereas hypertension was a significant predictor, especially in the 1995 cohort.

Possible explanations for less severe disease in the 1995 cohort include selection bias due to better access to rheumatologists and a more effective pharmacologic therapy. In 1995 compared to 1978, considerably more patients were currently treated with DMARDs and corticosteroids, the use of methotrexate had increased and the use of anti-malarials had decreased. In fact, the number of DMARDs ever taken was almost doubled. Despite this increase, only 20% were on methotrexate and none on anti-TNF therapy at baseline in the 1995 cohort. This is considerably less compared to more recent surveys of the RA population from this catchment area in which over 50% were on methotrexate therapy and 20% were on anti-TNF therapy in 2005 [194, 211]. Observational studies suggest that DMARD treatment may reduce the excess cardiovascular burden in RA patients. Antimalarials appear to have a favourable effect on lipids and antithrombotic effects [212], and methotrexate treatment has been associated with reduced CVD mortality [213] in a large observational study. Furthermore, recent reports from this geographical area [214] and England [215] demonstrate that anti-TNF treatment reduces CVD co-morbidity. Since TNF inhibitors were introduced in Sweden in 1999 and initially only used in a minority of patients, it is unlikely that anti-TNF treatment had any major impact on the development of CVD in the 1995 cohort.

Disease severity, including disability and markers of inflammation such as CRP and ESR, has been shown to predict CVD in subjects with RA [216].Patients with severe extra-articular RA manifestations are at an increased risk of developing CAD [33] as well as peripheral vascular disease [211], indicating that systemic inflammation is a major determinant of vascular co-morbidity in RA. These previous observations are supported by our findings of a high CRP and Steinbrocker's functional class as being predictors for CVD events. Since Steinbrocker's Functional Index probably is a more robust marker of long term disease severity than a single measure of CRP in most patients, it is not surprising that we found Steinbrocker's Functional Index to be a stronger risk factor for CVD.

In other recent studies, there is conflicting evidence on mortality due to CVD among patients with RA. In a study of the multi-centre ARAMIS cohort in the United States, a decline in mortality due to myocardial infarction over time (1980 to 1997) was observed [217], suggesting that improved management may protect patients with RA from cardiovascular death. By contrast, in a survey of patients from a British early RA cohort starting in 1986 with a follow up of 18 years, deaths from CVD were more frequent than expected, especially from CAD [132]. In a recently published study from Hong Kong, China, which lasted between 1999 and 2008, patients with RA and other inflammatory rheumatic disorders all had reduced life expectancy. Infection was the leading cause of death, followed by cardiovascular complications and malignancies [218]**.** Furthermore, a longitudinal study from Olmsted County, Minnesota, including all known incident cases of RA diagnosed between 1955 and 2000 in a defined area, showed constant mortality rates over time in the RA population up to 2007, whereas mortality rates in the background population decreased substantially, resulting in a widening mortality gap [219]**.** Previous surveys of RA patients from the same population have revealed an increased risk of CVD events compared to the general population [220] and an association between RA disease severity and cardiovascular death [110]**.**

Estimates of excess overall mortality and CVD mortality vary for both early and established RA [221]. The different study designs (inception early arthritis cohorts, prospective or cross sectional RA studies, clinic or population based cohorts) and variations in sample sizes, follow-up and geographic areas may explain some of these discrepancies.

In the present study, CVD mortality was increased compared to the general population in both cohorts, but overall mortality was only increased in the 1978 cohort. The lack of excess overall mortality in the 1995 cohort may be due to limited power, restricted follow up time or improved survival from non-CVD morbidity.

In previous surveys, traditional risk factors for vascular disease, such as smoking, hypertension, diabetes and hyperlipidemia, did not fully account for the increased risk of CVD in subjects with RA [222, 223]. In the present study, there was a trend towards fewer smokers and also higher cholesterol levels in the 1995 cohort. The latter finding could be due to better control of inflammation [224].

One potential problem in this study is that the patient cohorts recruited may have been selected differently. There were more rheumatologists serving the same population in the latter time period, and this could have increased the likelihood of milder cases being seen by a rheumatologist. Such differences in selection would bias the comparison towards lower disease severity and lower disease associated excess CVD morbidity in the 1995 RA cohort. However, analysis restricted to RF positive patients showed increased rates of CVD compared to the background population in both cohorts. Although it is difficult to exclude that differences in selection could have some impact, in our opinion these results indicate that there was no major change over time in excess CVD morbidity in comparable samples of patients from this area.

Reduced systemic endothelial activation (measured as HLA-DQ expression) in patients treated with adalimumab (paper III) - Discussion.

The major histocompatibility complex (MHC) class II subtypes HLA-DR, -DQ and –DP are regulators of T-cell dependent immune responses, and abberant expression of these tissue antigens in the endothelium has been demostrated in autoimmune disease such as RA and SLE [93]. In patients with RA, increased expression of HLA-DQ in synovial microvessels in joints with active arthritis has been reported [225]. By contrast, synovial HLA-DQ-expression in endothelial cells was not found in a group of patients with reactive arthritis [225], suggesting this finding to be specific for microvessels of RA-patients.

In a study of rheumatoid vasculitis, increased expression of HLA-DR, and of the adhesion molecules ICAM-1 and VCAM-1, was observed in muscle biopsy specimens from vasculitis patients with and without perivascular infiltrates, compared to controls with non-vasculitic RA and osteoarthritis [226]. Furthermore, our previous study of muscle biopsies from patients with severe extra-articular RA showed increased endothelial expression of HLA-DQ in the absence of local inflammation, compared to RA controls without extra-articular disease. Although the patients in the present study did not have severe extra-articular RA, they had active severe joint disease, which could also be associated with systemic inflammation and vascular endothelial activation. Our data suggest that treatment with adalimumab may decrease such activation. Since there was no comparison group, it is not known whether this effect is specific for adalimumab, or indeed for TNF-inhibitors, or if similar findings could be seen with other potent anti-rheumatic therapy.

Studies of myocarditis with dilated cardiomyopathy , suggest an association between endothelial expression of MHC class II and diffuse endothelial dysfunction [93, 227]. In addition, expression of MHC class II and other vascular endothelial markers in cardiac microvessel in patients with CVD may be greater among those with inflammatory rheumatic diseases compared to those without [228].

Inflammation is now generally accepted as a major component in all stages of atherogenesis, from endothelial stress, via vascular damage to plaque destabilization and plaque rupture subsequently leading to atherothrombosis [229].

Based on this, it would be expected that chronic systemic inflammation, as seen in many RA patients, could significantly aggravate atherogenesis. Indeed, striking similarities in the cellular and cytokine profiles of rheumatoid synovial lesions and atherosclerotic plaques have prompted speculations that shared inflammatory pathways in various rheumatic diseases may initiate and/or accelerate plaque formation [230]. Alternatively, inflammation and endothelial activation could lead to an increased risk of plaque rupture and aterothrombotic events that is partly independent from the extent of atherosclerosis.

What is the link between severe RA, systemic endothelial activation and complications such as severe extra-articular manifestations and CVD events ? Patients with severe RA, in particular those with severe extra-articular RA, are characterized by high levels of RF [77, 78], which correlate with levels of circulating immune complexes (CIC) [231]. Polyethylene glycol (PEG) precipitated CIC from synovial fluid induce TNF production in vitro [232], and, interestingly, a similar induction was seen using

PEG precipitated CIC from serum from patients with severe extra-articular disease - to a significantly greater extent than in RA controls without extra-articular involvement [231]. TNF and interferon-g (IFN-g) are well known inducers of MHC class II expression [93]. Based on this, circulating TNF, induced by CIC containing RF, may contribute to systemic HLA-DQ upregulation. The emergence of clonally expanded T cells with reduced expression of CD28 and high production of IFN-g has been shown to be dependent on an environment rich in TNF [233]. Such cells may therefore contribute to increased endothelial HLA-DQ expression based on effects of IFN-g. Such endothelial activation may be associated with endothelial dysfunction and/or increased local T cell activation, which could be part of the pathogenesis of extra-articular organ manifestations and possibly also CVD events (Figure 6).

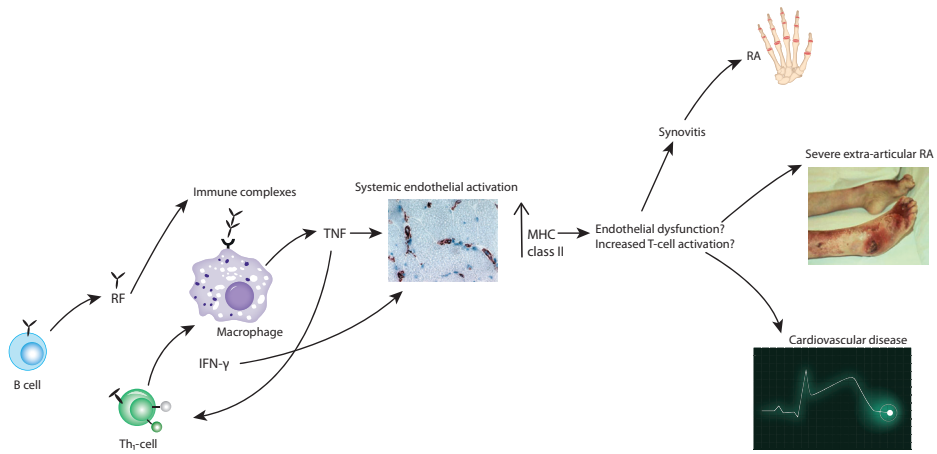


Figure 6. Model for the development of vascular endothelial activation and related complications in patients with severe RA. The model shows how RF, immune complexes, macrophages, and highly differentiated Th1 cells interact and how this through effects of TNF and IFN-γ leads to increased systemic MHC class II expression in endothelial cells. Previous observations suggest that increased endothelial HLA-DQ expression is a key feature in the development of destructive arthritis, severe extra-articular RA manifestations and possibly also CVD events.

Photo of capillaries with endothelium positive for HLA-DQ in a muscle biopsy from a patient with extra-articular RA by Carl Turesson. Original publication: [191]. Reproduced with permission from Oxford University Press.

Photo of systemic rheumatoid vasculitis with bilateral foot drop due to mononeuritis multiplex by Eric Matteson. Originally published in Turesson C, Matteson EL. Clinical features of rheumatoid arthritis: The patient with extra-articular manifestations. In Hochberg et al (Eds): Rheumatoid Arthritis.1st Edition. Mosby, London 2009. ISBN 978-0-323-05475-1. Reproduced with permission from Elsevier.

We also found a trend towards a decrease in expression of IL-1α , in particular among clinical responders. This is in line with previous studies that demonstrated that anti-TNF treatment may reduce IL-1 expression in inflammatory tissue [234].

IL-1α expression has been reported in arteriosclerotic lesions [235], specifically in endothelial cells of microvessels in atherosclerotic plaques [103], but not in normal blood vessels[235]. IL-1α induces tissue factor like procoagulant activity and

plasminogen activation inhibitor synthesis in human endothelial cell in vitro [236], indicating that increased IL-1α production may have a procoagulant effect on vascular endothelium. Reduction of IL-1α may thus also be important in the prevention of artherosclerotic vascular disease.

As previously noted, a growing body of evidence supports the concept that treatment with TNF inhibitors has beneficial effects on the vascular system. Anti-TNF blockade has been associated with a reduction of different vascular/inflammatory markers, such as serum concentrations of cellular adhesion molecules [237] and vascular endothelial growth factor (VEGF) [238]. A reduction of IMT has also been described [239] and, in addition, significantly reduced arterial stiffness [240], and improved endothelial function [241]. The beneficial effect of anti-TNF-agents may be exerted, at least in part, by activation of fibrinolytic system, which is usually inhibited in subjects with chronic inflammatory disorders [242]. We postulate that endothelial activation may be more important in the short term as a marker of anti-TNF treatment related effects on the risk of CVD than structural vascular changes.

The relation between such effects and other factors implicated in vascular disease is of major interest. Previous studies have also suggested that smokers with RA may be less likely to respond to anti-TNF treatment [243,244]. This needs to be interpreted in the context of findings indicating that in addition to being a strong predictor for developing RA, smoking may also aggravate outcome of established RA [245]. Rheumatoid arthritis patients who smoke have a higher need for DMARDs and feel worse, but they do not have more joint damage than non-smokers of the same serological group [245]. In the present study, we found a significant decrease in HLA-DQ expression in non-smokers, but not in current smokers, although baseline levels of expression were at least as high in smokers. This may indicate that some inflammatory pathways in smokers with RA are resistant to anti-TNF treatment. However, these results should be interpreted with caution due to the limited sample and the exploratory nature of this subanalysis.

How much of the CVD burden in patients with RA is due to traditional risk factors compared to disease specific inflammation? - Discussion.

The results from our study of CVD morbidity, together with other observations, suggest that coronary heart disease due to accelerated atherosclerosis is the main factor leading to the increased cardiovascular events in RA patients. The pathogenic mechanisms leading to accelerated atherosclerosis and increased risk of cardiovascular events in RA are complex and not yet completely elucidated. Most likely, however, multiple proatherogenic abnormalities can interact and result in the increased presence of atherosclerosis in RA. There is evidence that both established cardiovascular risk factors and RA manifestations can contribute for a significant proportion of atherosclerotic damage in RA. In addition, the drugs commonly employed to treat RA could negatively or positively affect the progression of atherosclerosis by favoring, for example, some traditional cardiovascular risk factors or by reducing disease activity.

A number of studies have concluded that the majority of classical cardiovascular risk factors are not seen more frequently than expected in RA patients [114]. However, the Framingham risk score, that identifies subjects at increased cardiovascular risk

within the general population, has recently been shown to be higher in patients with long-standing RA compared to both control subjects and patients with early disease [246]. Moreover, higher scores were independently associated with coronary calcification in these patients.

Another recent study demonstrated that metabolic syndrome features of hypertension, insulin resistance, and triglycerides represented risk factors for subclinical atherosclerosis, independent of previously identified determinants of CVD, in a cohort of 74 RA subjects [247].

The role of dyslipidemia in RA related CVD appears to be complex. Elevated total cholesterol may not be a good marker of the risk of CVD in patients with RA [247]. This may due to the fact that patients with active RA usually display decreased total cholesterol and high-density lipoprotein cholesterol levels [248-250]. However, elevated levels of lipoprotein (a) and small, dense low-density lipoprotein particles have been reported in RA patients, and these may represent independent atherosclerotic risk factors [248,251-253]. More recently, an increase of pro-inflammatory high-density lipoproteins has been shown in both SLE and RA [254]. The majority of these findings may be linked to the inflammatory background and immune dysregulation of RA. Their net effect on CVD events remaint to be determined.

As mentioned previously, smoking is a predictor of RA, and also associated with disease activity and some aspects of disease severity [49,255,256]. It has been shown that smoking increases cardiovascular risk before the onset of seropositive inflammatory polyarthritis [257] and that it is associated with subclinical atherosclerosis in RA. [258-260]. Taken together, these observations may suggest that the proatherosclerotic effect of smoking may be stronger in RA than in the general population, possibly through a synergic action with the pathogenic mechanisms of rheumatoid disease.

It has been suggested that the presence of established cardiovascular risk factors maybe necessary for systemic inflammation to promote atherosclerosis in RA patients [222]. Thus, the acceleration of arterial wall damage in RA could be the result of a strict interplay between well-known cardiovascular risk factors and rheumatoid manifestations. On the other hand, studies of CVD events have shown that RA in itself represents an independent cardiovascular risk factor [114]. The relative role of RA related inflammation and traditional risk factors could also vary over time. For example, we found a significant impact of hypertension on the risk of CVD only in the 1995 cohort, in which severe, active disease was less prevalent.

The increased cardiovascular mortality in these patients appears to be due not only to higher incidence of, but also a higher case fatality after, AMI [136]. Endothelial dysfunction, which precedes organic arterial wall damage and is strongly linked to the marker of inflammation, such as CRP. Endothelial dysfunction may also be seen in patients with RA and low clinical disease activity [261]. CRP levels and ESR are associated with accelerated atherosclerosis and CVD in RA [177,262-263]. Interestingly, a peak in the level of ESR immediately preceding new-onset heart failure in RA patients has been shown, thereby supporting the involvement of inflammatory stimuli in the initiation of CVD [264].

Finally, anti-CCP antibodies may be linked to CVD in RA. Anti-CCP antibodies are associated with severe disease, including the occurrence of severe extra-articular

manifestations [78], and the cardiovascular risk in RA is associated with the extent of inflammation as well as with the severity of the disease [132,265-266].

Analysis of carotid intima-media thickness (IMT) in two patient cohorts showed a higher IMT at the internal carotid arterial wall in the cohort of anti-CCP antibodie positive patients [267], suggesting that subclinical atherosclerosis is more evident in RA subjects with circulating anti-CCP. This does not clarify whether the more extensive arterial wall damage is due to a direct contribution of these antibodies or to other mechanisms associated with severe disease.

On the basis of the above described observations, it is conceivable that successful DMARD therapy should protect RA patients from CVD (see above). The relation between other treatments for RA and CVD is less clear. A deleterious effect on vascular health of oral corticosteroids has not been clearly demonstrated, but high-dose or long-term treatment might be expected to adversely affect the traditional cardiovascular risk factor profile by increasing body mass index (BMI) and blood pressure as well as worsening the lipid profile [268-269]. The use of some cytotoxic immunosuppressive drugs, including azathioprine, cyclosporine, and leflunomide, has been associated with significant increase in cardiovascular risk when compared to RA patients receiving methotrexate monotherapy [269]. It is difficult to distinguish direct negative effects of these drugs from a beneficial effect of methotrexate in this comparison, and confounding by other factors related to the indication for treatment could also play a role.

Results and discussions *Papers II* and *IV*.

Baseline characteristics (Table 19)

The total follow-up for the cohort in the MDCS was 313 425 person-years (pyr) (188 969 pyr in women, 124 455 pyr in men), which gives an estimated incidence of 55/100 000 pyr (72/100 000 in women; 29/100 000 in men). For the MPM cohort, the total follow-up was 731 703 pyr (211 262 pyr in women, 520 441 pyr in men), which gives an estimated incidence of 40/100 000 person-years (66/100 000 in women; 29/100 000 in men).

Table 19

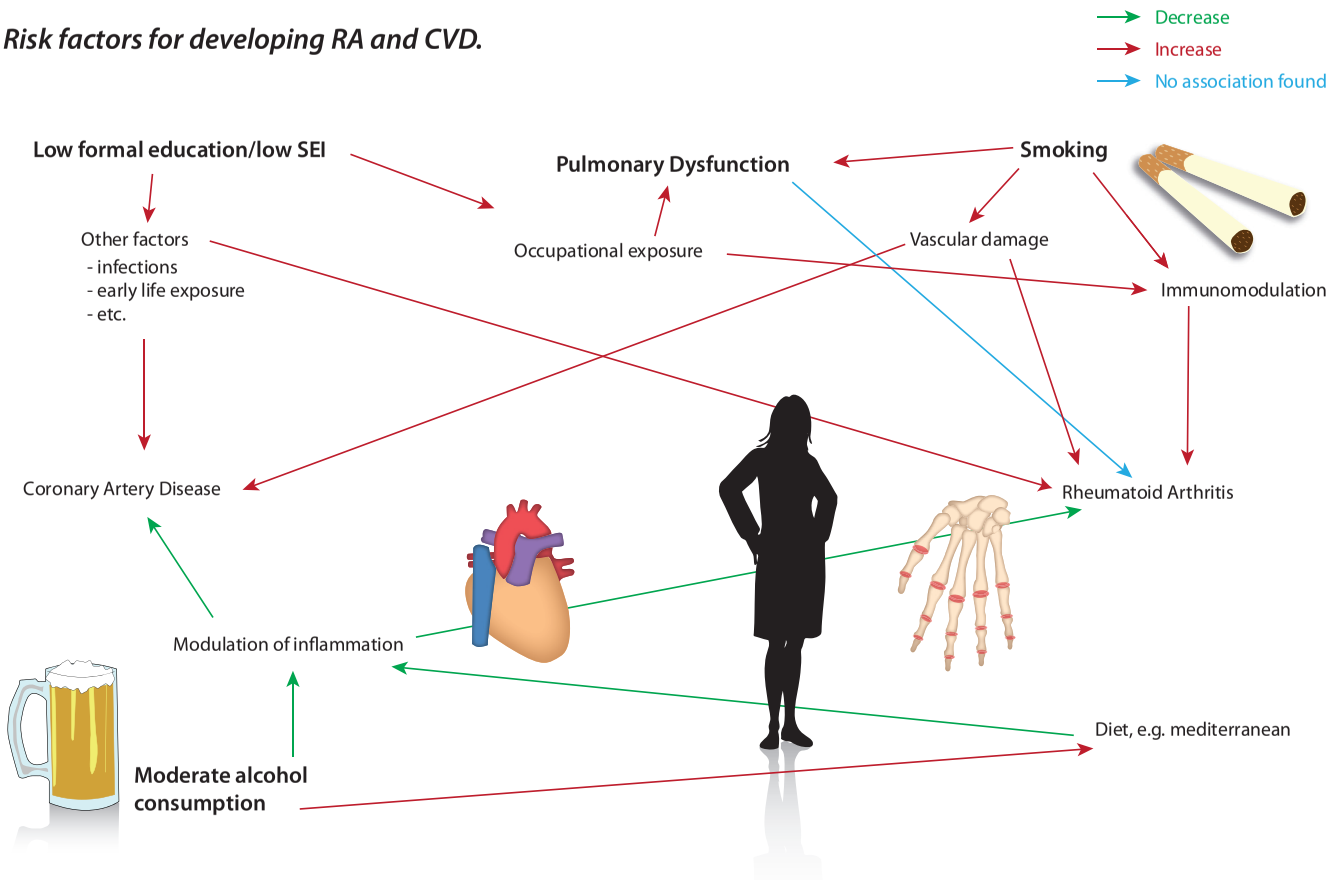
Baseline data for the pre-RA cases and controls in the MPMP and the MDCS

	MDCS Pre-RA cases (N =172)	**MDCS** Controls (N=688)	**MPMP** Pre-RA cases (N =290)	**MPMP** Controls (N=1160)
Age at disease onset (yrs)	63.4 (8.0)	NA	60.0 (8.7)	NA
Age at screening (yrs)	58.0 (7.2)	58.0 (7.2)	47.3 (7.1)	47.4 (7.1)
Female	136 (79 %)	544 (79 %)	139 (48 %)	556 (48 %)
Time from inclusion to RA diagnosis*	5 (1 to 13)	NA	12 (1 to 28)	NA
RF seropositive at diagnosis or later	69 %	NA	NAn	NA

*Values are given as means (standard deviation) unless otherwise noted. * Median in years (range). NA = Not applicable. NAn = Not Analyzed.*

Figure 7.

Risk factors for developing RA and CVD.



Smoking – Results (Table 20 and Figure 8)

Table 20

Impact of current smoking (at screening) on the risk of RA

	MDCS		MPMP	
	OR	95 % CI	OR	95 % CI
All *	1.81	1.15 to 2.83	1.79	1.32 to 2.42
Women*	ND	ND	1.61	1.04 to 2.49
Men*	ND	ND	1.97	1.29 to 3.02
Screened ≤ 12 years before RA diagnosis*	NA	NA	2.31	1.50 to 3.55
Screened > 12 years before RA diagnosis*	NA	NA	1.37	0.89 to 2.11
RF positive	1.95	1.14 to 3.32	ND	ND
RF negative	1.35	0.56 to 3.24	ND	ND
All, multivariate analysis I**	2.38	1.21 to 4.68	NA	NA
All, multivariate analysis II ***	1.87	1.17 to 2.98	NA	NA
RF positive, multivariate analysis I**	2.78	1.23 to 6.27	NA	NA
All, multivariate analysis III ****	NA	NA	1.72	1.22 to 2.42
All, multivariate analysis IV *****	NA	NA	1.95	1.34 to 2.83

** Bivariate analysis. NA = Not applicable. ND=Not Done.*
*** Adjusted for formal education and category of alcohol consumption measured in grams per day*
**** Adjusted for formal education and alcohol consumption pattern during the last year*
***** Adjusted for COPD and SEI (blue-collar vs white collar worker)*
****** Adjusted for RPD and SEI (blue-collar vs white collar worker)*

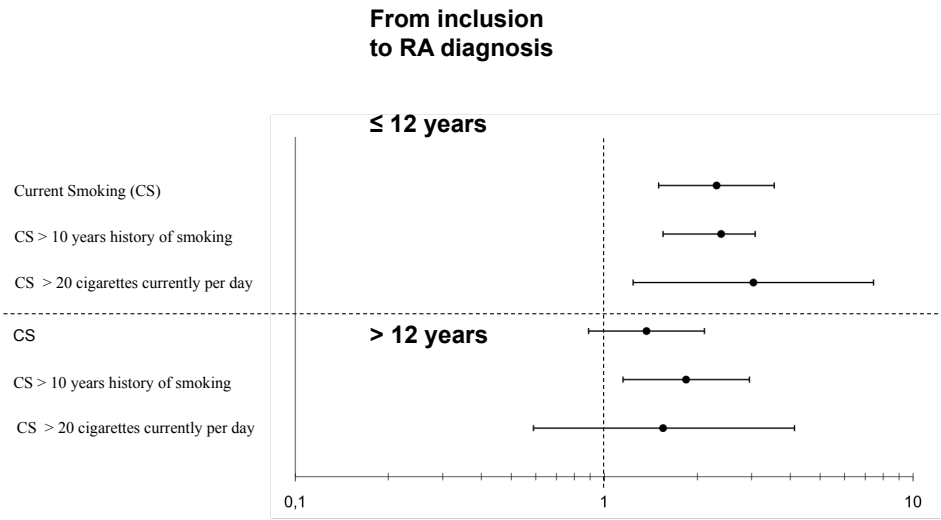


Figure 8. Smoking history as a predictor of RA. Bivariate analysis stratified by the time from inclusion to RA diagnosis in the cases; Odds ratio; 95 % CI. Logarithmic scale.

Current smoking at inclusion in the MDCS predicted subsequent development of RA. Stratifying for RF status, we found in bivariate analysis that smoking was significantly associated with future RF positive RA, but not RF negative RA. Overall and for RF positive RA, smoking was a robust predictor that remained highly significant in multivariate analyses, adjusted for both level of education and menu book reported alcohol consumption.

We also performed a bivariate analysis with smoking history defined in four categories with never smoked as reference. Again, current regular smoking was a significant predictor (OR 2.78; 95 % CI 1.21 to 6.07). Occasional smoking was also significantly associated with RA, although with a wider CI (OR 2.76; 95 % CI 1.04 to 7.27), whereas former smoking was not (OR 1.13; 95 % CI 0.69 to 1.86).

In the MPMP, current smoking was a strong predictor for developing RA overall as well as in separate analyses of men and women. Current smoking was also a stronger predictor among those screened ≤ 12 years before RA diagnosis than among those screened > 12 years before diagnosis. A reported history of long term smoking (> 10 years) among the current smokers was a significant risk factor in both subsets (Figure 8). Heavy smoking (> 20 cigarettes/day) and current smoking both predicted RA among those screened ≤ 12 years before RA diagnosis (p=0.015), but not among those screened > 12 years before diagnosis (Figure 8).

The association between RA and current smoking and remained significant in multivariate analysis adjusted for blue-collar worker status and COPD (Table 20). Similar results for current smoking were found in models adjusted for RPD instead of COPD (Table 20).

Smoking - Discussion

The association between smoking and development of RA is the most extensively studied link between the environment and the aetiology of this disease. The increased risk in smokers has been found to be confined to the subset of RA defined by the presence of antibodies to citrullinated peptides (ACPA) [59]. Interaction between smoking and the quantitatively most important genetic risk factor of RA— i.e., the HLA-DRB1 'shared epitope' (SE) [36], for the risk of developing ACPA-positive RA, has been described [59, 270–273], and also tissue studies have proposed smoking as an environmental factor that might lead to citrullination, potentially contributing to anti-citrulline autoimmunity in genetically susceptible individuals [60].

Previous studies have shown that cigarette smoking is associated with being RF seropositive also in individuals without RA [274]. It is clear that RF production may precede the development of RA, sometimes by many years [275]. This association between smoking on one hand and RF seropositivity and RA on the other may be causal or it may, at least partly, be due factors associated with smoking, such as differences in diet [276] or other exposures, which themselves may be partly responsible for the development of RA. For example, in a study of blood donors lower levels of antioxidants were found in those who later developed RA [277]. Finally, smoking is associated with other co-morbidities, including respiratory infections, which could be involved in the causation of RA [278].

Others have found that the increased risk for RA associated with smoking requires a long duration, but merely a moderate intensity, of smoking, and may remain for several years after smoking has stopped [279]. This may indicate that the mechanism behind the effect of smoking is complex, slow, or delayed [279]. The molecular pathways behind the increased risk of RA associated with smoking, and the interaction between smoking and genetic factors, need to be further investigated. Our observation of a lower impact of smoking on the risk of RA among those screened > 12 years before RA diagnosis suggests that individuals who quit smoking, even heavy smokers, may reduce their risk of RA. This is compatible with a slow and potentially reversible process. In a Swedish sample of early RA patients, ever smokers were more likely to present with rheumatoid nodules at RA diagnosis than those who had never smoked [280]. In the same cohort, current smokers at RA diagnosis were at an increased risk of developing severe extra-articular manifestations [281]. Previous studies of vasculitis and other severe exRA manifestations have shown similar results [162,282-283]. Taken together, this suggests that [smoking may be involved both in the pathogenesis and early manifestations of the disease, as well as the long term complications. The underlying mechanisms between these early and late events may be partly similar and related to microvascular damage.

Formal education and socioeconomic status - Results

Table 21

Formal education bivariate analysis.

	Cases (n)	Controls (n)	OR	95 % CI
University degree	15	93	1.00 (ref)	
Years of education				
: ≤8 years	78	262	2.46	1.20 to 5.02
: 9-10 years	44	182	1.75	0.84 to 3.62
: 11-12 years	12	56	1.52	0.59 to 3.62
: >12 years	11	54	1.36	0.52 to 3.58

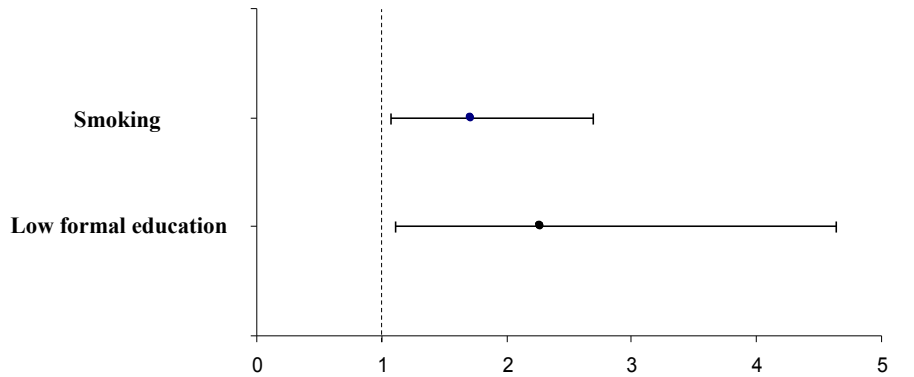


Figure 9. Current smoking (yes vs no) and low formal education level (≤8 years education vs university degree) were independent predictors for RA (Multivariate analysis; Odds ratio; 95 % CI).

Table 22

The impact of socio-economic index on the risk of RA

Bivariate analysis	OR	95 % CI
White-collar worker	1.00 (ref)	
Blue-collar worker	1.54	1.12 to 2.10
Women	1.45	0.97 to 2.27
Men	1.63	1.05 to 2.53
All, screened ≤12 years before diagnosis	1.71	1.10 to 2.61
All, screened >12 years before diagnosis	1.35	0.86 to 2.12
Multivariate analysis, adjusted for current smoking All subjects		
White-collar worker	1.00 (ref)	
Blue-collar worker	1.42	1.03 to 1.91

Individuals in the MDCS with elementary school education only (Table 21) had an increased risk of RA compared to those with a university degree. In multivariate models, the associations of smoking and low level of formal education with RA remained significant in a model adjusted for both factors (Figure 9). Blue-collar workers in the MPMP had an increased risk of RA compared to white-collar workers with similar results for men and women in analyses stratified by sex (Table 22). This association was also stronger among those screened ≤12 years before RA diagnosis compared to those with a longer time span from screening to RA diagnosis (Table 22). Current smoking (OR 1.72; 95 % CI 1.26 to 2.34) and blue-collar worker status (Table 22) remained significantly associated with RA in multivariate models including both factors .

Formal education and socioeconomic status – Discussion

A number of chronic diseases occur more frequently in individuals with limited formal education [61,284].An inverse association between level of education and clinical symptoms has been described in several studies of RA patients [285-287] and a lower level of formal education was associated with RA in a Swedish case-control study [288]. Furthermore in the same study, the combined group of manual labourers and assistant and intermediate non-manual workers had significantly higher risk of RF-positive RA, but not RF-negative RA, compared to more qualified non-manual workers [64]. A recent population-based case-control study, also from Sweden, found that individuals without a university degree had significantly higher risk of RA compared to individuals with a university degree [63]. This association was seen only for RF positive cases. Whether these associations are related to differences in lifestyle or other factors is unknown, but one possible explanation is that people with lower formal education also could have less access to health care for various reasons, present later and be more likely to develop the severe, chronic phenotype identified in these studies.

This pattern may also vary in different socioeconomic settings. A population based study in Norway found an inverse association between longer education and risk of RA, but this association was not statistically significant after adjustment for age, sex, marital status, body mass index, employment status, and current smoking [56].

In the present study from the MPMP, socioeconomic status was defined based on current occupation, which is related to formal education. In a national Swedish study from 2008, Li et al studied the impact of education and occupation on the risk of hospitalization with RA [62]. Their main finding was that men as well as women with an education level >12 years had significantly decreased standard incidence ratio (SIR) of hospitalization with RA. Among men, significantly increased SIR were present among farmers, miners and quarry workers, electrical workers, other construction workers, and engine and motor operators. Among women, assistant nurses and other social-science-related workers had significantly increased SIR. These results are supported by the present study, which was not limited to patients hospitalized with RA. Our sample size precluded analysis of the impact of specific occupations.

By contrast, a study based on a population from the United Kingdom found no association between social class, based on occupational status, and incidence of RA [289], although a later follow-up of the same cohort revealed that individuals with a low social status from deprived areas had a worse prognosis [290].

This suggests that not only is individual socioeconomic status and its impact on the personal way of life important to the outcome of arthritis, but also exposure related to the residential area you live in, and the people you associate with, may play a major role.

Such exposures may change over time, as individuals move to different environments and sometimes change occupation and related socioeconomic status. Even at population levels, changes occur when living standards get better for many people. Migration may lead to changes in the social context of certain neighborhoods, in particular in urban areas. Furthermore, due to major reforms in the education sys-

tem over the years, the implications of a low formal level of education may change substantially over time. The results of the present studies may therefore not apply to individuals who were educated or started their first job after those presently studied, i.e. those born in the 1950's or later.

Alcohol consumption- Results

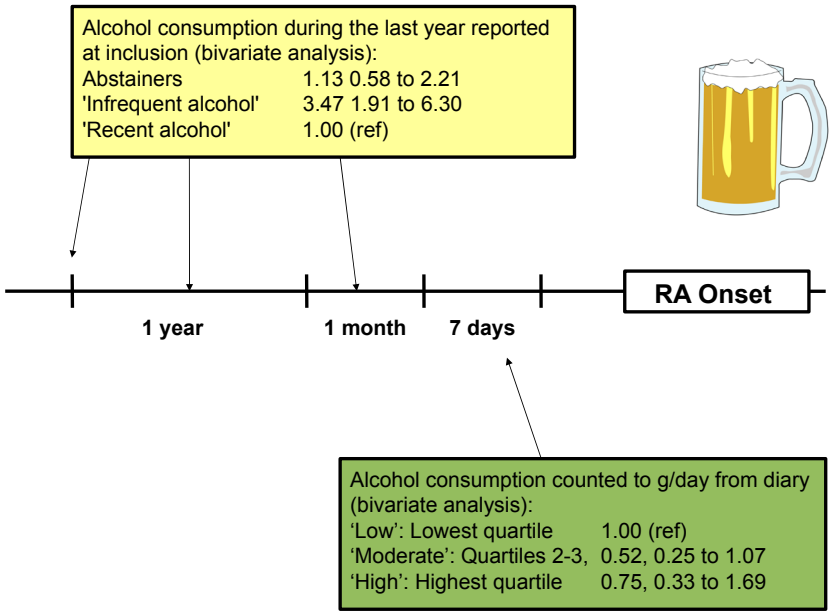


Figure 10. Results alcohol intake in MDCS (bivariate analysis).

Analyses of alcohol consumption in g/day during one week, restricted to adequate energy reporters (97 cases and 432 matched controls), showed in bivariate analyses that individuals with reported moderate alcohol intake had a tendency towards decreased risk of RA, whereas there was no major difference in the estimated risk for those with high intake, compared to those with low intake (Figure 10).

In multivariate analyses, adjusted for current smoking and formal level of education, individuals with reported moderate alcohol intake had a decreased risk of developing RA (OR 0.47; 95 % CI 0.22 to 0.99 vs. low intake). This effect was only statistically significant for patients with seropositive RA (adjusted OR 0.32; 95 % CI 0.12 to 0.85).

Looking at patterns of previous alcohol consumption, bivariate analyses showed that individuals with reported infrequent alcohol intake (within the last year but not the last month) had an increased risk of RA compared to those with reported recent intake (within the last month). There was no increased risk for abstainers (Figure 10). In multivariate models, adjusted for smoking and formal level of education, individuals with reported infrequent alcohol intake had an increased risk of RA (OR 3.52; CI 1.92 to 6.45 vs. recent intake).

Alcohol consumption – Discussion

In principle, alcohol could have a direct modulating effect on inflammation and autoimmunity in the pathogenesis of RA, or it could be a confounder for other exposure. Alcohol has been shown to diminish the response to immunogens in animals as well as in human [291,292]. Alcohol can down-regulate the production of pro-inflammatory molecules through its influence on innate immunity [293]. Notably, addition of alcohol to the drinking water for mice prone to develop collagen induced arthritis was recently shown to reduce subsequent clinical signs of arthritis as well as joint destruction [294]. An indication that alcohol consumption may also influence the risk of human RA has come from different studies of environmental factors in RA development. Several case control studies based on early RA samples have suggested that moderate drinking might reduce the risk for developing RA [46,47,50,71], whereas two prospective studies found no association between RA and reported alcohol consumption [68,295]. It is not clear whether these associations are causal or whether the observed protective effect among moderate alcohol consumers is due to confounders. One example of such a confounder is a higher intake of vegetables and fruits, which using MDCS data has previously been shown to be associated with alcohol intake [276].

Our results measuring alcohol consumption in grams per day support a protective effect against developing RA from moderate alcohol consumption (3.67-15.21 g/day), corresponding to approximately between 2 glasses of wine per week and 1 glass of wine daily. In contrast with two large retrospective case-control studies from Scandinavia, the Swedish EIRA study and the Danish CACORA study [71], there was no significant reduction of the risk of RA in the subgroup with the highest reported alcohol intake in the present study. Potential explanations include differences in the study design and in the validity of data on alcohol consumption. Due to potential misreporting, self-reports of alcohol intake must be used with caution when trying to establish a level where alcohol exerts a biologic effect. In the MDCS study, intakes of alcoholic beverages were recorded in the menu book during 7 consecutive days. The relative validity and reproducibility of the MDCS diet history for total alcohol and alcoholic beverages are high [296–298], suggest a good ability to rank individuals correctly.

Previous studies have suggested that misreporting of total energy intake may be associated with misreporting of other nutritients, including alcohol [198,199]. Like in previous studies using the MDCS cohort, we excluded individuals with a very high or very low reported energy intake compared to the reported physical activity level. By thus excluding likely misreporters of total energy intake, we probably reduced the impact of alcohol consumption misreported in absolute amounts.

Infrequent alcohol consumption (i.e. reported intake within the last year, but not within the last month) was a robust predictor of RA. This may be due to life style patterns and exposures associated with reporting recent alcohol consumption, rather than a direct biologic effect of alcohol itself. The multivariate analysis shows that this association was not due to differences in smoking or level of formal education, but unmeasured confounding by other exposures may play a role.

The risk of developing RA was similar in abstainers and recent alcohol users. In this context, abstainers probably constitute a mixed group containing people with previous alcohol problems and life long teetotallers.

Pulmonary function - Results

Table 23

Demographics and pulmonary function test (PFT) data in the pre-RA cases and controls

	Pre-RA cases (N =290)	Controls (N=1160)
Individuals with PFT data (n)	253	1006
$FEV_{1\%}$ (FEV_1/FVC)	77.9 (9.7)	78.4 (8.5)
Mild COPD (Stage I) *	17 (6.7 %)	56 (5.6 %)
Moderate to Very severe COPD (Stage II-IV)*	20 (7.9 %)	71 (7.1 %)
RPD	21 (9.7 %)	100 (11.4 %)

*Values are given as means (standard deviation) unless otherwise noted. * See Table 9 for definitions of stages in COPD.*

FEV_1, FVC and the FEV_1/FVC ratio (expressed as $FEV_{1\%}$; FEV_1 as percentage of FVC) were similar in cases and controls, both measured in lung volume (L) and percentage of predicted lung volume. Similar results for FEV_1 and FVC (Table 24), as well as $FEV_{1\%}$ (Figure 11), were found in separate analyses of four groups, stratified for time from screening to RA diagnosis.

Table 24

Pulmonary function tests in pre RA cases and controls, stratified by the time from inclusion to RA diagnosis in the cases.

From inclusion to RA diagnosis		Case	Controls
		% of predicted	
1-8 years	FEV_1	91.9 (16.6)	93.0 (17.7)
	FVC	98.2 (18.1)	99.7 (17.5)
8-12 years	FEV_1	94.0 (20.0)	96.2 (16.6)
	FVC	100.3 (18.9)	101.5 (15.4)
12-18 years	FEV_1	95.1 (19.5)	93.5 (15.7)
	FVC	102.6 (19.9)	98.5 (15.6)
18-28 years	FEV_1	96.0 (17.7)	92.5 (15.1)
	FVC	102.0 (14.1)	97.8 (14.6)

Values are given as means (standard deviation) of lung volume measured in % of predicted for FEV_1 and FVC.

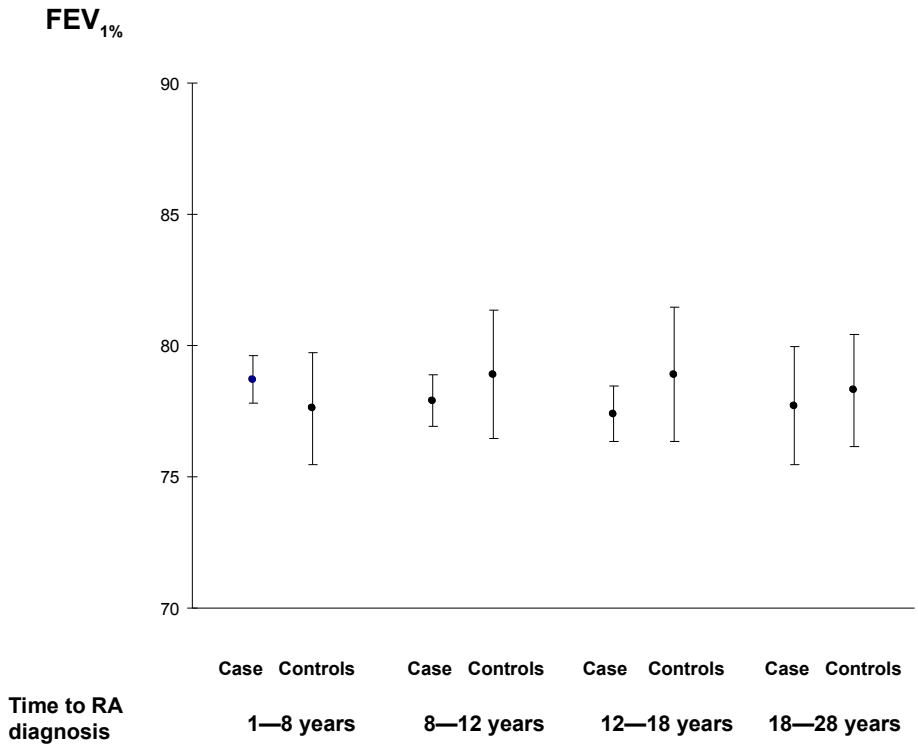


Figure 11. $FEV_{1\%}$ in four groups stratified for time from screening to RA diagnosis. Values are given as means, 95 % CI.

COPD

In bivariate analysis, there was no association between mild COPD (OR 1.35; 95 % CI 0.68-2.66) or moderate to very severe COPD (OR 1.22; 95 % CI 0.67-2.22) and subsequent development of RA.

There was no significant association between COPD and RA in analyses stratified for sex or for time from screening to RA diagnosis (both in those screened ≤ 12 and > 12 years before diagnosis). COPD was less frequent in women (cases 10.9 % vs controls 6.3 %) than in men (cases 17.5 % vs controls 17.2 %), leading to less precise estimates for the impact of COPD in women (Table 25). Although ORs for COPD in women were higher than in men, there was no significant interaction between sex and COPD in logistic regression analysis with RA as the dependent variable (p=0.16). We found no association between mild COPD (stage I; OR 1.34; CI 0.67-2.68) or moderate to very severe COPD and subsequent development of RA in this multivariate model.

Table 25

COPD as a predictor of RA in bivariate analysis; stratified by sex.

	Number of cases	Number of controls	OR	CI
Women				
No COPD	98	399	1.00 (ref)	
COPD stage I *	7	16	2.49	0.73 to 8.48
COPD stage II-IV *	5	11	3.26	0.84 to 12.70
Men				
No COPD	118	480	1.00 (ref)	
COPD stage I *	10	40	1.02	0.45 to 2.33
COPD stage II-IV *	15	60	0.96	0.49 to 1.88

**See Table 9 for definitions of stages in COPD.*

RPD

RPD did not predict RA (OR 0.69; 95% CI 0.37 -1.27) in bivariate analysis. Although ORs were different for restrictive pulmonary disease among those screened ≤ 12 and > 12 years before diagnosis (1.04 vs 0.39), confidence intervals overlapped, and there was no significant interaction between RPD and stratum of time to diagnosis in the analysis of the risk of RA (p=0.13). These differences may therefore be due to chance.

We also performed a multivariate analysis including current smoking, blue collar worker status and RPD. RPD did not predict RA in this analysis (OR 0.62; CI 0.33-1.15).

Pulmonary function and related exposures – Discussion

Exposure to crystalline silica is another well defined inhalation exposure, reported, for example, from industries involving mining, construction, ceramics, glass, agriculture, but also in sectors such as electronics. Silica exposure has been observed to be linked to RA [65], with a twofold increased risk of developing RA in analysis adjusted for smoking [65]. The lack of association between reduced pulmonary function and a future diagnosis of RA in the present study suggests that possible effects of inhalation exposures leading to immune modulation and chronic inflammation is related to at most minor tissue injury, and not to clinically recognizable lung damage.

Alternatively, smoking related damage to vascular endothelium (see Smoking section above) may be a driver of different aspects of RA pathogenesis and have only a modest correlation with effects of smoking on pulmonary function.

Although ILD may occur in early RA, sometimes preceding the clinical diagnosis of RA [65], we found no association between restrictive pulmonary dysfunction and subsequent development of RA. This most likely reflects that only a small proportion of RA patients have very early ILD involvement that has an effect on pulmonary function, and that such a small subset would not have a major impact on the overall results. In patients with established RA, suggested risk factors for developing ILD include male sex, older age at RA onset, a high "inflammatory burden", low functional capacity, and being treated with glucocorticosteroids or methotrexate [299]. In a recent study of an early RA cohort, the occurrence of severe extra-articular disease manifestations, a major proportion of which were cases of ILD, was predicted by high disease activity and extensive disability burden over the first two years after RA diagnosis [65].This suggests that such manifestations are part of the most severe RA phenotype. We can not exclude, however, that subclinical ILD involvement not leading to reduced pulmonary function could be substantially more frequent in the pre-clinical phase of RA.

Conclusions

Cardiovascular morbidity and mortality in two community based RA cohorts established in 1978 and 1995 was increased compared to the general population in the corresponding area.

Treatment with adalimumab (a TNF-inhibitor) was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA.

Smoking and a low level of formal education were independent risk factors for developing RA. Moderate alcohol consumption was associated with a reduced risk for developing RA.

Reduced pulmonary function was not associated with future RA, but smoking and low socioeconomic status were independent predictors of RA.

Final comments and future perspectives

Smoking

In these and other studies, smoking was a predictor of RA. Will changes in smoking habit on the population level affect the incidence of RA and disease outcomes? More than half a century ago, smoking was more common among men: in 1946, 50 % of Swedish men were regular smokers but only 9 % of women [300]. Since then smoking has decreased, in particular among men. In 2008/2009, the proportion of overall daily smokers in Sweden was 13 % for men and 16 % for women [301]. These national data are compatible with our own findings among Malmö residents in the control samples from the MPMP and the MDCS.

Most smokers begin their habit at a rather young age. In a Swedish survey among second-year upper-secondary pupils (aged 17) in 2009, the average proportion of self-reported daily smokers was 13 % for boys and 17 % for girls [302], confirming the long term trend towards a higher prevalence of smoking among women compared to men in Sweden.

Smoking has not decreased uniformly across all strata of society. While half a century ago the very highest proportions of smokers were found in better-off groups, the present situation is the reverse: daily smoking is more frequent among blue-collar workers, the financially vulnerable and people on low incomes [300].

If smoking and low formal education or low socioeconomic status related to present occupation indeed reflect different exposures related to RA development, as indicated in the present thesis, the risk of RA would in the future be particularly elevated in women with a poor socioeconomic background. Due to effects of smoking and chronic inflammation, an increased burden of CVD would also be expected in this subset.

What do we know about current smoking among patients with established RA? A survey of a community based sample in Malmö in 2008 showed that almost 22 % of those who responded were current smokers. Obviously, more has to be done about smoking, both for prevention among the general population, especially younger women, but also among patients with established RA.

Obesity

Obesity is defined as a BMI of ≥30 kg/m2. BMI is calculated from a person's weight and height and provides a reasonable indicator of body fatness and weight categories that may lead to health problems. Obesity is a major risk factor for CVD, certain types of cancer, and type 2 diabetes. During the past 20 years there has been a dramatic increase in obesity in many countries [303], in particular in the United States. In 2009, in the majority of states, over 25 % of the adult population was obese, and only Colorado and the District of Columbia had a prevalence of obesity less than 20%. Similar trends are seen in Sweden. The proportion of individuals aged 16-84 with overweight (BMI >25 kg/m2) has increased from 31% overall in 1980 to 44% in 2007 [301].

A recent study from the United States [169], suggests that a similar trend appears among RA patients. The prevalence of obesity within a year from RA diagnosis was 47 % among those diagnosed between 1995 and 2007, compared to 22 % among those diagnosed between 1985 and 1994. This could in the future contribute to a further increase in the CVD burden for RA patients. Previous studies have showed a paradoxical association between low BMI and CVD among patients with RA [304,305]. This most likely reflects an association with cachexia related to severe inflammation in some patients. With improved early management of inflammation and reduced physical activity in the general population, in particular among those with musculoskeletal pain, this pattern is likely to change. Abdominal obesity [306,307] and physical inactivity [308] may be better markers of the risk of CVD than BMI in the general population, but their impact has not been specifically studied in patients with RA. The possible importance of abdominal obesity in RA is supported by recent research demonstrating an increase of such abnormal fat distribution both in RA patients with short [309] and long disease duration [310]. This is an important field for further research and preventive interventions.

In contrast to the increase in obesity, surveys indicate a global decrease in age-standardized mean blood pressure [311] and a decrease in the mean cholesterol level in most Western countries [312]. The impact of such changes on the risk of CVD in the general population and among patients with RA is unknown.

CVD burden

Interventions with drug therapy to prevent CVD should be based on a comprehensive risk assessment such as the SCORE system [152], the Framingham risk score [313] or similar algorithms. Since such algorithms do not take into account the increased risk of CVD due to RA in itself, and modified algorithms that do have not been systematically evaluated, further work is necessary in this area. The elevated relative risk of CVD in patients with RA on the population level is well established, but the absolute risk and the contribution of RA related and traditional risk factors in individual patients are difficult to estimate. Based on this, the development of a risk score based on actual patient data from longitudinal studies of RA patients has been proposed, and a current EULAR collaboration is exploring this issue. Such work could be based on new, prospective studies, or existing cohorts with long term follow-up. An example of such a cohort is the Olmsted County RA sample, in which it has been found that the absolute risk of CVD within 10 years from RA onset is > 10 % in a substantial proportion of patients, and that age, sex and traditional risk factors have a major impact on the absolute risk [220]. It should be remembered, however, that such patterns could change over time with variations in the distribution of certain risk factors (see Smoking and Obesity above), and with improved control of inflammation due to more efficient therapy.

Environmental factors, genetics and biomarkers

The etiology of RA is still far from completely understood, but it is presumed to be an immunological disease with contributing genetic and environmental factors. Evidence suggests that RA develops in 3 phases: an asymptomatic period when the individual is already predisposed because of genetic factors and early life events, a pre-clinical period in which RA-related antibodies and other biomarkers can be detected in some cases, and a clinical phase with symptoms and signs of arthritis. Although the discovery of autoantibodies such as anti-CCP several years before RA diagnosis was a major step forward in our understanding of the disease, it should be kept in mind that such antibodies are detected at this stage only in a minority of those who later develop the clinical RA phenotype [25,26]. We have recently demonstrated that a subset of pre-RA cases who are anti-CCP negative during the pre-clinical stage have elevated levels of cartilage oligomeric matrix protein (COMP), a marker of increased cartilage turnover, a few years before RA diagnosis [26]. This suggests that early development of anti-CCP antibodies and early changes in the cartilage may be part of distinct pathways in the development of RA. The relation between biomarker patterns and genetic and environmental risk factors deserves further study, and may be key concepts in understanding RA pathogenesis. Furthermore, such patterns may also be important for prognosis and for the outcome of very early, even preventive, treatment. Together with longitudinal studies of patients with recently identified early arthritis, studies of the pre-clinical phase may lead to development of new complex models involving several biomarkers and clinical features that could result in a biomarker signature capable of predicting and monitoring a variety of clinical outcomes. In a future of personalized medicine, we could use such biomarker signatures to tailor treatment regimens to groups of patients.

Smoking, the lung and RA

In our present study we found no association of reduced pulmonary function measured by spirometry and subsequent development of RA. This means that the association between RA and smoking is most likely explained by mechanisms not directly related to lung damage, such as vascular damage or systemic immunomodulatory effects of smoking. However, our findings do not exclude that subclinical local effects in the lungs may be important.

COPD is characterised by an inflammatory response by the lungs to inhaled substances such as cigarette smoking and air pollutants. In addition to the pulmonary features of COPD, several systemic effects have been recognised even after controlling for common aetiological factors such as smoking or steroid use. These include skeletal muscle dysfunction, CVD, osteoporosis and diabetes. Individuals with COPD have significantly raised levels of several circulating inflammatory markers indicating the presence of systemic inflammation. This raises the issue of cause and effect. The role of TNF in COPD is thought to be central to both lung and systemic inflammation. It has been hypothesised that inflammation in the lung results in 'overspill' into the circulation causing systemic inflammation. There is supportive evidence that protein movement can occur from the lung surface to the systemic

circulation [314,315]. Evidence from inhaled substances such as air pollutants and cigarette smoke has demonstrated a temporal link between the inflammatory process in the lung and systemic inflammation [314,316]. Also, studies have shown alterations in circulating inflammatory cells in patients with COPD compared with controls which may reflect the effects of inflammatory mediators (derived from the lung) on circulating cells or the bone marrow [314]. The relevance of such alterations in smokers with RA and no clinical COPD should be studied. For a detailed understanding of the mechanisms involved, direct studies of the lung using tissue biopsies, broncho-alveolar lavage (BAL) or very sensitive imaging techniques would be necessary. The work by Makrygiannakis et al on citrullination in lung tissue and BAL cells is an example of this concept [60], and supports that increased citrullination and subsequent immune reaction against citrullinated peptides may be part of the RA specific "overspill" from the challenged lung.

Populärvetenskaplig sammanfattning

Reumatoid artrit (RA) är en kronisk inflammatorisk ledsjukdom som företrädesvis förekommer hos kvinnor och som obehandlad leder till leddestruktion med nedsatt funktion och rörlighet, trötthet samt smärtor. Risken att dö för tidigt, speciellt i hjärt/kärlsjukdom (HKS) och då företrädesvis hjärtinfarkt, är också ökad jämfört med befolkningen i övrigt. Ålder för insjuknande är i genomsnitt 60 år men även yngre personer drabbas. Sjukdomen drabbar 0,5-1 % av befolkningen världen över med högst förekomst bland indianer i Nordamerika och lägst förekomst i Afrika. Behandlingen av RA har gjort stora framsteg sedan 1970-talet, då det mest använda läkemedlet var ett antimalaria läkemedel, kortison i höga doser under längre tid, samt medel med huvudsakligen smärtstillande effekt. Idag används läkemedel som påverkar immunsystemet, bl a Methotrexate, ibland tillsammans med ett av de nya biologiska preparaten med stor framgång, vilket lett till markant förbättrad livskvalitet och ökad överlevnad bland patienterna.

Den bäst karakteriserade och egentligen enda helt säkert kända miljöfaktorn som associerats med att drabbas av RA är rökning. Om man då dessutom är född med en speciell genuppsättning har man en mångfalt ökad risk att utveckla RA.

Utifrån denna kunskap ställde vi oss ett antal frågor:
- Har förekomst av hjärt/kärlsjukdom hos RA patienter förändrats över tid i takt med att behandlingen förbättrats?
- Påverkas kärlförändringar i kroppen, undersökta med mikroskopi av småkärl i muskelprov från benet, över tid hos patienter med RA vid behandling med ett biologiskt anti-TNF läkemedel?
- Vilka livsstilsfaktorer t ex rökning, alkohol, kost, infektioner eller utbildning kan associeras med en ökad eller minskad risk att utveckla RA?

I arbete I studerade vi uppkomsten av HKS vid långtidsuppföljning av två grupper individer med RA insamlade fortlöpande från specialistmottagningar i Malmö 1978 respektive 1995. Uppgifter om dödliga och icke dödliga hjärt/kärlhändelser (hjärtinfarkt, slaganfall etc) hämtades från nationella slutenvårds- och dödsorsaksregister. Incidensen av HKS och dödsfall jämfördes med befolkningen i Malmö under motsvarande tidsperiod för båda grupperna. Vi såg en signifikant förhöjd risk för såväl sjukhusinläggning pga HKS som död i HKS jämfört med befolkningen i allmänhet i båda grupperna. Vi tittade också på riskfaktorer för HKS. Högt blodtryck och uttalad funktionsnedsättning pga RA-sjukdomen var starkt förknippat med utvecklande av HKS. Fynden har betydelse för aktuella diskussioner om behov av screening och särskilt förebyggande åtgärder mot traditionella hjärt/kärlriskfaktorer hos patienter med RA.

Delarbete III handlar om att undersöka hur behandling med anti-TNF läkemedlet adalimumab (Humira©) påverkar markörer för kärlskada vid RA. Denna kärlskada är troligen en mycket viktig pusselbit i utvecklandet av åderförkalkning. Studien bygger vidare på observationer av min huvudhandledare i tidigare studier av kärlmarkörer hos patienter med svår RA som har en markant ökad risk för HKS. Prover togs från skelettmuskel i benet före behandlingsstart och efter tre månaders

behandling. Fjorton patienter deltog i studien som registrerades som en läkemedelsprövning fas IV. Resultaten visade ett minskat uttryck av protein HLA-DQ i kärlväggen efter tre månaders behandling. Arbetet har betydelse för vår förståelse för relationen mellan HKS och anti-TNF behandling, där observationsstudier har visat på en minskad sjuklighet i HKS hos behandlade patienter.

I delarbete II och IV har vi studerat riskfaktorer för att utveckla RA bland individer som dels deltagit i hälsoundersökningen Malmö-Kost-Cancer innefattande 30 447 Malmöbor undersökta under perioden 1991 till 1996 och dels hälsoundersökningen Malmö- Förebyggande-Medicin, i vilken 32 906 Malmöbor undersöktes under perioden 1974-1992. För att identifiera fall som utvecklat RA efter deltagande samkörde vi Malmös lokala RA register med andra lokala register och nationella. I dessa båda hälsoundersökningar har deltagarna svarat på omfattande enkäter om bl a livsstil m m samt utfört enstaka undersökningar, däribland lungfunktionstest samt slutligen också lämnat en hel del blodprover att sparas för framtida bruk. I Malmö-Kost-Cancer fyllde deltagarna också i en dagbok över intag av mat och dryck under sju dagar. Den stora fördelen med dessa studier jämfört med många andra är att alla data samlades in innan RA sjukdomen debuterat. Studierna visar entydigt att aktuell rökning vid deltagande i båda hälsoundersökningarna både för kvinnor och män var en oberoende riskfaktor för att utveckla RA. Låg formell utbildning (≤ 8 år jämfört med universitetsexamen) var också en oberoende riskfaktor i Malmö-Kost-Cancer studien, likaså lågt socioekonomiskt status (definierat som registrerat yrke som arbetare jämfört med tjänstemän och akademiker) i Malmö-Förebyggande-Medicin. Utbildning och yrke är starkt kopplat till varandra varför detta fynd var väntat. Detta fynd kan tolkas så att det finns andra faktorer som dessa personer exponeras för, t ex infektioner eller annan exponering under uppväxten, som är de verkliga riskfaktorerna som bidrar till den ökade risken att utveckla RA.

Sjävrapporterat infrekvent intag av alkohol (senaste året, men inte senaste månaden) var en signifikant riskfaktor för RA jämfört med frekvent intag (senaste månaden) och rapporterat måttligt alkoholintag taget från 7-dygnsdagboken var associerat med lägre risk för RA i analyser med hänsyn tagit till rökning och utbildningsnivå. Analogt med resonemanget för låg utbildningsnivå kan exponering eller något i livsstilen som hänger ihop med måttligt alkoholintag, t ex viss kost, vara en skyddande faktor för att utveckla RA. Alternativet är att måttligt och regelbundet alkoholintag kan ha direkt skyddande effekt.

Lungfunktion utifrån spirometriundersökningarna visade inte på några betydande skillnader i lungfunktion mellan fall som senare utvecklar RA eller övriga och ingen signifikant påverkan på risken att utveckla RA utifrån tecken på obstruktiv (t ex astma eller kronisk obstruktiv lungsjukdom) eller restriktiv (t ex stendammlunga) lungfunktionsnedsättning. Detta talar för att rökning bidrar till uppkomst av RA genom någon annan mekanism än allvarlig lungskada.

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Cardiovascular morbidity and mortality remain similar in two cohorts of patients with long-standing rheumatoid arthritis seen in 1978 and 1995 in Malmö, Sweden

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Objective. Patients with RA have an increased risk of cardiovascular disease. Management of RA has changed substantially over time. Our aim was to evaluate changes in cardiovascular morbidity and mortality over the period of 1978–2002.
Methods. Two cohorts of consecutive patients with RA seen at outpatient clinics in Malmö, Sweden, were started in 1978 ($n = 148$) and 1995 ($n = 161$) and compared with the corresponding background population. Patients were followed for 8 years, and fatal and non-fatal cardiovascular first events were identified using two national registers, hospital discharge and cause of death. Standardized morbidity ratio (SMoR) and standardized mortality ratio (SMR), adjusted for age and sex were calculated.
Results. Sex distribution, age at disease onset and disease duration were similar in both groups. The 1995 cohort was more extensively treated with DMARDs and had less disease activity and disability. Total cardiovascular morbidity was increased in the 1978 cohort (SMoR 158; 95% CI 111, 225) as well as in the 1995 cohort (SMoR 168; 95% CI 118, 232). This was mainly due to an increased risk of coronary artery disease. Overall mortality was elevated in the 1978 cohort but not in the 1995 cohort. There was no change in cardiovascular excess mortality (SMR 175; 95% CI 100, 284; and 172; 100, 276 for the two cohorts, respectively).
Conclusions. There were similar elevations in the incidence of cardiovascular comorbidity in RA patients, identified two decades apart compared with the general population, in spite of more extensive treatment and reduced disease severity in the more recent cohort.

Key words: Cardiovascular, Mortality, Morbidity, Rheumatoid arthritis, Epidemiology, DMARDs, Disability evaluation, Disease activity, Malmö, Sweden.

Introduction

RA is a chronic systemic inflammatory disease that leads to joint destruction and has a major effect on quality of life. RA-related chronic inflammation not only affects the joints but also the vascular system, which contributes to the increased comorbidity and premature mortality compared with the general population, especially from coronary artery disease (CAD) [1–4].

There is some evidence for secular changes in the incidence and severity of RA. Prevalence estimates have varied between 0.5 and 1.0% in the Caucasian population, but recent studies from Scandinavia suggest that the prevalence is close to 0.5% in the adult population [5, 6]. A decreasing incidence over time as well as a shift towards older age at RA onset has been reported [7–10].

The treatment of RA has changed substantially over the last three decades, with earlier and more extensive use of DMARDs, and during the last decade introduction of TNF inhibitors and other biologic agents [11]. We have previously reported data indicating a reduced disease activity and disability in patients with RA [12], and other studies corroborate these findings [13, 14]. A corresponding decrease in the risk of comorbidity and premature mortality over time might be expected, and this concept has been supported by observational studies of patients with RA treated with MTX [15, 16]. However, in a long-term population-based study, there was no change in RA-associated excess mortality over several decades [17, 18].

The main aim of this article was to estimate the relative risk for cardiovascular comorbidity and mortality in two cohorts of patients with RA initiated in 1978 and 1995 [12, 19] compared with the background population, and to investigate changes over time in RA-associated comorbidity. We also evaluated traditional risk factors for cardiovascular disease (CVD), as well as disease severity markers, and studied their effect on the cardiovascular burden in these patients.

Methods

Patients in the 1978 and 1995 cohorts

The cohort from 1978 consisted of 148 consecutive patients who fulfilled the 1958 American Rheumatism Association (ARA) criteria for RA [20], and were seen during 1 month at the outpatient rheumatology clinic at Malmö University Hospital [19]. This is the only hospital serving the population of Malmö (237 000 inhabitants in 1978 and 246 000 inhabitants in 1995). In 1978, this was the only outpatient clinic for rheumatology in the city.

The patient cohort from 1995 consisted of 161 consecutive patients (10 patients were included in both cohorts) who fulfilled the 1987 ARA criteria for RA [21] and who, during 1 month, visited either the outpatient rheumatology clinic at Malmö University Hospital or the outpatient clinics of two board-certified rheumatologists working outside the hospital. Previous surveys indicate that >90% of all patients with RA in the city were seen at these clinics [22, 23].

All patients were systematically evaluated at baseline, using a structured protocol, including joint tenderness, disability, CRP and RF analysis as part of a previous study [12], evaluating differences in disease severity and treatment between 1978 and 1995.

Disease activity was clinically evaluated using Ritchie's articular index, which is a score for joint tenderness based on the examination of 53 joints with scoring of 26 joint areas (score range 0–78) [24]. Functional disability was evaluated according to the Steinbrocker functional class index, which categorizes the patients into four classes based on the patient's ability to perform everyday activities [25].

The serum levels of CRP were analysed by the routine method at the Department of Clinical Chemistry, Malmö University Hospital, which had the same reference values for normal/abnormal values in both periods. RF was analysed at the

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Department of Microbiology using the bentonite flocculation test (BFT) and the SCAT on both occasions [26]. In this study, the patients were considered as RF seropositive when they had a SCAT titre of $\geqslant 1/16$ or BFT $\geqslant 1/40$. In both patient groups, information on present and previous treatment with DMARDs and glucocorticosteroids was obtained through a structured review of all the clinical records.

Through a second structured review in 2007 of both cohorts, we registered information on diabetes (diagnosed before inclusion or on treatment at inclusion), blood pressure (at inclusion or 1 year before or after inclusion, registered in subsequent case records) and current pharmacological treatment focusing on heart and anti-hypertensive agents, anti-thrombotic drugs and on NSAIDs at inclusion. For definition of hypertension diagnosis see Table 2. The process of data extraction and availability of these data were similar for both cohorts.

Smoking habits (mentioned at inclusion, in case records after inclusion indicating that they smoked at inclusion or mentioned in the case records $\leqslant 1$ year before inclusion) and lipid levels (for detailed data availability, see 'Results' section) were also registered. For most patients, data on smoking and lipid levels were not available at inclusion, but could be extracted from other visits. At the time of this review, five medical records (two in the 1978 cohort and three in the 1995 cohort) could not be obtained.

Standard population and cardiovascular event definition

The general population of Malmö, aged $\geqslant 16$ years, served as the standard population. The Swedish population register [27] was used to obtain the number of residents in Malmö during the study periods.

Data on cardiovascular events were retrieved from the Swedish National Hospital Discharge Register and the Causes of Death Register [27]. The underlying assumption is that, virtually, all clinical significant CAD, cerebrovascular disease (CEVD) or peripheral artery disease (PAD) events would lead to either hospitalization or death. These registers are both administered by the Swedish National Board of Health and Welfare. The hospital discharge register is based on reports from local registers, and includes information on age, sex and place of residence for each individual, as well as the time for hospitalization and discharge diagnoses (primary and secondary), classified according to the International Classification of Diseases, 8th, 9th and 10th revision (ICD-8, -9 and -10) [28–30] for each inpatient episode. ICD-9 uses the same codes as ICD-8 for diagnoses used in these analyses. The proportion of hospital discharges not reported to the register has been estimated to be 1–2% [27]. In evaluations of the accuracy of registered diagnoses conducted in 1987 and 1995, 86% of the episodes classified as being due to acute myocardial infarction (AMI) fulfilled predefined criteria for definite AMI, whereas 9% was considered to have had possible AMI [27]. The Causes of Death Register is based on compulsory reporting of underlying and contributing causes of death, and contains information on age, sex, place of residence and date of death. In 1996, the register was estimated to include data on 99.64% of all deaths [27]. Previous validation studies of death certification for myocardial infarction have reported confirmation rates of 92–96% [31].

For both cohorts, data on all hospital episodes with a registered diagnosis (both underlying and contributing causes) of CAD (ICD-10: I21–I25; ICD-9: 410–414), CEVD (ICD-10: I61–I69; ICD-9: 431–438) or PAD (ICD-10: I70–I72, I73.9 and I74; ICD-9: 440–442, 443× and 444), and all deaths using the same ICD codes were retrieved from the Hospital Discharge Register and Causes of Death Register. For the period 1 January 1974 to 1 January 1997 (for the causes of Death Register) and 1 January 1998 (Hospital Discharge Register) ICD-8 and -9 codes were used; and for the period after these dates to the end of the study, the corresponding ICD-10 codes were used. To get an estimation of prevalent CVD, events for a period of 4 years before baseline, i.e. 1974–78 and 1991–95, were captured in a similar fashion through linkage with the Hospital Discharge Register. The period of 4 years before baseline was used, since the starting date for the Hospital Discharge Register in Malmö was 1 January 1974. Patients with prevalent CVD were excluded from the analysis of first-ever CVD events.

Statistical analyses

The groups were compared using the Student's *t*-test for normally distributed variables, the Mann–Whitney U-test for variables without a normal distribution and the chi-square test for categorical variables. Cardiovascular event rates were calculated for the time periods 1978–85 for the 1978 cohort and 1995–2002 for the 1995 cohort. To enable age- and sex-specific cardiovascular event rates, the male and female subgroup of patients and controls were stratified into 15 age groups (i.e. fourteen 5-year periods: e.g. 15- to 19-year olds, 20- to 24-year olds, etc.; and one $\geqslant 85$-year-old group). We also stratified the events into time intervals of 4 years for the 1978 cohort (1978–81 and 1982–85) and the 1995 cohort (1995–98 and 1999–2002). Based on indirect standardization to the Malmö general population, the expected number of events, based on information from the same national registers, i.e. Hospital Discharge Register and the Causes of Death Register, for each group in the RA cohorts was estimated. Age-adjusted standardized mortality rates (SMRs) and standardized morbidity rates (SMoRs) with 95% CIs were calculated for each sex separately for CAD, CEVD and PAD and also for CVD overall. Both fatal and non-fatal events were included in SmoR. In these analyses, a single patient can contribute to all three subgroups (CAD, CEVD and PAD), if the patient has been discharged (or died) with several diagnoses during the follow-up period.

In each of the RA cohorts, the impact of disease severity markers and traditional CVD risk factors on the risk of new-onset cardiovascular morbidity, during the study period, was examined separately using Cox regression, adjusted for age at baseline and sex. The study was approved by the ethics committee at the Medical Faculty at Lund University, Sweden.

Results

Baseline characteristics for the two cohorts

The sex distribution, age at disease onset and disease duration were similar in the two cohorts (Table 1). Lower disease activity was found in the 1995 cohort, measured by CRP ($P < 0.001$) and Ritchie's articular index for joint tenderness ($P < 0.001$). Functional disability classified by Steinbrocker functional class index showed a pattern with twice as many patients in Class I and only half as many in Classes III and IV in the 1995 cohort compared with the 1978 cohort. In the 1978 cohort, a higher proportion was RF-positive cases compared with the 1995 cohort.

Patients in the 1995 cohort were found to take DMARDs and glucocorticosteroids more frequently at baseline, whereas NSAID use did not differ significantly (Table 1). Patients in the 1995 group had, on average, taken almost twice as many DMARDs during the course of their disease. Combination therapies were not used in any patient in the 1978 cohort and in only one patient in the 1995 cohort.

Occurrence and treatment of traditional risk factors for CVD

In both cohorts, ~25% of the patients were treated for hypertension. Blood pressure measured at inclusion was slightly lower in the 1995 cohort (Table 2). Overall, the proportion treated for hypertension was similar in the two groups, although angiotensive-converting enzyme inhibitors ($n = 3$) and calcium

blockers ($n=10$) were only used in 1995, due to lack of availability of these drugs in 1978. Lipid-lowering drugs were not used at all in either of the cohorts. Anti-platelet drugs were used by only nine patients in the 1995 cohort, and none by any in the 1978 cohort. A major proportion of the patients in both cohorts used NSAIDs (Table 1). The occurrence of diabetes was similar in the two cohorts. Cholesterol was analysed between 1972 and 1993 in 98 (66%) patients for the 1978 cohort and between 1981 and 2007 in 62 (39%) patients for the 1995 cohort. Cholesterol levels were higher in the 1995 cohort compared with the 1978 cohort (mean 5.68 *vs* 4.75 mmol/l; $P=0.001$).

Data on smoking were available for 104 (70%) patients in the 1978 cohort and 82 (51%) patients in the 1995 cohort. There was a trend towards fewer current smokers in the 1995 cohort (24 *vs* 31%).

TABLE 1. Baseline data for the two RA cohorts

	1978 cohort, $n=148$	1995 cohort, $n=161$
Age at disease onset, years	46.3 (14.2)	48.3 (16.4)
Female, *n* (%)	117 (79.1)	125 (77.6)
Disease duration, years	13.97 (10.3)	13.38 (11.5)
RF seropositive, %	88**	73
CRP, *n* (%), mg/l†		
0–9	41 (28)	85 (53)
10–49	46 (31)	39 (24)
⩾50	61 (41)	37 (23)
Steinbrocker functional class index†, *n* (%)		
Class 1	26 (18)	59 (37)
Class 2	82 (55)	81 (50)
Class 3	31 (21)	19 (12)
Class 4	9 (6)	2 (1)
RAI, median (IQR)	7 (3–15)***	4 (2–8)
Number of DMARDs ever taken	1.46 (0.98)***	2.66 (2.00)
Current NSAIDs, *n* (%)	133 (90)	123 (76)
Current DMARDs, *n* (%)	75 (51)	108 (67)
Current MTX, *n* (%)	0	33 (20.4)
Current anti-malarials, *n* (%)	36 (24)	17 (10)
Current biologics, *n* (%)	0	0
Current glucocorticosteroids, *n* (%)	18* (12)	35 (22)

Values are given as mean (S.D.) unless otherwise indicated. *$P=0.03$; **$P=0.001$; ***$P<0.001$ *vs* 1995 cohort; †$P<0.001$ for the distribution of CRP levels and Steinbrocker functional class index in the 1978 cohort *vs* 1995 cohort (chi-square test). RAI: Ritchie's articular index. IQR: inter quartile range.

TABLE 2. Cardiovascular risk factors and preventive treatment at inclusion

	1978 cohort, $n=148$	1995 cohort, $n=161$
Hypertension, *n* (%)	81 (55)	90 (56)
Systolic blood pressure, mm Hg	145 (23.6)	144 (18.2)
Diastolic blood pressure, mm Hg	84 (9.4)	81 (9.9)
Anti-hypertensive treatment, *n* (%)	35 (24)	37 (23)
Diabetes	5 (3.4)	4 (2.5)
Smoking, *n* (%)		
Current	33 (31)	20 (24)
Former	8 (8)	15 (18)
Non-smoker	63 (61)	48 (58)
Cholesterol, mmol/l**	4.75 (1.13)*	5.68 (1.22)
Lipid-lowering drug, *n*	0	0
Anti-platelet treatment, *n* (%)	0	9 (4.5)

Values are given as mean (S.D.) unless otherwise indicated. Hypertension was defined as blood pressure over 140/80 at inclusion, treatment with anti-hypertensive agents or diagnosis-predating inclusion. Diabetes was defined as a documented clinical diagnosis at the time of assessment. *$P=0.001$ *vs* the 1995 cohort. **Based on 98 subjects in the 1978 and 62 subjects in the 1995 cohorts, respectively.

Cardiovascular morbidity

Sixty-eight patients had a first-ever cardiovascular event during the study period (31 in the 1978 cohort and 37 in the 1995 cohort). In the 1978 cohort, 24 first-ever events of CAD, 6 of CEVD and 8 of PAD occurred; whereas in the 1995 cohort, the corresponding figures were 23 for CAD, 15 for CEVD and 7 for PAD. Overall CVD morbidity was elevated compared with the background population in the 1978 cohort (SMoR 158; 95% CI 111, 225) as well as in the 1995 cohort (SMoR 173; 95% CI 117, 228). This was mainly due to a significantly increased risk of CAD in both RA cohorts. Hospitalization due to PAD occurred more frequently than expected in both cohorts, although fewer events resulted in less precise estimates for PAD compared with CAD (Fig. 1).

Stratification by sex revealed a significantly increased morbidity from CVD overall and CAD in women with RA compared with the general population (Table 3). The estimated SMoRs were slightly higher in the 1995 cohort than in the 1978 cohort, suggesting that RA-associated excess CVD morbidity did not decrease over time in women (Table 3). Statistical power is limited for the male subset, as indicated by the wide CIs.

Predictors for CVD events

Substantial RA-related disability (Steinbrocker's functional classes III and IV) was associated with an increased age- and sex-adjusted risk of CVD in the 1995 cohort, as well as in the 1978 cohort (Table 4). A high baseline CRP ($\geqslant 50$ mg/l) level also tended to predict CVD in both cohorts, although the association did not reach statistical significance. Hypertension at inclusion was a predictor for CVD events, in particular in the 1995 cohort (Table 4).

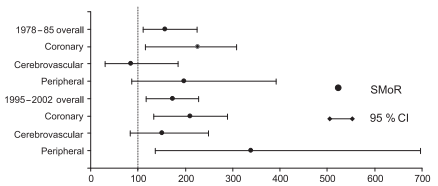

FIG. 1. Cardiovascular morbidity over a period of 8-year follow-up for the two cohorts of RA patients identified in 1978 and 1995, for overall CVD and its subsets. SMoR with 95% CIs.

TABLE 3. Cardiovascular morbidity (fatal and non-fatal) during the 8-year follow-up in patients with RA compared with the background population

	1978 cohort, $n=148$			1995 cohort, $n=161$		
	Observed	Expected	SMoR (95% CI)	Observed	Expected	SMoR (95% CI)
CVD overall women	23	13.9	165 (105, 248)	28	15.0	186 (124, 269)
CVD overall men	8	5.7	141 (61, 277)	9	6.4	140 (64, 266)
CAD women	16	7.3	219 (125, 355)	17	7.6	224 (130, 359)
CAD men	8	3.3	240 (104, 473)	6	3.3	181 (66, 393)
CEVD women	4	5.3	75 (20, 193)	11	7.2	153 (76, 274)
CEVD men	2	1.7	115 (14, 414)	4	2.8	145 (39, 371)
PAD women	6	2.9	205 (75, 446)	5	1.0	485 (157, 1133)
PAD men	2	1.1	180 (22, 652)	2	1.0	193 (23, 697)

TABLE 4. Impact of baseline factors on the risk of cardiovascular events (fatal and non-fatal) during the 8-year follow-up, adjusted for age at inclusion and sex

	1978 cohort, $n=148$ HR (95% CI)	1995 cohort, $n=161$ HR (95% CI)
RF		
Negative	1.00	1.00
Positive	1.80 (0.42, 7.60)	1.03 (0.50, 2.14)
CRP, mg/l		
0–9	1.00	1.00
10–49	1.88 (0.64, 5.51)	1.03 (0.44, 2.43)
⩾50	1.86 (0.67, 5.15)	1.43 (0.68, 3.02)
Steinbrocker functional class index		
Classes I and II	1.00	1.00
Classes III and IV	1.60 (1.01, 2.53)	3.72 (1.76, 7.86)
Hypertension*		
No	1.00	1.00
Yes	1.65 (0.63, 4.32)	3.36 (1.27, 8.85)

*Hypertension was defined as a documented clinical diagnosis at the time of assessment or as blood pressure over 140/80 at inclusion or finally using anti-hypertensive treatment. HR: hazard ratio.

TABLE 5. Mortality during the 8-year follow-up in patients with RA compared with the background population

	Observed	Expected	SMR	95% CI
1978 cohort, $n=148$				
Total mortality	36	22.4	161	116, 223
CVD-related mortality	16	9.2	175	100, 284
1995 cohort, $n=164$				
Total mortality	34	29.7	115	82, 160
CVD-related mortality	17	9.9	172	100, 276

Mortality

Excess CVD mortality over time was similar in the two cohorts (SMR 175; 95% CI 100, 284 and 172; 95% CI 100, 276 for the 1978 and 1995 cohorts, respectively) (Table 5). On the other hand, the overall mortality during the 8-year follow-up period was significantly increased in the 1978 cohort, but not in the 1995 cohort (Table 5). However, the 95% CIs overlap, indicating that the difference in mortality between the cohorts was not significant. This difference was mainly due to a lower number of deaths attributed to other causes than CVD. In the 1978 cohort, there were 20 deaths due to other causes (2 malignances, 3 infections, 5 arthritis and 10 miscellaneous) compared with 13.2 expected. In the 1995 cohort, there were 17 deaths due to other causes (three malignances, three infections, six arthritis and five miscellaneous) compared with 19.8 expected.

Discussion

In the present study, we found a significantly increased cardiovascular morbidity and mortality in patients with RA recruited in 1978 as well as in 1995. The pattern was similar in both cohorts in spite of more intensive treatment and more favourable markers of inflammation and disability in the 1995 cohort. Substantial RA-related disability (Steinbrocker's functional classes III and IV) was associated with an increased age- and sex-adjusted risk of CVD in both cohorts, whereas hypertension was a significant predictor in especially the 1995 cohort.

In other recent studies, there is conflicting evidence on mortality due to CVD among patients with RA. In a study of the ARAMIS cohort, a decline in mortality due to myocardial infarction over time (1980–97) was observed [32], suggesting that improved management may protect patients with RA from cardiovascular death. In contrast, in a recently published survey of patients from a British early RA cohort starting in 1986 with a follow-up of 18 years, deaths from CVD were more frequent than expected, especially from CAD [33]. Estimates of excess and CVD mortalities vary for both early and established RA [34]. The different study designs (inception early arthritis cohorts, prospective or cross-sectional RA studies and clinic- or population-based cohorts) and variations in sample sizes, follow-up and geographic areas may explain some of these discrepancies.

It has been suggested that RA is becoming a milder disease [35–38] and that the incidence is declining [7, 10, 38, 39]. The particular trends in incidence and severity are, however, difficult to disentangle, since the ACR criteria for RA contain several elements, which reflect disease severity such as the presence of RF and radiological erosions. Furthermore, such temporal trends may be secondary to an earlier and more intensive treatment [40] with DMARDs or flawed by methodological problems [12].

Possible explanations for less severe disease in the 1995 cohort include selection bias due to better access to rheumatologists and a more effective pharmacological therapy. In the 1995 cohort, compared with 1978, considerably more patients were treated with DMARDs and corticosteroids, the use of MTX had increased and the use of anti-malarials had decreased. In fact, the number of DMARDs ever taken had almost doubled. Despite this increase, only 20% were on MTX and none was on TNF inhibitor therapy at baseline in the 1995 cohort. This is considerably less compared with more recent surveys of the RA population from this catchment area, in which >50% were on MTX therapy and 20% were on anti-TNF therapy in 2005 [11, 41]. Observational studies suggest that DMARD treatment may reduce the excess cardiovascular burden in RA patients. Anti-malarials appear to have a favourable effect on lipids and anti-thrombotic effects [42], and MTX treatment has been associated with reduced CVD mortality [16] in a large observational study.

Furthermore, recent reports from this geographical area [43] and England [44] demonstrate that anti-TNF treatment reduces CVD comorbidity. Since TNF inhibitors were introduced in Sweden in 1999 and initially used only in a minority of patients, it is unlikely that anti-TNF treatment had any major impact on the development of CVD in the 1995 cohort.

Disease severity, including disability and markers of inflammation such as CRP and ESR, has been shown to predict CVD in subjects with RA [45]. Patients with severe extra-articular RA manifestations are at an increased risk of developing CAD [46] as well as peripheral vascular disease [47], indicating that systemic inflammation is a major determinant of vascular comorbidity in RA. These previous observations are supported by our findings of a high CRP and Steinbrocher functional class index as being predictors for CVD events. Since Steinbrocker functional class index probably is a more robust marker of long-term disease severity than a single measure of CRP in most patients, it is not surprising that we found Steinbrocker functional class index to be a stronger risk factor for CVD.

Taken together, despite the fact that disability and CRP levels were lower, and treatment with DMARDs was more active in the 1995 cohort compared with the 1978 cohort, CVD incidence was not reduced in the latter cohort. This may be due to the fact that many patients were still not adequately treated in 1995.

CVD mortality was increased compared with the general population in both cohorts, but overall mortality was only increased in the 1978 cohort. The lack of excess overall mortality in the 1995 cohort may be due to limited power, restricted follow-up time or improved survival from non-CVD morbidity.

In previous surveys, traditional risk factors for vascular disease, such as smoking, hypertension, diabetes and hyperlipidaemia, did not fully account for the increased risk of CVD in subjects with RA [48, 49]. In the present study, there was a trend towards fewer smokers and also higher cholesterol levels in the 1995 cohort. The latter finding could be due to better control of inflammation [50].

In the past few years, the risk of adverse vascular events associated with NSAID treatment, in particular with selective COX-2 inhibitors, has been recognized [51].

In the present study, NSAID treatment at baseline was common in both cohorts. Given the lack of longitudinal data regarding exposure, we were unable to estimate the impact of NSAID treatment on CVD.

One potential problem in this study is that the patient cohorts recruited may have been selected differently. There were more rheumatologists serving the same population in the latter time period, and this could have increased the likelihood of milder cases being seen by a rheumatologist. Such differences in selection would bias the comparison towards lower disease severity and lower disease-associated excess CVD morbidity in the 1995 RA cohort.

Another possible limitation of this study concerns the validity of cardiovascular events. Validation studies have demonstrated a high level of accuracy for the Hospital Discharge Register and the Cause of Death Register, but misclassification is still likely to occur in some cases. There is, however, no intuitive reason to believe that this should have a different effect in the RA cohorts than in the general population. Random misclassification would only decrease the likelihood of detecting true differences between the groups. Another possible limitation relates to left censorship. It would have been advantageous if the cohorts had been based on incident and not prevalent RA cases. In addition, longitudinal data on disease severity and traditional risk factors would probably have been more informative than results from a single structured examination, and would thus have given more precise estimates for the risk factors evaluated. Furthermore, the available data did not enable us to control for differences in life style factors between the RA cohort and the standard population. Smoking [52] and possibly obesity [53] are associated with RA, and this may account for part of the increase of CVD morbidity in this group, although previous surveys do not indicate that this is the major explanation [48].

A major strength of this study is the uniform follow-up of well-defined cohorts and comparison groups. The availability of detailed national statistics for the background population is crucial for these comparisons and makes it possible to adjust for temporal trends in the general population. Investigation of first-ever CVD events in consecutive patients, followed using national registers, reduces the risk of selection bias and uncertainty on right censorship.

In conclusion, we have demonstrated an increased first-ever incidence of cardiovascular morbidity and mortality in two community-based RA cohorts over time, compared with the general population in the corresponding area. This suggests that RA-associated CVD remained a substantial health problem in the community, at least up to the beginning of the 21st century. Heightened awareness of the risk of cardiovascular disease, as well as adequate preventive strategies, are of major importance in the management of patients with RA.

Rheumatology key messages

- Elevation in the incidence of cardiovascular comorbidity in RA patients has been identified two decades apart.
- More has to be done to improve the handling of cardiovascular comorbidity in RA patients.
- Disability is a major predictor of cardiovascular events in patients with RA.

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Smoking, Low Formal Level of Education, Alcohol Consumption and the Risk of Rheumatoid Arthritis.

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Objective. Suggested predictors of rheumatoid arthritis (RA) include environmental exposure, such as smoking. Our purpose was to investigate potential predictors of RA in a nested case-control study based on a prospective cohort.

Methods. Between 1991 and 1996, 30447 persons were included in the Malmö Diet Cancer Study (MDCS). Individuals who developed RA after inclusion were identified by linking the database to different registers. Four controls for every case were selected. Data on life style factors were collected in the MDCS.

Results. We identified 172 incident cases of RA (36 men/136 women, mean age at diagnosis 63 years, 69 % RF positive, median time from inclusion to diagnosis 5 years; range 1–13). In bivariate analyses, current smoking [Odds ratio (OR) = 1.77; 95% confidence interval (CI): 1.13 to 2.78] and low level of formal education, i.e. ≥8 yrs, (OR = 2.46; 95% CI: 1.20 to 5.02 vs University degree) predicted subsequent RA development. Infrequent alcohol consumption was a predictor of RA (OR = 3.63; 95% CI: 1.97 to 6.70) compared to recent use (within the last month), and moderate alcohol consumption (3.5-15.2 g/day vs < 3.5 g/day) was associated with a reduced risk of RA (OR = 0.45;95% CI: 0.21 to 0.97) in multivariate analyses, adjusted for smoking and level of education. High intake was not associated with a further reduction of the risk.

Conclusion. Smoking and a low level of formal education were independent predictors of RA. In addition, moderate alcohol consumption may be associated with a reduced risk.

Introduction

Some genetic factors and many environmental factors have been suggested to be involved in the etiology of rheumatoid arthritis (RA). HLA-DRB1 alleles featuring the shared epitope are found in most patients with RA [1], and genetic factors have an effect on disease progression [2,3]. Analyses of twin studies suggest that over 40% of the etiological fraction is due to environmental factors [4].

The only well established environmental risk factor for developing RA today is Smoking [5-13]. Many studies have demonstrated a link between smoking and the development of RA, in particular seropositive RA, especially in men [8,9,13-16]. Most studies have also shown an association between smoking and RA in women [9,13], although there are exceptions [6]. Smoking individuals who carry the shared epitope have a multifold increased risk of developing RA [17]. This interaction may be restricted to RA patients with anti-citrullinated peptide antibodies (ACPA) [17]. This is of particular interest, since ACPA may be detected years before RA onset [18,19].

Diet and hormonal factors may play a role in the etiology of RA. Some prospective data indicate that red meat consumption may be associated with an increased risk of developing RA [20], although conflicting results have been published [21]. Coffee consumption was a significant predictor for RF positive RA in a prospective study from Finland [22], but not in the US Nurses' Health Survey [23]. Several other factors have been reported to reduce the

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Running title: RA and risk factors

risk for developing RA, such as vitamin D intake [24] and long term breast feeding [25, 26] in prospective studies, and oral contraceptives [27] and regular alcohol intake [6,28] in case control studies.

A number of case control studies based on either incident or prevalent cohorts of RA patients have shown that low level of formal education is associated with an increased risk of developing RA [29-31] and a similar pattern is seen for other chronic diseases [32]. This may be a marker for occupational exposures [33,34] or other factors.

Many case-control studies assess nutritional exposures retrospectively when relating them to chronic diseases, which may lead to methodological problems. Exposure is sometimes affected by recall bias after diagnosis or onset of disease such as denial of exposure or selective recall of presumed etiologic factors. Furthermore, there may be changes in behaviour in cases after onset of symptoms, and this could affect the reported exposure in retrospective surveys. Finally, the control participants often represent a more health-conscious group than the study population, and this may in itself explain some differences in life style factors. In prospective studies recall bias is not a problem, when exposure is measured before disease onset.

In this nested case-control study based on a prospective health survey, we have examined the effect of smoking, alcohol consumption and level of education on the risk of developing RA.

Patients and Methods

Malmö Diet and Cancer Study (MDCS).
This nested case–control study used information from the MDCS which is a prospective cohort study performed in Malmö, a city in the south of Sweden (approximately 245,000 inhabitants in 1995). The MDCS source population was, in 1991, defined as all persons living in the city of Malmö and born during 1926–1945. However, in May 1995, the cohort was extended to include all women born during 1923–1950 and all men born during 1923–1945. With this extension, 74,138 persons constituted the source population. Inadequate Swedish language skills and mental incapacity were the only exclusion criteria. During the baseline examinations from March 1991 to October 1996 a total of 30447 persons joined the MDCS. Details of recruitment and the cohort are described elsewhere [35]. Participants visited the MDCS screening centre twice. During the first visit, they were informed about the study and instructed how to register meals in the menu book and how to fill in the diet questionnaire. Participants completed all questionnaires at home. During the second visit, approximately 2 weeks after the first, the socioeconomic questionnaire was checked for completeness and the dietary interview conducted.

Case and control selection.
From the MDCS cohort we identified persons who developed RA (from inclusion until 2004-12-31) after inclusion in the MDCS by linking the MDCS register to a community based RA register [36], the local outpatient clinic administrative register for Malmö University Hospital, the National Hospital Discharge Register [37] and the National Cause of Death Register [38]. In a structured review of the medical records, patients were classified according to the 1987 American College of Rheumatology (ACR) criteria for RA [39], and the year of RA diagnosis was noted together with Rheumatoid Factor (RF) status (ever vs never positive). Four controls for every case, matched for sex, year of birth and year of screening, who were alive and free of RA when the index person was diagnosed with RA, were selected from the MDCS register.

Variables.
Data on level of formal education, smoking (including cigarettes, cigars, cigarillos and pipe tobacco) and alcohol consumption were collected from the self administered MDCS questionnaire. Formal education was classified into five levels: ≤ 8 years of elementary school, 9-10 years, 11-12 years, >12 years and university degree. University degree was used as the reference. Based on the smoking data from the questionnaire patients were classified into four categories : current regular smoker, occasional smok-

er, former smoker and never smoker. We created a dichotomous variable, comparing current regular smokers to a reference group containing all other. Three patterns of reported alcohol consumption were registered from one single questionnaire item requesting information during the preceding year: No alcohol consumption within the last year (abstainers); alcohol consumption within the last year but not the last month (infrequent alcohol consumption) and alcohol consumption within the last month (recent alcohol consumption). The pattern variable has been used in previous studies, and when used together with follow-up questions on alcohol consumption habits, it is a valid method for assessing alcohol intake [40].

Alcohol exposure in the cohort was also assessed from the menu book, completed during 7 consecutive days following the first visit to the screening centre [41,42], and expressed in grams/day. Among those who reported alcohol intake in the menu book, we used quartiles to define them in three categories, the middle category containing quartiles two and three: 'Low' 0.05-3.66 g/day, 'Moderate' 3.67-15.21 g/day, 'High' 15.22-194.00 g/day. For comparison, one glass containing approximately 150 millilitres of wine with a alcoholic strength of about 12 % corresponds to 14.0 g of alcohol.

The two alcohol variables, i.e., alcohol consumption patterns during the last year from the questionnaire and alcohol intake from the menu book, were treated as independent exposures. For example, an individual could report no alcohol within the last year, but some alcohol consumption in the menu book, or individuals reporting no consumption in the menu book may have reported some alcohol intake during the last year.

In logistic regression models, recent alcohol consumption was used as reference for the analyses of alcohol consumption pattern. All models of quantified alcohol intake were adjusted for total energy intake.[41,42] In the analyses concerning alcohol consumption per day we excluded persons who were considered to be misreporters of total energy intake. Energy misreporting was defined as having a ratio of energy intake to basal metabolic rate outside the 95% confidence interval (CI) limits of the calculated physical activity level [42]. This definition (i.e., of low-, adequate-, and high-energy reporters in the MDCS cohort) was previously described in detail by Mattisson *et al.* using the approach described by Goldberg *et al.* [43] and later refined by Black *et al.* [44]. The exclusions left 97 cases and 432 controls to be included in these analyses. Low alcohol intake was used as the reference.

Statistics

Potential predictors were examined in conditional logistic regression analyses. Each case and the corresponding controls were assigned a group number, and this was entered into the logistic regression models as a categorical variable. Analyses were performed bivariately and also adjusted for the other risk factors evaluated in multivariate models. The analyses were also stratified by RF status (positive vs negative) at diagnosis or later in the cases.

All patients gave their informed consent to be included in the Malmö RA register and the MDCS database. No informed consent was obtained specifically for the present study. This procedure, and the study protocol, was approved by the regional research ethics committee in Lund, Skåne, Sweden.

Results

We identified 172 patients (36 men/136 women, mean age at RA diagnosis 63 years, 69 % RF positive) (Table 1), who were diagnosed with RA after inclusion in the MDCS. The median time from participation in the survey in which exposure data were collected to RA diagnosis was 5 years (range 1–13). The total follow-up for the cohort was 313 425 person-years, which gives an estimated incidence of 55/100 000 person-years (72/100 000 in women; 29/100 000 in men).

Current smoking at inclusion (Table 2) predicted subsequent development of RA (odds ratio (OR) 1.81; 95 % CI 1.15 to 2.83). Stratifying for RF status, we found in bivariate analysis that smoking was

significantly associated with future RF positive RA (OR 1.95; 95 % CI 1.14 to 3.32), but not RF negative RA (OR 1.35; 95 % CI 0.56 to 3.24) (Table 3). Overall and for RF positive RA, smoking was a robust predictor that remained highly significant in multivariate analyses, adjusted for both level of education and menu book reported alcohol consumption (OR = 2.78, 95% CI: 1.23-6.27 for RF positive RA). We also performed a bivariate analysis with smoking history defined in four categories with never smoked as reference. Again, current regular smoking was a significant predictor (OR 2.78; 95 % CI 1.21 to 6.07). Occasional smoking was also significantly associated with RA, although with a wider CI (OR 2.76; 95 % CI 1.04 to 7.27), whereas former smoking was not (OR 1.13; 95 % CI 0.69 to 1.86). Individuals with elementary school education only (Table 2) had an increased risk of RA compared to those with a university degree (OR 2.46; CI 1.20 to 5.02) in bivariate analyses. In multivariate models, the associations between RA and smoking (OR 1.71; CI 1.08 to 2.69) and low formal education (OR 2.27; CI 1.11 to 4.64) remained significant in a model adjusted for both factors (Figure 1).

Analyses of alcohol consumption in g/day during one week, restricted to adequate energy reporters (97 cases and 432 matched controls), showed in bivariate analyses that individuals with reported moderate alcohol intake had a tendency towards decreased risk of RA (OR 0.52; CI 0.25 to1.07 vs. low intake), whereas there was no major difference in the estimated risk for those with high intake, compared to those with low intake (Table 2).

In multivariate analyses, adjusted for current smoking and formal level of education, individuals with reported moderate alcohol intake had a decreased risk of developing RA (OR 0.47; CI 0.22 to 0.99 vs. low intake) (Table 4). This effect was only statistically significant for patients with seropositive RA (not shown).

Looking at patterns of alcohol consumption, bivariate analyses (Table 2) showed that individuals with reported infrequent alcohol intake had an increased risk of RA (OR 3.47; CI 1.91 to 6.30 vs. recent intake). There was no increased risk for abstainers.

In multivariate models, individuals with reported infrequent alcohol intake had an increased risk of RA (OR 3.52; CI 1.92 to 6.45 vs. recent intake).

Discussion

In this nested case-control study based on a prospective health survey, smoking and low level of formal education were independent risk factors for RA. In addition moderate alcohol consumption tended to be protective for RA development in multivariate analyses and infrequent alcohol intake pattern was associated with an increased risk of developing RA. Previous studies have shown that cigarette smoking is associated with being RF seropositive also in individuals without RA [45]. The exact role of RF in RA pathogenesis is unknown, although it is clear that RF production may precede the development of RA, sometimes by many years [46]. This association between smoking on one hand and and RF seropositivity and RA on the other may be causal or it may, at least partly, be due factors associated with smoking, such as differences in diet [47] or other exposures, which themselves may be partly responsible for the development of RA. For example, in a study of blood donors lower levels of antioxidants were found in those who later developed RA [48]. Finally, smoking is associated with other co-morbidities, including respiratory infections, which could be involved in the causation of RA[49].

A number of chronic diseases occur more frequently in individuals with limited formal education [32,50].An inverse association between level of education and clinical symptoms has been described in several studies of RA patients [51-53] and a lower level of formal education was associated with RA in a Swedish case-control study [54]. Furthermore, the combined group of manual labourers, and assistant and intermediate non-manual workers had significantly higher risk of RF-positive RA, but not RF-negative RA, compared to more qualified non-manual workers [33]. A recent population-based case-control

study, also from Sweden, found that individuals without a university degree had significantly higher risk of RA compared to individuals with a university degree [31]. This association was seen only for rheumatoid factor (RF) positive cases. Whether these associations are related to differences in lifestyle or other factors is unknown, but one possible explanation is that people with lower formal education also could have less access to healthcare for various reasons, present later and be more likely to develop the severe, chronic phenotype identified in these studies.

This pattern may also vary in different socioeconomic settings. A population

based study in Norway found an inverse association between longer education and risk of RA, but this association was not statistically significant after adjustment for age, sex, marital status, body mass index, employment status, and current smoking. [15] Another population based study in the United Kingdom found no association between social class, based on occupational status, and incidence of RA [55].

Alcohol has been shown to diminish the response to immunogens in animals as well as in human [56,57]. Alcohol can down-regulate the production of proinflammatory molecules through its influence on innate immunity [58]. Notably, addition of alcohol to the drinking water for mice prone to develop collagen induced arthritis was recently shown to reduce subsequent clinical signs of arthritis as well as joint destruction [59]. An indication that alcohol consumption may also influence the risk of human RA has come from different studies on environmental factors in RA development. Several case control studies based on early RA samples have suggested that moderate drinking might reduce the risk for developing RA [5,6,9,28], whereas two prospective studies found no association between RA and reported alcohol consumption [22,60]. It is not clear whether these associations are causal or whether the observed protective effect among moderate alcohol consumers is due to confounders. One example of such a confounder is a higher intake of vegetables and fruits, which using MDCS data has previously been shown to be associated with alcohol intake [47].

Our results measuring alcohol consumption in grams per day (table 3) support a protective effect against developing RA from moderate alcohol consumption (3.67-15.21 g/day), corresponding to approximately between 2 glasses of wine per week and 1 glass of wine daily. In contrast with two large retrospective case-control studies from Scandinavia, the Swedish EIRA study and the Danish CACORA study [28], there was no significant reduction of the risk of RA in the subgroup with the highest reported alcohol intake in the present study. Potential explanations include differences in the study design and in the validity of data on alcohol consumption. Due to potential misreporting, self-reports of alcohol intake must be used with caution when trying to establish a level where alcohol exerts a biologic effect. In the MDCS study, intakes of alcoholic beverages were recorded in the menu book during 7 consecutive days. The relative validity and reproducibility of the MDCS diet history for total alcohol and alcoholic beverages are high [61–63], suggest a good ability to rank individuals correctly.

Previous studies have suggested that misreporting of total energy intake may be associated with misreporting of other nutritients, including alcohol [41,42]. Like in previous studies using the MDCS cohort, we excluded individuals with a very high or very low reported energy intake compared to the reported physical activity level. By thus excluding likely misreporters of total energy intake, we probably reduced the impact of alcohol consumption misreported in absolute amounts.

Infrequent alcohol consumption (i.e. reported intake within the last year, but not within the last month) was a robust predictor of RA. This may be due to life style patterns and exposures associated with reporting recent alcohol consumption, rather than a direct biologic effect of alcohol itself. The multivariate analysis shows that this association was not due to differences in smoking or level of formal education, but unmeasured confounding by other exposures may play a role.

The risk of developing RA was similar in abstainers and recent alcohol users. In this context, abstainers probably constitute a mixed group containing people with previous alcohol problems and life long teetotallers.

One limitation of the present study is due to the relatively low number of cases, with limited precision of estimates, especially in multivariate analyses. Due to the design of the MDCS, which included only women aged 44-74 and men aged 45-74 at study entry, our results apply only to prediction of RA with onset in the late 40's or later. The relative importance of early life events and genetic factors may be greater for RA with onset in younger individuals. Furthermore, the relatively low participation rate in the MDCS may slightly hamper the ability to generalise our results to the source population. Alcohol consumption pattern can also change over time, although it is plausible that cases and controls will make comparable changes.

Strengths of this study include the community-based approach, the well-defined catchment area, and the comprehensive effort to identify incident RA cases using multiple sources. The estimated incidence of RA in this cohort of 72/100 000 person-years in women and 29/100 000 in men is slightly higher than recent findings in corresponding age groups in a population-based study from the UK (annual incidence 54–65 per 100 000 in women aged 44–74; corresponding figures in men: 16–29 per 100 000 aged 45–74) [64]. This suggests that we identified virtually all cases of incident RA in the cohort, indicating that our cases are likely to be a representative sample of patients with RA in the area. Data on predictors were collected before disease onset, which means that recall bias in individuals who developed RA or possible effects of RA on lifestyle factors could not influence our results.

In conclusion, smoking and a low level of formal education are independent risk factors for developing RA. In addition, moderate alcohol consumption may be associated with a reduced risk. The underlying mechanisms require further study.

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Disclosure statement/Competing interests

The authors have no potential conflicts of interest regarding this paper.

1. Key messages

Smoking and a low level of formal education are independent risk factors for developing RA.
Moderate alcohol consumption may be associated with a reduced risk of developing RA.

2. Key words for internet

Smoking
Risk factors
Education
RA
Alcohol
Malmö Diet Cancer Study
Sweden

Table I
Baseline data for the pre-RA cases and controls.

	Pre-RA cases (N =172)	Controls (N=688)
Age at disease onset (yrs)	63.4 (8.0)	NA
Age at screening (yrs)	58.0 (7.2)	58.0 (7.2)
Female	136 (79.1 %)	544 (79.1 %)
Time from inclusion to RA diagnosis *	5 (1 to 13)	NA
RF seropositive	69 %	NA
Current smoking	52 (32.3 %)	155 (23.9 %)
Formal education		
: ≤ 8 years	78 (48.8 %)	262 (40.5 %)
: 9-10 years	44 (27.5 %)	182 (28.1 %)
: 11-12 years	12 (7.5 %)	56 (8.7 %)
: > 12 years	11 (6.9 %)	54 (8.3 %)
: University degree	15 (9.4 %)	93 (14.4) %
Alcohol consumption last year**		
: Abstainers	17 (10.6 %)	70 (10.8 %)
: 'Infrequent alcohol'	32 (19.9 %)	58 (8.9 %)
: Recent alcohol'	112 (69.6 %)	521 (80.3 %)
Alcohol consumption (one week) *** (g/day)	10.5 (8.8)	12.6 (15.1)
: 'Low': Lowest quartile	28 (28.9 %)	90 (20.8 %)
: 'Moderate': Quartiles 2-3	41 (42.3 %)	230 (53.2 %)
: 'High': Highest quartile	28 (28.9 %)	112 (25.9 %)

Values are given as means (standard deviation) unless otherwise noted. * Median in years (range). NA = Not applicable. RF = Rheumatoid factor. **Abstainers: No consumption during the last year, 'Infrequent alcohol': Some consumption during the last year but not the last month, 'Recent alcohol': Consumption during the last month. *** Only adequate energy reporters (97 cases, 432 controls).'Low': 0.05-3.66 g/day, 'Moderate': 3.67-15.21 g/day , 'High': 15.22-194.00 g/day.

Table II
Smoking, formal education and alcohol consumption as predictors of RA in bivariate analysis.

	Cases	Person-years at risk	Odds Ratio	CI *
Smoking				
No current smoking	109	3268	1.00 (ref)	
Current smoking	52	1131	1.81	1.15 to 2.83
Formal education				
University degree	15	570	1.00 (ref)	
Years of education	44			
: ≤ 8 years	78	1874	2.46	1.20 to 5.02
: 9-10 years	44	1274	1.75	0.84 to 3.62
: 11-12 years	12	381	1.52	0.59 to 3.62
: > 12 years	11	287	1.36	0.52 to 3.58
Alcohol consumption last year**				
: Abstainers	17	494	1.13	0.58 to 2.21
: 'Infrequent alcohol'	32	420	3.47	1.91 to 6.30
: 'Recent alcohol'	112	3483	1.00 (ref)	
Alcohol consumption (one week)***				
: 'Low': Lowest quartile	28	674	1.00 (ref)	
: 'Moderate': Quartiles 2-3	41	1418	0.52	0.25 to 1.07
: 'High': Highest quartile	28	811	0.75	0.33 to 1.69

* CI = 95 % Confidence intervall. ** Abstainers: No consumption during the last year, 'Infrequent alcohol': Some consumption during the last year but not the last month, 'Recent alcohol': Consumption during the last month. *** Only adequate energy reporters (97 cases, 432 controls).'Low': 0.05-3.66 g/day, 'Moderate': 3.67-15.21 g/day, 'High': 15.22-194.00 g/day.

Table III
Predictors of RA, stratified by RF status at diagnosis or later in the cases; bivariate analysis.

	RF positive				RF negative			
	Cases	**Person-years at risk**	**OR***	**CI****	**Cases**	**Person-years at risk**	**OR***	**CI****
Smoking								
No current smoking	69	2039	1.00		37	1109	1.00	
Current smoking	38	737	1.95	1.14 to 3.32	13	379	1.35	0.56 to 3.24
Formal Education								
: University degree	10	347	1.00		5	214	1.00	
: ≤ 8 years	55	1111	2.22	0.96 to 5.10	22	727	3.19	0.74 to 13.84
Alcohol consumption ***								
: 'Recent'	77	2242	1.00		32	1139	1.00	
: Abstainers	8	281	0.73	0.30 to 1.76	8	195	2.40	0.77 to 7.52
: 'Infrequent'	22	253	3.81	1.82 to 7.97	10	152	3.42	1.18 to 9.91
Alcohol consumption (one week)******								
: 'Low'	18	395	1.00		9	246	1.00	
: 'Moderate'	27	957	0.32	0.12 to 0.85	13	407	0.80	0.20 to 3.11
: 'High'	19	542	0.58	0.21 to 1.58	8	245	0.61	0.12 to 3.26

* OR = Odds Ratio. ** CI = Confidence intervall.*** Abstainers: No consumption during the last year, 'Infrequent alcohol': Some consumption during the last year but not the last month, 'Recent alcohol': Consumption during the last month.**** Only adequate energy reporters (97 cases, 432 controls). 'Low': Lowest quartile: 0.05-3.66 g/day. 'Moderate': Quartiles 2-3: 3.67-15.21 g/day. 'High': Highest quartile: 15.22-194.00 g/day.

Table IV
Smoking, formal education and alcohol consumption measured in grams per day as predictors of RA in multivariate analysis, adjusting for the other factors in the table.

	ALL	
	OR*	**CI****
Smoking		
No current smoking	1.00	
Current smoking	2.38	1.21 to 4.68
Formal Education		
: University degree	1.00	
: ≤ 8 years	1.99	0.70 to 5.67
Alcohol consumption (one week)***		
: 'Low'	1.00	
: 'Moderate'	0.47	0.22 to 0.99
: 'High'	0.74	0.32 to 1.74

* OR = Odds Ratio. ** CI = 95 % Confidence intervall. *** Only adequate energy reporters (97 cases, 432 controls). 'Low': Lowest quartile: 0.05-3.66 g/day. 'Moderate': Quartiles 2-3: 3.67-15.21 g/day. 'High': Highest quartile: 15.22-194.00 g/day.

Table V
Smoking, formal education and alcohol consumption pattern as predictors of RA in multivariate analysis, adjusting for the other factors in the table.

	ALL	
	OR*	**CI****
Smoking		
No current smoking	1.00	
Current smoking	1.87	1.17 to 2.98
Formal Education		
: University degree	1.00	
: ≤ 8 years	2.15	1.03 to 4.46
Alcohol consumption ***		
: 'Recent'	1.00	
: Abstainers	1.26	0.63 to 2.51
: 'Infrequent'	3.52	1.92 to 6.45

* OR = Odds Ratio. ** CI = 95 % Confidence intervall. *** Abstainers: No consumption during the last year. 'Infreqent alcohol': Some consumption during the last year but not the last month. 'Recent alcohol': Consumption during the last month.

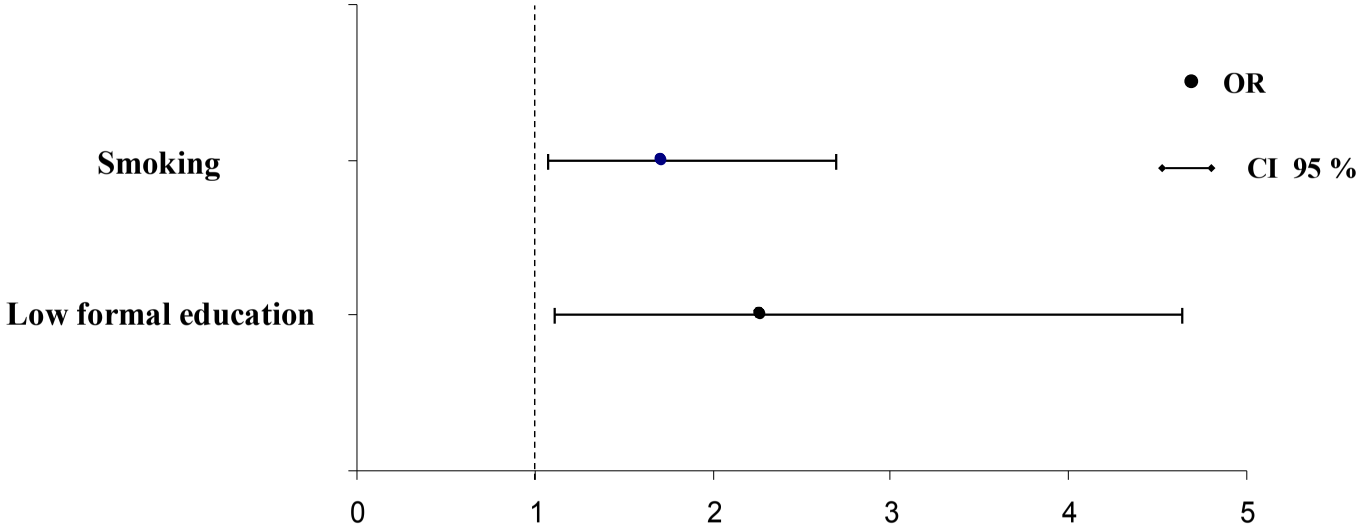

Figure 1. *Current smoking (yes vs no) and low formal education level (≤8 years education) vs university degree are independent predictors for RA (Multivariat analysis; Odds ratio; 95 % CI).*

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Effects of Adalimumab Treatment on Endothelial Cell Activation markers in Skeletal Muscle of Patients with Rheumatoid Arthritis.

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Abstract

Background. Patients with rheumatoid arthritis (RA), and particularly those with severe disease, have increased risk of cardiovascular disease (CVD). Patients with severe extra-articular RA have signs of endothelial cell activation, e.g. by expression of HLA-DQ and interleukin-1α (IL-1α) in microvessels of skeletal muscles. This may indicate a systemic vascular involvement that might contribute to the increased risk for CVD in these patients. Treatment with tumor necrosis factor (TNF) inhibitors in patients with severe RA was associated with a lower risk to develop CVD. The aim of this study was to investigate treatment with adalimumab on endothelial cell activation markers in muscle tissue of patients with RA.

Methods. Fourteen patients with active RA started treatment with adalimumab 40 mg every two weeks. Muscle biopsies were taken before and 3 months after start of treatment.. Eleven patients [9 females, 2 males; mean age 54.2 years, median disease duration 6.5 years, 91 % anti-CCP positive] had muscle biopsies available for analysis from both visits. Seven of these patients were on methotrexate (median dose 20 mg/week). None of the patients had clinical signs of myopathy. IL-1α and HLA-DQ were investigated by immunohistochemistry. Quantification was performed by computer assisted image analysis.

Results. Disease activity, measured by DAS28, decreased (mean 5.5, vs 4.1, p=0.018). A good or moderate EULAR response was seen in 6/11 patients. HLA-DQ was mainly expressed in endothelial cells in capillaries, whereas IL-1α was mainly seen in larger vessels. HLA-DQ expression decreased significantly after treatment (median (M) 0.073 %, interquartile range (IQR) 0.027-0.121 vs M vs 0.023 %, IQR 0.009-0.040; p=0.041). There was a similar trend for IL-1α (mean difference 0.049 %; 95 % confidence interval -0.069-0.168). Decreased expression of IL-1α was seen in EULAR good/moderate responders, but not in non-responders (mean difference 0.114 % vs -0.028 %). HLA-DQ expression decreased in both groups (median difference 0.046 % vs 0.036 %).

Conclusion. Adalimumab treatment was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA. Our findings could indicate reduced endothelial activation in patients treated with anti-TNF drugs. This might contribute to a lower risk of cardiovascular co-morbidity.

This study is registered with ClinicalTrials.gov, number NCT01270087.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, which affects 0.5-1% of the population. Without successful treatment, it leads to joint damage, impaired activity of daily living and increased mortality, especially from cardiovascular disease (CVD). It is well established that there is an association between RA and cardiovascular events (1), mainly due to an increased risk of myocardial infarction (2). The mechanisms behind the increased risk for CVD in patients with RA are not fully understood. However, patients with a more severe form of RA, those with extra-articular manifestations, seem to have a higher risk than others suggesting that the load of inflammation is one risk factor (3,4). Notably, in previous study, patients with extra-articular RA had an increased expression of Interleukin-1α (IL-1a) and Human Leukocyte Antigen DQ (HLA-DQ) in the endothelium of small vessels in skeletal muscle compared to RA patients without extra-articular manifestations, matched for age, sex and disease duration (5). Such endothelial activation has been suggested to contribute to systemic inflammation and vascular disease (6) which might confer a risk to develop premature atherosclerosis. Several vascular abnormalities have been reported to be more common among patients with RA compared to healthy controls, such as increased thickness of the intima and media of the carotid artery (7,8) increased vascular stiffness (9) and endothelial cell dysfunction (10). These findings fit well with the concept of an inflammatory process that leads to activation of vascular endothelium and damage to the vessel wall, which may lead to cardiovascular events. The expression of IL-1α and HLA molecules in endothelial cells of skeletal muscle may indicate a systemic inflammatory reaction and thus could decrease after effective anti-inflammatory treatment.

Treatment with tumor necrosis factor (TNF) inhibitors has led to major clinical improvement in many cases of severe RA (11,12). Adalimumab is a fully human monoclonal anti-TNF antibody, which has been efficacious in clinical trials of methotrexate non-responders as well as methotrexate naïve patients (13,14). In addition to reduced joint inflammation and protection from radiographic damage, TNF inhibitors may also have an effect on RA associated vascular co-morbidity. In a population based cohort, patients with RA treated with biologics who had no previous history of cardiovascular disease had a reduced risk by an estimated 50% of developing cardiovascular disease compared to RA patients treated with non-biologic DMARDs when adjusted for disease severity (15). A recent metaanalysis of published cohort studies showed a decreased risk of myocardial infarction and cardiovascular events in patients with RA treated with anti-TNF drugs (16). However, to this date there have been no controlled trials evaluating the effect of TNF inhibitors on the risk of CVD in patients with RA.

Taken together, observations from earlier reports are compatible with a generalized blood vessel and endothelial cell involvement in at least subgroups of RA patients. Microvessel involvement with endothelial cell activation could play a role in the pathogenesis of cardiovascular disease and potentially be reversible by aggressive anti-inflammatory treatment. The aim of this study was to investigate markers of endothelial cell activation in muscle biopsies from patients with RA before and after 3 months of treatment with adalimumab and to correlate these data to clinical outcome variables.

Patients and methods

Patients who fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA (17), and for whom treatment with adalimumab (Humira) was indicated according to their rheumatologist, were enrolled. They had to have been non-responders to at least one DMARD. Additional inclusion criteria were: At least six swollen joints in 28-joint index, and a CRP > 8 mg / L within the last three months. Patients were excluded if they had been treated with anti-TNF drugs in the last three months prior to inclusion, received intravenous corticosteroids within fourteen days before inclusion, and if they

had ongoing treatment with oral high-dose corticosteroids (equivalent to ≥ 20 mg of prednisolon daily) or had completed such treatment less than fifteen days before inclusion. Patients with contraindications to muscle biopsy, such as severe bleeding disorder, extensive or refractory leg ulcers or severe peripheral vascular disease were also excluded.

Tissue samples, clinical evaluation and laboratory parameters.

Muscle biopsies were performed before and after three months of treatment with adalimumab. Biopsies were taken from the tibialis anterior muscle and were obtained under local anesthesia using a semi-open technique (18). At least three biopsy samples were taken from each patient, snap frozen in isopentane chilled with liquid nitrogen, and stored at -70°C. The second biopsy was taken in the contralateral leg.

All muscle samples were assessed without knowledge of the patient history for histopathological changes by an experienced neuropathologist Dr I Nennesmo at the Division of Pathology, Karolinska University Hospital Huddinge, Sweden. Muscle biopsies were evaluated using conventional histopathology and immunohistochemistry on serial sections to identify pathological changes. The first and last section of each series of consecutive sections were stained with Mayer's haematoxylin and eosin (19), to confirm that the histopathology of the biopsies remained unchanged in the consecutive series of sections. These sections were also used for evaluation of the presence of degeneration, regeneration, atrophy, central nuclei and inflammatory cell infiltrates.

Patients were evaluated at baseline and after 3 months of treatment with adalimumab for RA disease activity, using standard measures [number of swollen joints, number of tender joints, rheumatoid factor (RF), C-reactive protein (CRP), health assessment questionnaire (HAQ) disability index, patient's assessment of pain , patient's global assessment of disease activity and physician's assessment of disease activity]. In addition, a standard physical examination was performed, and data on medications and cardiovascular risk factors such as smoking, current hypertension and history of cardiovascular events were recorded using a structured clinical interview.

Immunohistochemistry studies

The skeletal muscle biopsy specimens were frozen in pre-cooled isopenthane, embedded in OCT compound (Tissue-Tek, Sakura Finetek BV, Zoeterwoude, The Netherlands) and stored at -70°C until sectioning was performed. Cryostat sections from the biopsies (6-8μm) were placed on chrome gelatin-coated slides (Novakemi AB, Enskede, Sweden) and air dried for 30 minutes. The sections were initially fixed for 20 minutes with freshly prepared 24% formaldehyde (Sigma Chemicals, St Louis, MO, USA) at +4°C, washed twice in phosphate buffered saline (PBS) and then left to air-dry before storage at -70°C. Immunohistochemical staining was performed using standard avidin-biotin-peroxidase complex technique, as previously described (20) (for details about primary antibodies, see table 1). As secondary antibody, a biotinylated horse anti-mouse IgG1 antibody (Vector Laboratories, Burlingame, CA, dilution 1/320) was used.

Evaluation of muscle biopsy stainings

The sections were coded, and the investigator was blinded to the clinical information for each section. Whole tissue sections were assessed using computer assisted image analysis. The method has been described in detail previously (5). Analysis of an entire tissue section typically involved 10-30 microscopic fields. The area of specific immunostaining was expressed as a percentage of the total tissue area evaluated.

Statistical analysis

In the comparison of baseline findings and after three months treatment, the paired T test was used for parameters with a normal distribution (i.e. IL-1α and CD31 expression by computer assisted image analysis); and the results were presented as mean pairwise differences with 95 % confidence intervals. For parameters without a normal distribution (i.e. HLADQ expression

by computer assisted image analysis), the Wilcoxon sign rank test was used, and the results were presented as median pairwise differences and p-values for between group differences. These comparisons were stratified by EULAR good/moderate responder status at 3 months after start of adalimumab. In an additional post-hoc exploratory analysis, the analyses were stratified by current smoking status at baseline.

We analyzed correlations between changes (from inclusion to after three months of treatment) in clinical parameters (i.e. Disease Acivity Score 28 (DAS28)) and endothelial markers (i.e. IL-1α and HLA-DQ expression by computer assisted image analysis). For parameters with a normal distribution, Pearson's correlation test was used, and for parameters without a normal distribution we used Spearman's correlation test.

The study was approved by the regional research ethics committee in Lund, Sweden, and also approved as a phase IV clinical trial by the Swedish Medical Products Agency. The study was monitored according to a standard protocol by an independent agent. All participating patients gave their written informed consent to participate.

This study is registered with ClinicalTrials.gov, number NCT01270087.

Results

Clinical baseline characteristics

Fourteen patients with active RA were started on treatment with adalimumab 40 mg subcutaneously every two weeks. Muscle biopsies taken before and after three months of treatment with adalumimab were available for evaluation from 11 patients, who were enrolled in this study [table 2]. Seven of these patients were on methotrexate (MTX); median dose 20 mg/week, range (10-25 mg/week). The other four patients had previously been treated with MTX. Two of the patients had been treated with anti-TNF drugs before, but not within the last three months. Both had discontinued anti-TNF treatment due to adverse events. One of these patients had been treated with two different anti-TNF drugs. Six patients were current smokers and three had never smoked. None of the patients reported a history of cardiovascular events, but two had previously been diagnosed with hypertension. Six of the patients had a blood pressure over 140/80 mm Hg [mean 135/78; standard deviation (SD) for systolic pressure = 15; SD for diastolic pressure = 13] when examined at inclusion. Three patients used antihypertensive drugs. Diabetes or hyperlipidemia had not been diagnosed in any patient, but two patients had HbA1c just over above the normal range, and three had moderate hypercholesterolemia, at inclusion. None were treated with statins. None of the patients had clinical signs of myositis or myopathy at baseline or after three months. Three of the patients had extra-articular involvement in the form of rheumatoid nodules at inclusion, but no current or previous history of vasculitis or other severe manifestations was recorded.

RA clinical and laboratory outcomes

A good or moderate EULAR (21) response was seen in 6 out of the 11 patients. A clinically significant decrease in disease activity, measured by DAS28, was seen after the three-month period (mean change 1.40; p = 0.018). Disability measured by HAQ (mean change 0.27; p=0.11) and CRP (median change 11 mg/L; p=0.29) were also reduced after 3 months, but the differences did not reach statistical significance.

Muscle histopathological assesment

In the hematoxylin-eosin stained sections two samples showed centrally located nuclei (one at baseline and one other patient after three months of treatment). Minor inflammatory cell infiltrates surrounding or invading non-necrotic muscle fibers were seen in two patients at baseline, but in none of the patients after three months of treatment. No signs of fiber degeneration, regeneration or atrophy were seen.

HLA-DQ, IL-1α, and CD31 expression in endothelial cells of muscle tissue

HLA-DQ was mainly expressed in endothelial cells in capillaries, whereas IL1-α was mainly seen in larger vessels. Staining for HLA-DQ decreased significantly after

treatment (median (M) 0.073 %, IQR 0.027-0.121 vs M vs 0.023%, IQR 0.009-0.040; p=0.041) (Fig 1, illustrated by representative examples in Fig 2a and Fig 2b). There was a similar trend for IL-1α measured from baseline to three months follow up (mean difference 0.049 %; 95 % confidence interval(-0.069-0.168). Capillairy density, measured as the percentage of CD31 positive area (Figure 3), also tended to be reduced after adalimumab treatment (Table 3).

Decreased expression of IL-1α was seen in EULAR good/moderate responders, but not in non-responders (mean difference -0.114% vs 0.028%) (Table 3). HLA-DQ expression decreased in both groups (median difference -0.046 % vs -0.036 %). There were no significant correlations between changes in DAS28 and changes in HLA-DQ or IL-1α (data not shown).

There was no major difference in baseline HLA-DQ expression between current smokers and non-smokers (M 0.084% vs 0.073%). There was a significant reduction of HLA-DQ expression in non-smokers (median difference 0.059 %; p=0.043), but not in current smokers (median difference 0.019%, p=0.345).

Other molecules in muscle tissue

ICAM-1, VCAM-1, IL-1β and TNF expression in endothelium and infiltrating cells was extremely limited, and detected in only occasional patients. There was no difference between baseline and follow-up samples (data not shown).

Discussion

Treatment with adalimumab was associated with decreased expression in muscle tissue of endothelial markers previously associated with extra-articular RA. Our findings could indicate reduced systemic endothelial activation in patients treated with anti-TNF drugs. Since there was no comparison group, it is not known whether this effect is specific for adalimumab, or indeed for TNF-inhibitors, or if similar findings could be seen with other potent anti-rheumatic therapy.

Atherosclerosis is the main cause of CVD, and inflammation is now generally accepted as a major component in all stages of atherogenesis, from endothelial stress, via vascular damage to plaque destabilization and plaque rupture subsequently leading to atherothrombosis (22). Based on this, it would be expected that chronic systemic inflammation, as seen in many RA patients, could significantly aggravate atherogenesis. Indeed, striking similarities in the cellular and cytokine profiles of rheumatoid synovial lesions and atherosclerotic plaques have prompted speculations that shared inflammatory pathways in various rheumatic diseases may initiate and/or accelerate plaque formation (23). In fact, the increased risk of CVD events in patients with RA compared to the general population seems not to be explained by the traditional CVD risk factors (24).

Immunological pathways associated with chronic inflammation are important in autoimmune diseaese. The major histocompatibility complex (MHC) class II subtypes HLA-DR, -DQ and –DP are regulators of T-cell dependent immune responses, and abberant expression of these tissue antigens in the endothelium has been demostrated in autoimmune disease such as RA and systemic lupus erythematosus (SLE) (6). Endothelial activation has been defined as "a quantitative change in the level of expression of specific gene products (i.e. proteins), which in turn endow endothelial cells with new capabilities that cumulatively allow endothelial cells to perform new functions" (25). Studies suggest an association between endothelial expression of MHC class II and diffuse endothelial dysfunction (26). In addition, expression of MHC class II and other vascular endothelial markers in cardiac microvessel in patients with CVD may be greater among those with inflammatory rheumatic diseases compared to those without (27). Endothelial up-regulation of MHC class II molecules in systemic autoimmune diseases may be a local phenomenon in affected organs, due to cytokine productions in inflammatory lesions, or a generalized vasular phenomenon, due to circulating cytokines. In patients with RA, increased expression of HLA-DQ in synovial microvessels in joints with active arthritis has been re-

ported (28). By contrast, synovial HLA-DQ-expression in endothelial cells was not found in a group of patients with reactive arthritis (28), suggesting this finding to be specific for microvessels of RA-patients.

In a study of rheumatoid vasculitis, increased expression of HLA-DR, and of the adhesion molecules ICAM-1 and VCAM-1, was observed in muscle biopsy specimens from vasculitis patients with and without perivascular infiltrates, compared to controls with non-vasculitic RA and osteoarthritis (29). Furthermore, our previous study of muscle biopsies from patients with severe extra-articular RA showed increased endothelial expression of HLA-DQ in the absence of local inflammation, compared to RA controls without extra-articular disease. Although the patients in the present study did not have severe extra-articular RA, they had active severe joint disease, which could also be associated with systemic inflammation and vascular endothelial activation. Our data suggest that treatment with adalimumab may decrease such activation.

We also found a trend towards a decrease in expression of IL-1α , in particular among clinical responders. This is in line with previous studies that demonstrated that anti-TNF treatment may reduce IL-1 expression in inflammatory tissue (30).

IL-1α expression has been reported in arteriosclerotic lesions (31), specifically in endothelial cells of microvessels in atherosclerotic plaques (32), but not in normal blood vessels (31). IL-1α induces tissue factor like procoagulant activity and plasminogen activation inhibitor synthesis in human endothelial cell in vitro (33), indicating that increased IL-1α production may have a procoagulant effect on vascular endothelium. Reduction of IL-1α may thus also be important in the prevention of artherosclerotic vascular disease.

A growing body of evidence supports the concept that treatment with TNF inhibitors has beneficial effects on the vascular system. Anti-TNF blockade has been associated with a reduction of different vascular/inflammatory markers, such as serum concentrations of vascular endothelial growth factor (VEGF), which is elevated in patients with RA and correlates with disease activity (34), as well as reduced angiogenesis in the synovium (34). A reduction of intima-media thickness has also been described (35) and, in addition, improved vascular elasticity expressed as significantly less arterial stiffness (36). We postulate that endothelial activation may be more important in the short term as a marker of anti-TNF treatment related effects on the risk of CVD than structural vascular changes.

Previous studies have suggested that smokers with RA may be less likely to respond to anti-TNF treatment (37, 38). In the present study, we found a significant decrease in HLA-DQ expression in non-smokers, but not in current smokers, although baseline levels of expression were at least as high in smokers. This may indicate that some inflammatory pathways in smokers with RA are resistant to anti-TNF treatment. However, these results should be interpreted with caution due to the limited sample and the exploratory nature of this subanalysis.

Major strengths of this study are the repeated biopsies and the well-established set of methods of immune histochemistry used, and also the careful characterization of endothelial marker expression with computer assisted image analysis.

The main limitations of the present study are due to the small sample size, in particular in analyses stratified by clinical response.

In conclusion, treatment with adalimumab was associated with decreased expression of endothelial markers previously associated with severe systemic inflammation in RA. Our findings could indicate reduced systemic endothelial activation in patients treated with anti-TNF drugs, which might reduce the risk to develop cardiovascular co-morbidity.

Abbreviations

RA: Rheumatoid arthritis; OR: Oddsratio; CI: Confidence Intervall; RF: Rheumatoid factor; ACR: AmericanCollege of Rheumatology; HLA-DRB1: Human leukocyte antigen – DRB1. ACPA: anti-citrullinated peptide antibodies; vs: versus; US: United States of America; IL-1α: Interleukin-1α; IL-1β: Interleukin-1β; TNF: Tumor necrosis factor; ICAM-1: Intracellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; CD31: Endothelial cell marker. SLE: Systemic lupus erythematosus; VEGF: Vascular endothelial growth factor; CVD: Cardiovascular disease. IQR: interquartile range. Hba1c: Glycosylated hemoglobin. MHC: Major histocompatibility complex. HAQ: **Health Assessment Questionnaire.** EULAR: European League Against Rheumatism.

Authors' contributions

UB performed part of the muscle biopsies, participated in the collection of clinical data, participated in the immunohistochemistry procedures, performed the conventional microscopic evaluation and the compuer assisted image analysis and the statistical analysis and drafted the manuscript. CG designed the immunohistochemistry protocols and participated in the immunohistochemistry procedures and the conventional microscopic evaluation and the computerized image analysis. IEL participated in the design of the study, in the development of the immunohistochemistry protocols and in manuscript preparation. LTH participated in the design of the study, in the collection of clinical data and in the analysis and interpretation of the results. KN coordinated the study and participated in the collection of clinical data and the muscle biopsy procedures. CT conceived of the study, was responsible for correspondence with the regional research ethics committee and the Swedish Medical Products agency, performed part of the muscle biopsies, participated in the collection of clinical data and the statistical analysis and helped draft the manuscript. All authors read and approved the final manuscript.

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Disclosure statement/Competing interests

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*Table 1. Antibodies used for immunohistochemical stainings**

Antigen	**Clone**	**Dilution or concentration used for tissue staining**	**Isotype**	**Supplier**
HLA-DQ	SK10	1/80	Mouse IgG_1	Becton-Dickinson, San Jose, CA
IL-1α	1277-89-7	1 mg/ml	Mouse IgG_1	Immunokontakt, Bioggo, Switzerland
IL-1β	2D8 combined with 1437-96-5	1 mg/ml	Mouse IgG_1	Immunokontakt, Bioggo, Switzerland
TNF *	2C8	5 mg/ml	Mouse IgG_1	Biodesign, Saco, ME
TNF *	Mab1 combined with Mab11	500 µg/ml	Mouse IgG_1	BD, PharMingen, San Diego, CA
ICAM-1	84H10	1 mg/ml	Mouse IgG_1	AbD Serotec, UK
VCAM-1	51-01C9	0,5 mg/ml	Mouse IgG_1	BD, PharMingen, San Diego, CA
CD31	6002-1	1/400	Mouse IgG_1	Monosan, Uden, The Netherlands
Negative control	X 0931	100 µg/ml	Mouse irrelevant IgG_1	Dakocytomation A/S, Glostrup, Denmark

** HLA = Human leukocyte antigen, IL-1α=interleukin-1α, IL-1β=interleukin-1β, TNF=tumor necrosis factor (clone 2C8 is a non-neutralizating antibody and Mab1 combined with Mab11 is neutralizing antibodies). ICAM-1=Intracellular adhesion molecule, VCAM-1= vascular cell adhesion molecule-1, CD31=endothelial cell marker.*

Table 2. Baseline characteristics

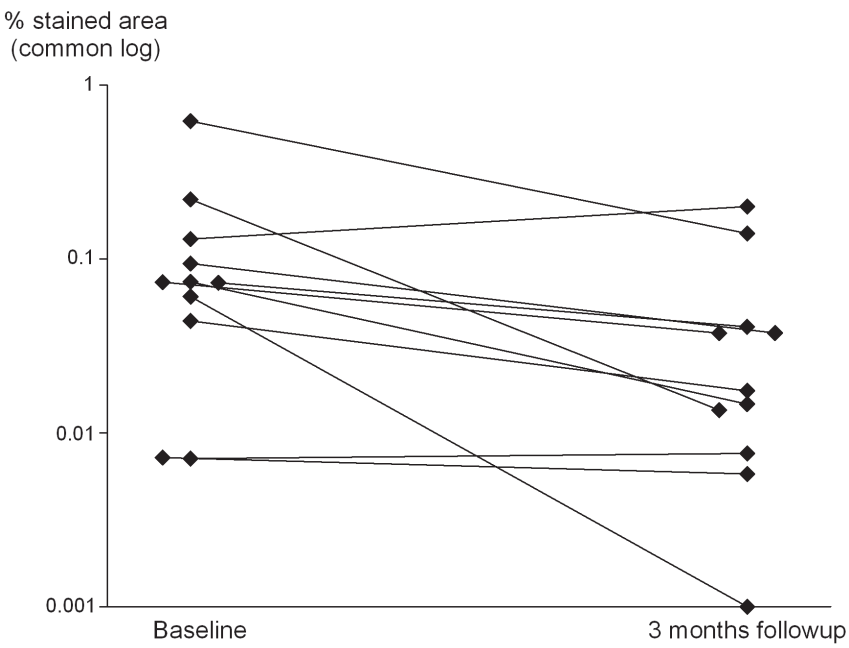
N	11
Sex	9 female / 2 male
Age at inclusion (mean years; SD)	54.2 (10.9)
Disease duration (median years; IQR)	6.5 (1.5 to 15)
Rheumatoid factor seropositive	9/11 (82 %)
Anti-CCP positive	10/11 (91 %)
Methotrexate treated at inclusion	7/11 (64 %)
DAS28 (mean; SD)	5.5 (1.4)
HAQ (mean;SD)	1.44 (0.76)
CRP (mg/L) (median; IQR)	20 (13 to 43)

IQR = Interquartile range. SD = Standard deviation. DAS =Disease activity score. HAQ = Health assessement questionnaire CRP = C-reactive protein.

Table 3. Endothelial markers; % of total tissue area

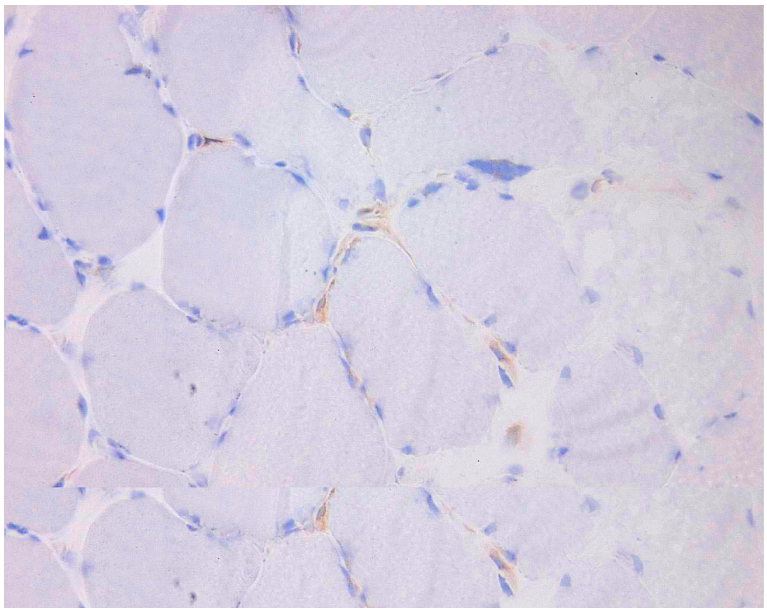
		Baseline	Change from baseline to 3 months follow up	P
IL-1α	Total n=11	0.122 (0.161)	-0.049 (0.176)	0.38
(mean;SD)	Responder n= 6	0.184 (0.200)	-0.114 (0.206)	0.23
	Non responders n=5	0.047 (0.042)	+0.028 (0.105)	0.58
HLA-DQ	Total n= 11	0.073 (0.044-0.130)	-0.036 (-0.061 to -0.001)	0.04
(median;IQR)	Responder n=6	0.073 (0.047-0.152)	-0.046 (-0.097 to 0.018)	0.34
	Non responders n=5	0.073 (0.026-0.357)	-0.036 (-0.268 to -0.014)	0.04
CD31	Total n=11	1.16 (1.36)	-0.51 (1.41)	0.26
(mean;SD)	Responder n=6	1.09 (0.91)	-0.44 (1.36)	0.46
	Non responders n=5	1.25 (1.89)	-0.58 (1.35)	0.47

Figure 1.



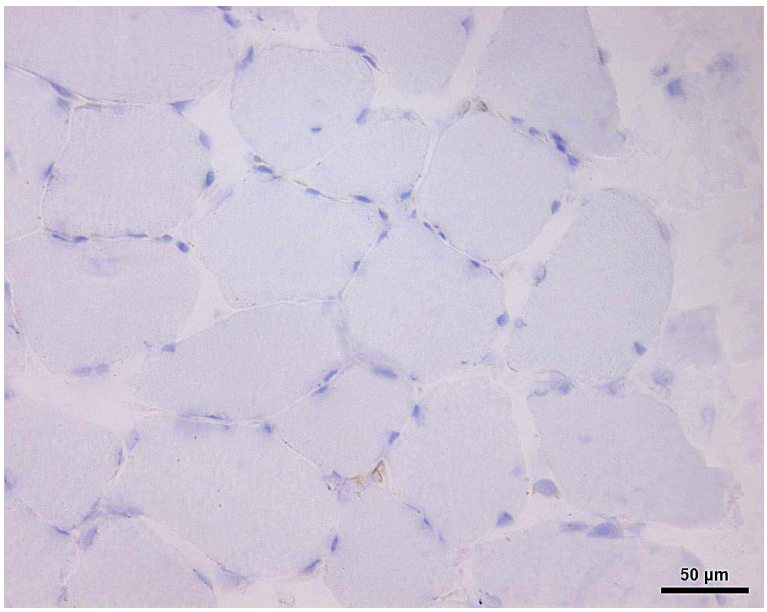
Scattter diagram for endothelial HLA-DQ expression for computer assisted image analysis, baseline data and after three months of treatment for 11 patients.

Figure 2a.



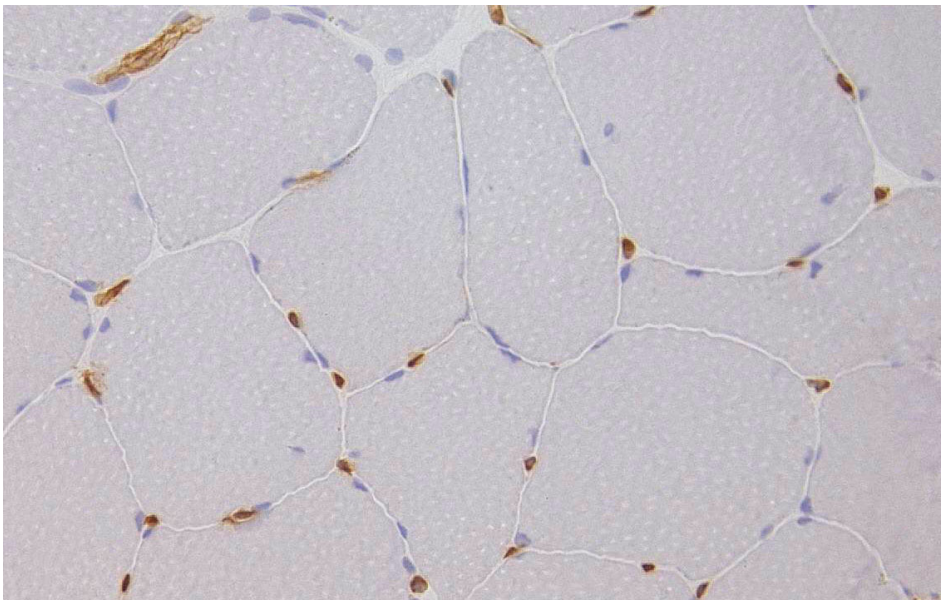
Immunohistochemical staining of a skeletal muscle with capillaries with endothelium positive for HLA-DQ in a patient at baseline. Magnification x20.

Figure 2b.



Skeletal muscle with capillaries with positive HLA-DQ-staining after three months of adlimumab treatment in the same patient as Figure 2a. Magnification x20.

Figure 3.



Representitive example of CD31 staining (endothelial cell marker) of mainly small vessles. Magnification x20.

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Pulmonary Dysfunction, Smoking, Socioeconomic Status and the Risk of Developing Rheumatoid Arthritis

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Abstract

Objectives. Environmental risk factors are of potential interest for both prevention and treatment of rheumatoid arthritis (RA). The purpose of this study was to examine the effect of pulmonary function, smoking and socioeconomic status on the future risk of RA.

Methods. Between 1974 and 1992, 22444 men and 10902 women were included in the Malmö Preventive Medicine Program (MPMP). Pulmonary function was assessed by a standard screening spirometry. Chronic obstructive pulmonary disease (COPD) and restrictive pulmonary dysfunction were defined based on pulmonary function tests. Individuals who developed RA were identified by linking the MPMP database to national and local RA registers. The patients were classified according to the 1987 ACR criteria for RA. Four matched controls for every case were selected.

Results. We identified 290 cases of incident RA (151 men/139 women; mean age at diagnosis 60 years). The median time from inclusion to diagnosis was 12 years. FVC and FEV1 values were similar in cases and controls, overall and also in separate analysis of those screened ≤ 8 years before diagnosis. There was no association between COPD or restrictive pulmonary dysfunction and subsequent development of RA. Current smoking was a strong predictor for RA [odds ratio (OR) 1.79; 95 % confidence interval (CI) 1.32-2.42]. Blue-collar workers had an increased risk of RA (OR 1.54; 95 % CI 1.12-2.10), independent of smoking.

Conclusions. Pulmonary dysfunction did not predict RA, but smoking and low socio-economic status were independent risk factors for RA. Other effects of smoking may be important for RA susceptibility.

Introduction

Rheumatoid arthritis (RA) is a systemic disease, which may involve the lungs. The nature of the relation between inflammation in the lungs and in the joints is incompletely understood. In 1948, Ellman and Ball described three cases with classic manifestations of RA and extensive pulmonary involvement [1]. Since then a number of studies have focused on interstitial lung disease (ILD) among patients with established RA, with widely varying estimates of incidence and prevalence depending on the methods used [2-8]. There is limited data on obstructive pulmonary disease in RA. Geddes et al found that 32 out of 100 studied patients with established RA had an obstructive lung disease, based on standard pulmonary function tests [9]. To our knowledge, no previous studies have investigated pulmonary function before the onset of RA.

Some genetic factors and many environmental factors have been suggested to be involved in the etiology of RA. The most established environmental risk factor for developing RA today is smoking. Individuals with many years of heavy consumption (i.e. a large number of pack-years) are at the greatest risk, both among men and women [10-14] . Smoking individuals who carry the shared epitope have a multifold increased risk of developing RA, suggesting a gene-environment interaction [15]. This has led to the hypothesis that the autoimmune response in RA may be induced in the lungs [15]. Such hypothesis would be supported by the presence of abnormal lung function prior to the clinical onset of RA.

Most chronic diseases are reported more frequently by individuals with fewer than 12 years of formal education [16]. Obviously, level of formal education may be a marker for socio-economic status or occupation. Certain occupations have previously been associated with an increased risk of developing RA [17,18]. This may be a marker for specific occupational exposures [19,20] or other factors.

The object of this study was to investigate the relation between pulmonary function and the risk of RA, and in the same sample analyze the impact of current and previous smoking habits and socio-economic status on RA development. For this purpose, we used a nested case-control study approach, based on a large prospective health survey.

Patients and Methods

Malmö Preventive Medicine Program

This nested case–control study used information from the Malmö Preventive Medicine Program (MPMP), which was a preventive case-finding program, focusing on cardiovascular risk factors and alcohol abuse and started in 1974 at the Department of Preventive Medicine, Malmö University Hospital in the city of Malmö, Sweden (population 235 000 in 1974). The aim was to screen large strata of the adult population in order to find high risk individuals for preventive interventions [21]. Subjects were invited to participate in health screening, including a physical examination and laboratory tests [21]. In addition, every participant filled out a self-administered questionnaire, including questions on lifestyle. Between 1974 and 1992, a total of 22,444 males and 10,902 females attended the screening program with an overall attendance rate of 71%, differing somewhat between years (range 64–78%). Males were mostly screened in the first part of the period (1974–82), and females in the latter part (1981–92), thus implying different length of follow-up time. Various interventions (lifestyle modification, drug therapy) engaged nearly 25% of the screened subjects [21].

Selection of cases and controls.

We identified persons who developed RA after inclusion in this cohort by linking the MPMP register to a community based RA register [22], the local outpatient clinic administrative register for Malmö University Hospital, the National Hospital Discharge Register [23] and the National Cause of Death Register [24]. In a structured review of the medical records, patients were classified according to the 1987 American College of Rheumatology criteria for RA [25], and the year of RA diagnosis was noted.

Four controls for every case, matched

for sex, year of birth and year of screening, who were alive and free of RA when the index person was diagnosed with RA, were selected from the MPMP register.

Variables.

Pulmonary function tests were assessed by a screening spirometry performed by a spirotron apparatus according to a standard protocol as part of the health survey [26].

Obstructive pulmonary dysfunction was defined using the Global initiative for obstructive lung disease (GOLD) criteria for chronic obstructive pulmonary disease (COPD) diagnosis using measured spirometry values of the forced vital capacity (FVC) and the forced expiratory volume within 1 second (FEV1), without previous bronchial dilatation.. Mild COPD (stage I) was defined as FEV1/FVC < 70 % and FEV1 ≥80 % of predicted, and moderate to very severe COPD (stage II-IV) as FEV1/FVC < 70 % and FEV1 < 80% of predicted [27]. Restrictive pulmonary dysfunction was defined as reduced FVC without major reduction of the FEV1/FVC ratio [28], with cut-offs of ≤ 80 % of predicted FVC and ≥ 70% for the FEV1/FVC ratio. Predicted FEV1 and FVC were calculated using the European Respiratory Society standard [29].

Based on the smoking data from the survey questionnaire, subjects were classified using three dichotomous variables: current smoker (yes vs no), current smoker with a history of smoking for more than 10 years (yes vs no), and current smoker with reported smoking of more than 20 cigarettes per day (yes vs no).

Data on socioeconomic status was derived from self-reported job titles in the Swedish national censuses carried out in the years 1960, 1970, 1980, 1985, and 1990

[30]. In the 1975 census no job title was asked for. Occupation was coded using national adaptations of the Nordic Occupational Classification. Three-digit codes were combined into 53 occupational groups and 1 group of people who were economically active but whose occupations were unknown [31], and converted into standardized social class categories expressed as a socioeconomic index (SEI) [32]. Both men and women were classified according to their reported occupation in the census immediately before or following inclusion in the MPMP. Retired individuals were classified according to the last reported occupation in a previous census. Housewives, students, and unemployed without any classification during the study period were excluded due to low numbers and the fact that quite variable socio-economic backgrounds may be present in such individuals [33].

Subjects were classified as blue-collar workers, white-collar workers and "other". Blue-collar workers included manual workers, both skilled and unskilled. White-collar workers included non-manual employees of high-level, medium-level and low-level, as well as self-employed professionals such as lawyers and architects.

Finally, the "other" category covered other self-employed individuals, including farmers.

Statistics

Potential predictors were examined in conditional logistic regression models, taking into account the matched design of the study. Each case and the corresponding controls were assigned a group number, and this was entered into the logistic regression models as a categorical variable. Analyses were performed bivariately and also adjusted for the other risk factors evaluated in multivariate models. FVC, FEV1 and FVC/FEV1 values in cases and controls were compared using Student's T test. In order to assess whether there was any particular pattern for pulmonary function in pre-RA cases included close to RA diagnosis, separate analyses of four groups, stratified for time from screening to RA diagnosis (1-8 years, 8-12 years, 12-18 years and 18-28 years) were performed.

All patients gave their informed consent to be included in the Malmö RA register and the MPMP database. No informed consent was obtained specifically for the present study. This procedure, and the study protocol, was approved by the regional research ethics committee in Lund, Skåne, Sweden.

Results

We identified 290 patients (151 men/139 women, mean age at RA diagnosis 60 years) (Table I), who were diagnosed with RA after inclusion in the MPMP. The median time from participation in the survey in which exposure data were collected to RA diagnosis was 12 years (total range 1–28). The total follow-up for the cohort was 731 703 person-years, which gives an estimated incidence of 40/100 000 person-years (66/100 000 in women; 29/100 000 in men).

Pulmonary function tests

Spirometry data were available for 253 (87 %) of cases and 1006 (87%) of controls. FEV1, FVC and the FEV1/FVC ratio (expressed as FEV1%; FEV1 as percentage of FVC) were similar in cases and controls, both measured in lung volume (L) and percentage of predicted lung volume (Figure I). Similar results were found in separate analyses of four groups, stratified for time from screening to RA diagnosis, including those screened only 1-8 years before RA diagnosis (Table II). In bivariate analysis, there was no association between mild COPD (stage I) [odds ratio (OR) 1.35; 95 % confidence interval (CI) 0.68-2.66 or moderate to very severe COPD (stage II-IV) (OR 1.22; 95 % CI 0.67-2.22) and subsequent development of RA. Restrictive pulmonary dysfunction did not predict RA (OR 0.69; 95% CI 0.37 -1.27) (Table III). There was no significant association between COPD or restrictive pulmonary dysfunction and RA in analyses stratified for sex (Table IV) and time from screening to RA diagnosis (both in those screened ≤ 12 and > 12 years before diagnosis) (Table III). Although ORs were different for restrictive pulmonary disease among those screened ≤ 12 and > 12 years before diagnosis (1.04 vs 0.39), confidence intervals overlapped, and there was no significant interaction between restrictive pulmonary dysfunction and stratum of time to diagnosis in the analysis of the risk of RA (p=0..13). These differences may therefore be due to chance. COPD was less frequent in women (cases 10.9 % vs controls 6.3 %) than in men (cases 17,5 % vs controls 17,2 %), leading to less precise estimates for the impact of COPD in women (Table IV). Although ORs for COPD in women were higher than in men, there was no significant interaction between sex and COPD in logistic regression analysis with RA as the dependent variable (p=0.16).

Smoking and socioeconomic index in bivariate models.

In bivariate analysis (Table III) , current smoking was a strong predictor for developing RA overall (OR 1.79; 95% CI 1.32 to 2.42), as well as in men and women when studied separately (Table III). Current smoking was also a stronger predictor among those screened ≤ 12 years before RA diagnosis (OR 2.31; 95% CI 1.50 to 3.55) than among those screened > 12 years before diagnosis (OR 1.37; 95% CI 0.89 to 2.11). A reported history of long term smoking (> 10 years) among the current smokers was a significant risk factor in both subsets (Figure II). Heavy smoking (> 20 cigarettes/day) and current smoking both predicted RA among those screened ≤ 12 years before RA diagnosis (p=0.015), but not among those screened > 12 years before diagnosis (Figure II).

Blue-collar workers had an increased risk of RA compared to white-collar workers (OR 1.54; CI 1.12 to 2.10), with similar results for men and women in analyses stratified by sex (Table IV). This association was also stronger among those screened ≤ 12 years before RA diagnosis compared to those with a longer time span from screening to RA diagnosis (Table III).

Multivariate models: Pulmonary function tests, smoking and socioeconomic index.

The associations between RA and current smoking (OR 1.72; CI 1.22 to 2.42) and blue-collar worker status (OR 1.59; CI 1.12 to 2.26) remained significant in multivariate models including these two factors and COPD (Table V). We found no association between mild COPD (stage I) or moderate to very severe COPD (stage II-IV) and subsequent development of RA in this multivariate model (Table V).

Similar results for current smoking (OR 1.95; 95 % CI 1.34 to 2.83) and blue collar worker status (OR 1.72; 95 % CI 1.16 to 2.55) were found in models adjusted for restrictive pulmonary dysfunction instead of COPD. Restrictive pulmonary dysfunction did not predict RA in this analysis (OR 0.62; CI 0.33-1.15).

Discussion

Our results do not support a role for smoking induced pulmonary dysfunction in the pathogenesis of RA. Smoking and low socioeconomic status, defined as blue-collar worker status based on occupation, were independent risk factors for developing RA in this nested case-control study based on a prospective health survey. This suggests that smoking exposure leads to RA through mechanisms that are not directly related to pulmonary dysfunction. These unique data provide new insights on the role of environmental factors for disease susceptibility.

The association between smoking and development of RA is the most extensively studied link between the environment and the aetiology of this disease. The increased risk in smokers has been found to be confined to the subset of RA defined by the presence of antibodies to citrullinated peptides (ACPA) [15]. Interaction between smoking and the quantitatively most important genetic risk factor of RA— i.e., the HLA-DRB1 'shared epitope' (SE) [34], for the risk of developing ACPA-positive RA, has been described [15, 35–38], and also tissue studies have proposed smoking as an environmental factor that might lead to citrullination, potentially contributing to anti-citrulline autoimmunity in genetically susceptible individuals [39].

Exposure to crystalline silica is another well defined inhalation exposure, reported, for example, from industries involving mining, construction, ceramics, glass, agriculture, but also in sectors such as electronics. Silica exposure has been observed to be linked to RA [20]., with a twofold increased risk of developing RA in analysis adjusted for smoking [20]. The lack of association between reduced pulmonary function and a future diagnosis of RA in the present study suggests that possible effects of inhalation exposures leading to immune modulation and chronic inflammation is related to at most minor tissue injury, and not clinically recognizable lung damage.

Although ILD may occur in early RA, sometimes preceding the clinical diagnosis of RA [40], we found no association between restrictive pulmonary dysfunction and susbsequent development of RA. This most likely reflects that only a small proportion of RA patients have very early ILD involvement that has an effect on pulmonary function, and that such a small subset would not have a major impact on the overall results. In patients with established RA, suggested risk factors for developing ILD include male sex, older age at RA onset, and a severe disease course [41]. In a recent study of an early RA cohort, the occurrence of severe extra-articular disease manifestations, a major proportion of which were cases of ILD, was predicted by high disease activity and extensive disability burden over the first two years after RA diagnosis [40].This suggests that such manifestations are part of the most severe RA phenotype. We can not exclude, however, that subclinical ILD involvement not leading to reduced pulmonary function could be substantially more frequent in the pre-clinical phase of RA.

Others have found that the increased risk for RA associated with smoking requires a long duration, but merely a moderate intensity, of smoking, and may remain for several years after smoking has stopped [42]. The molecular pathways behind the increased risk of RA associated with smoking, and the interaction between smoking and genetic factors, need to be further investigated. Since some individuals quit smoking in middle age, and a higher proportion would be expected to quit smoking over a longer period of time, our observation of a lower impact of smoking on the risk of RA among those screened > 12 years before RA diagnosis suggests that individuals who quit smoking, even heavy smokers, may reduce their risk of RA. This is compatible with a slow and potentially reversible process.

We have recently shown that individuals with low level of formal education (< 8 years) have an increased risk of developing RA compared to those with a university degree [43]. In the present study, socioeconomic status was defined based on current occupation, which is related to formal education. In a national Swedish study from 2008, Li et al studied the impact of education and occupation on the risk of hospitalization with RA [17]. They found a negative association between RA and an education level > 12 years in women as wellas in men, and there were also associations with certain occupations. These results are supported by the present study, which was not limited to patients hospitalized with RA. By contrast, a study based on a population from the United Kingdom found no association between social class, based on occupational status, and incidence of RA [44], although a later follow-up of the same cohort revealed that individuals with a low social status from deprived areas had a worse prognosis [45].

Unusually, this study included a majority of male cases with RA, due to the sex distribution of the source cohort. Most results were similar in analyses stratified by sex, but the low number of women with COPD is a limitation for this subanalysis. Due to the design of the MPMP, where the majority of participants were in their 40'ies and 50'ies (mean age at screening 44 years in men and 49 years in women), our results apply only to prediction of RA with onset in this age group or later. The relative importance of early life events and genetic factors may be greater for RA with onset in younger individuals. As pulmonary dysfunction may change over time, in particular in smokers, the fact that we had only access to spirometric values from a single time point is a limitation for this study, and we could not exclude an association with COPD in women, possibly due to confounding by other exposures in women with COPD. Spirometry data were only available for 87 % of cases and 87 % of controls, and we did not have any data on treatment with systemic corticosteroids for COPD, which might affect PFT values and possibly also the time of diagnosis of RA. In addition, since we excluded housewives, students and unemployed individuals from the analysis of SEI, the impact of socio-economic factors related to these groups could not be assessed in the present study.

Strengths of this study include the community-based approach, the well-defined catchment area, and the comprehensive effort to identify incident RA cases using multiple sources. The estimated incidence of RA in this cohort of 66/100 000 person-years in women and 29/100 000 in men is slightly higher than recent findings in corresponding age groups in a population-based study from the UK (annual incidence 54–65 per 100 000 in women aged 44–74; corresponding figures in men (16–29 per 100 000 aged 45–74) [46]. This suggests that we identified virtually all cases of incident RA in the cohort, indicating that our cases are likely to be a representative sample of patients with RA in the area. Data on predictors were collected before disease onset, which means that recall bias in individuals who developed RA or possible effects of RA on lifestyle factors could not influence our results.

In conclusion, reduced pulmonary function was not associated with future RA, but smoking and low socioeconomic status were independent predictors of RA. This suggests that several different exposures are important through RA development. Potential underlying underlying mechanisms, including modulation of the immune system and low grade chronic inflammation, require further study.

Table I
Demographics, lung function test, smoking and socio-economic index data in the pre-RA cases and controls.

	Pre-RA cases (N =290)	Controls (N=1160)
Age at disease onset (years)	60.0 (8.7)	NA
Age at screening (years)	47 (7.1)	47 (7.1)
Female	139 (48 %)	556 (48 %)
Time from inclusion to RA diagnosis in years (median, IQR) Total range in years	12 (8 to 18) 1 to 28	NA NA
Duration ≤ 12 years from inclusion to RA diagnosis (n)	149 (51 %)	596 (51 %)
Current smoking (n)	155 (53.8 %)	486 (42.9 %)
Current smoking; > 10 years history of smoking (n)	136 (50.0 %)	394 (36.9 %)
Current smoking; > 20 cigarettes per day (n)	25 (8.6 %)	67 (5.8 %)
$FEV_{1\%}$ (FEV_1/FVC) *	77.9 (9.7)	78.4 (8.5)
Mild COPD (Stage I) **	17 (6.7 %)	56 (5.6 %)
Moderate to Very severe COPD (Stage II-IV)***	20 (7.9 %)	71 (7.1 %)
Restrictive pulmonary dysfunction ****	21 (9.7 %)	100 (11.4 %)
Socioeconomic index		
: Blue collar workers (n) *****	146 (50.3 %)	496 (42.8 %)
: White collar workers (n)******	107 (36.9 %)	511 (44.1 %)
: Others (n) *******	37 (12.8 %)	153 (13.1 %)

Values are given as means (standard deviation) unless otherwise noted.
* Pulmonary function test (spirometry) was performed by 253 cases (87 %) and 1006 controls (87%).
** COPD = Chronic obstructive pulmonary disease. Mild disease FEV_1/FVC < 70 % and FEV_1 predicted ≥ 80 %.
*** Moderate to very severe disease: FEV_1/FVC < 70 % and FEV_1 predicted < 80 %.
**** Restrictive pulmonary dysfunction: ≤ 80 % of predicted FVC and FEV_1/FVC ≥ 70%
***** Blue collar workers (se methods section).
****** White collar workers (se methods section).
******* Others (se methods section).

Table II
Pulmonary function tests in pre RA cases and controls, stratified by the time from inclusion to RA diagnosis in the cases.

From inclusion to RA diagnosis		Case	% of predicted	Controls	% of predicted	P-value for % of predicted
1-8 years	$FEV_{1\%}$	78.7 (8.7)	NA	77.6 (9.5)	NA	0.38 *
Cases = 61 Controls = 238	FEV_1	2.91 (0.66)	91.9 (16.6)	2.95 (0.73)	93.0 (17.7)	0.66
	FVC	3.78 (0.95)	98.2 (18.1)	3.81 (0.90)	99.7 (17.5)	0.56
8-12 years	$FEV_{1\%}$	77.9 (10.2)	NA	78.9 (9.0)	NA	0.48 *
Cases = 61 Controls = 237	FEV_1	3.07 (0.88)	94.0 (20.0)	3.12 (0.72)	96.2 (16.6)	0.38
	FVC	3.93 (1.02)	100.3 (18.9)	3.97 (0.93)	101.5 (15.4)	0.61
12-18 years	$FEV_{1\%}$	77.4 (9.1)	NA	78.9 (8.0)	NA	0.19 *
Cases = 63 Controls = 255	FEV_1	3.05 (0.84)	95.1 (19.5)	3.07 (0.76)	93.5 (15.7)	0.50
	FVC	3.93 (1.03)	102.6 (19.9)	3.88 (0.99)	98.5 (15.6)	0.08
18-28 years	$FEV_{1\%}$	77.7 (9.9)	NA	78.3 (8.4)	NA	0.60 *
Cases = 68 Controls = 276	FEV_1	3.36 (0.72)	96.0 (17.7)	3.19 (0.71)	92.5 (15.1)	0.09
	FVC	4.37 (0.99)	102.0 (14.1)	4.08 (0.91)	97.8 (14.6)	0.03

Values are given as means (standard deviation) of lung volume measured in litres (except for $FEV_{1\%}$ and % of predicted for FEV_1 and FVC).
P-values are given for comparison of % of predicted values, except where otherwise indicated
*** P-values for comparison of measured $FEV_{1\%}$.**

Table III
Pulmonary dysfunction, smoking and socio-economic index as predictors of RA in bivariate analysis

	Number of cases	Number of controls	OR	95 % CI
All				
No current smoking	133	646	1.00 (ref)	
Current smoking	155	486	1.79	1.32 to 2.42
White-collar worker	107	511	1.00 (ref)	
Blue-collar worker	146	496	1.54	1.12 to 2.10
No COPD	216	879	1.00 (ref)	
COPD stage I *	17	56	1.35	0.68 to 2.66
COPD stage II-IV **	20	71	1.22	0.67 to 2.22
No restrictive pulmonary dysfunction	195	779	1.00 (ref)	
Restrictive pulmonary dysfunction***	21	100	0.69	0.37 to 1.27
Duration ≤ 12 years from inclusion to RA diagnosis				
No current smoking	62	329	1.00 (ref)	
Current smoking	86	244	2.31	1.50 to 3.55
White-collar worker	53	280	1.00 (ref)	
Blue-collar worker	72	247	1.71	1.10 to 2.61
No COPD	110	427	1.00 (ref)	
COPD stage I *	7	28	1.35	0.68 to 2.66
COPD stage II-IV **	9	36	1.22	0.67 to 2.22
No restrictive pulmonary dysfunction	97	382	1.00 (ref)	
Restrictive pulmonary dysfunction***	13	45	1.04	0.47 to 2.32
Duration > 12 years from inclusion to RA diagnosis				
No current smoking	242	317	1.00 (ref)	
Current smoking	69	71	1.37	0.89 to 2.11
White-collar worker	54	231	1.00 (ref)	
Blue-collar worker	74	249	1.35	0.86 to 2.12
No COPD	106	452	1.00 (ref)	
COPD stage I *	10	28	1.67	0.68 to 4.07
COPD stage II-IV **	11	35	1.44	0.63 to 3.30
No restrictive pulmonary dysfunction	98	397	1.00 (ref)	
Restrictive pulmonary dysfunction***	8	55	0.39	0.14 to 1.06

* COPD = Chronic obstructive pulmonary disease. Mild disease: $FEV_1/FVC < 70$ % and FEV_1 predicted ≥ 80 %.

** Moderate to very severe disease $FEV_1/FVC < 70$ % and $FEV_1 < 80$ % of predicted.

*** Restrictive pulmonary dysfunction: ≤ 80 % of predicted FVC and $FEV_1/FVC \geq 70$ %.

Table IV
Pulmonary dysfunction, smoking and socio-economic index as predictors of RA in bivariate analysis stratified by sex.

	Number of cases	Number of controls	OR	95 % CI
Women				
No current smoking	73	331	1.00 (ref)	
Current smoking	64	197	1.61	1.04 to 2.49
White-collar workers	55	253	1.00 (ref)	
Blue-collar workers	67	230	1.45	0.97 to 2.27
No COPD	98	399	1.00 (ref)	
COPD stage I *	7	16	2.49	0.73 to 8.48
COPD stage II-IV **	5	11	3.26	0.84 to 12.70
No restrictive pulmonary dysfunction	93	375	1.00 (ref)	
Restrictive pulmonary dysfunction ***	5	24	0.54	0.16 to 1.89
Men				
No current smoking	60	315	1.00 (ref)	
Current smoking	91	289	1.97	1.29 to 3.02
White-collar workers	52	258	1.00 (ref)	
Blue-collar workers	79	266	1.63	1.05 to 2.53
No COPD	118	480	1.00 (ref)	
COPD stage I *	10	40	1.02	0.45 to 2.33
COPD stage II-IV **	15	60	0.96	0.49 to 1.88
No restrictive pulmonary dysfunction	102	404	1.00 (ref)	
Restrictive pulmonary dysfunction ***	16	76	0.74	0.36 to 1.51

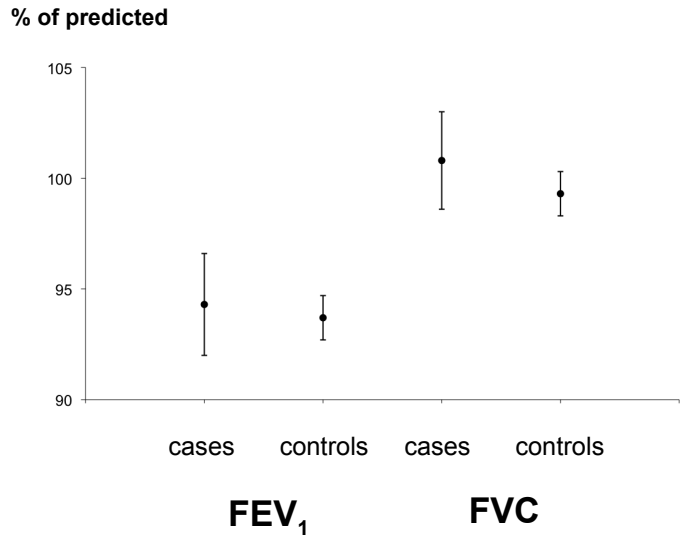
* COPD = Chronic obstructive pulmonary disease. Mild disease: $FEV_1/FVC < 70$ % and FEV_1 predicted ≥ 80 %.
** Moderate to very severe disease $FEV_1/FVC < 70$ % and $FEV_1 < 80$ % of predicted.
*** Restrictive pulmonary dysfunction: ≤ 80 % of predicted FVC and $FEV_1/FVC \geq 70$ %.

Table V
Obstructive pulmonary dysfunction, current smoking and socio-economic index as predictors of RA in multivariate analysis adjusted for all variables in the table .

	OR	95 % CI
All		
No current smoking	1.00 (ref)	
Current smoking	1.72	1.22 to 2.42
White-collar worker	1.00 (ref)	
Blue-collar worker	1.59	1.12 to 2.26
No COPD	1.00 (ref)	
COPD stage I *	1.34	0.67 to 2.68
COPD stage II-IV **	1.08	0.59 to 1.99

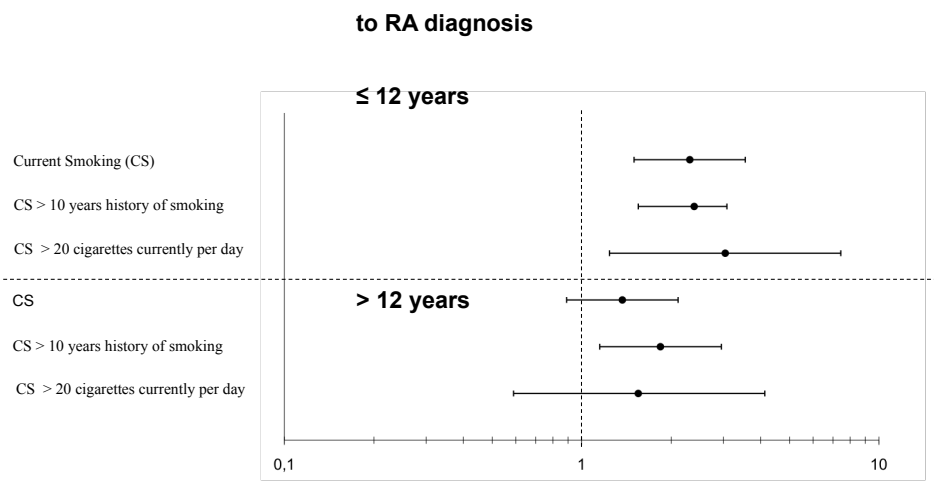
* COPD = Chronic obstructive pulmonary disease. Mild disease: $FEV_1/FVC < 70$ % and FEV_1 predicted ≥ 80 %.
** Moderate to very severe disease $FEV_1/FVC < 70$ % and $FEV_1 < 80$ % of predicted.

Figure 1.



Pulmonary function tests FEV1 and FVC in pre RA cases and controls. Values are given as % of predicted; Means, 95 % confidence intervals.

Figure 2.



Smoking history as a predictor of RA. Bivariate analysis stratified by the time from inclusion to RA diagnosis in the cases; Odds ratio; 95 % confidence intervals. Logarithmic scale.

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Disclosure statement/Competing interests

The authors have no potential conflicts of interest regarding this paper.

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Key messages:

- Reduced pulmonary function was not associated with future RA development.
- Smoking and low socioeconomic status were independent predictors of RA

Key words for internet:

Smoking
Risk factors
Pulmonary dysfunction
Rheumatoid arthritis
Occupation
Spirometry
Malmö Preventive Medicine Program
Socioeconomic status
Sweden

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