Epidemiological and Pathogenic aspects on Cardiovascular Disease in Rheumatoid Arthritis

Anna Södergren
ABSTRACT

Epidemiological and pathogenic aspects on cardiovascular disease in rheumatoid arthritis
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Rheumatoid arthritis (RA) is a chronic disabling disease that is associated with a shortened life span. Cardiovascular disease (CVD) contributes to this increased mortality, and also to a great extent to the co-morbidity observed in patients with RA. This thesis aimed to investigate these issues further.

The incidence of, and prognosis after an acute myocardial infarction (AMI) or stroke in a cohort of RA patients was compared with that in the general population within the northern Sweden MONICA register. The standard incidence ratio (SIR) for AMI was 2.9 and for stroke 2.7 in RA patients compared with the general population (p<0.05 for both). During the first 10 years following an event, RA patients had a higher overall case fatality (CF) compared with controls (HR for AMI=1.67, 95%CI [1.02, 2.71], HR for stroke=1.65, 95%CI [1.03, 2.66]).

An elevated level of homocysteine is regarded to be a risk marker for CVD. The effects of treatment with B vitamins on the homocysteine level in patients with RA were studied in a consecutive cohort of patients with RA. Sixty-two patients with RA having a homocysteine level of ≥12 μmol were randomized to receive either a placebo or a combination of the vitamins B6, B12 and folic acid. The patients were treated and evaluated in a double-blind manner over 12 months. The homocysteine level was found to be significantly decreased in the B-vitamin treated patients compared with the placebo group (p<0.0001).

To evaluate the progression of sub-clinical atherosclerosis in patients with very early RA compared with controls, all patients from the three most northern counties of Sweden newly diagnosed with RA and aged ≤60 years were consecutively recruited. Age and sex matched controls from the general population were also included. Intima media thickness (IMT) of the common carotid artery and endothelium dependent flow mediated dilation (ED-FMD) of the brachial artery were measured using ultrasonography. After 18 months the same measurements were undertaken in a sub-group of the patients with early RA and the relevant controls. There were no differences between patients with early RA and controls in terms of IMT or ED-FMD at inclusion into the study. However, after 18 months there was a significant increase in the IMT among the patients with early RA (p<0.05); no such increase occurred in the control group.

Biomarkers of endothelial activation that may reflect the early atherosclerosis that occurs in RA were also evaluated. At inclusion, both IMT and ED-FMD among the patients with early RA related significantly to several of the biomarkers of endothelial activation. Furthermore, markers of inflammation (e.g., DAS28) were significantly related to biomarkers of endothelial activation.

In conclusion, RA patients had a higher incidence of CVD and a higher CF after a CV event. The increased homocysteine level among patients with RA was as easy to decrease as in the general population. At the time of diagnosis of RA there were no differences in atherosclerosis between patients and controls, however the patients with RA had a more rapid progression of atherosclerosis than the control subjects. Moreover, there were implications of endothelial activation already in patients with very early RA. Taken together, these results emphasize the necessity of optimizing the preventive, diagnostic and caring strategies for CVD in patients with RA.
LIST OF ORIGINAL PAPERS

The thesis is based on the following papers that will be referred to by their roman numerals:


II  Södergren A, Stegmayr B, Öhman ML, Wållberg-Jonsson S. Increased incidence of and impaired prognosis after stroke among patients with seropositive rheumatoid arthritis. Submitted


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* Yxfeldt is the maiden name of Anna Södergren
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<tbody>
<tr>
<td>ACPA</td>
<td>Anti-cyclic citrullinated peptide/protein antibodies</td>
</tr>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
</tr>
<tr>
<td>AIx</td>
<td>Augmentation Index</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CCA</td>
<td>Common carotid artery</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CK-MB</td>
<td>Creatine kinase-MB</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DAS28</td>
<td>Disease activity score 28 joint count</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease modifying anti-rheumatic drug</td>
</tr>
<tr>
<td>ED-FMD</td>
<td>Endothelium dependent flow mediated dilation</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>HAQ</td>
<td>Health assessment questionnaire</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>IL-1 receptor antagonist</td>
</tr>
<tr>
<td>IL-2 sRα</td>
<td>IL-2-soluble receptor alpha</td>
</tr>
<tr>
<td>IMT</td>
<td>Intima media thickness</td>
</tr>
<tr>
<td>IQR</td>
<td>Inter quartile range</td>
</tr>
<tr>
<td>IRR</td>
<td>Incidence rate ratio</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoproteins</td>
</tr>
<tr>
<td>MCP</td>
<td>Metacarpophalangeal</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein -1</td>
</tr>
<tr>
<td>MONICA</td>
<td>Multinational Monitoring of Trends and Determinants of Cardiovascular Disease</td>
</tr>
<tr>
<td>MTP</td>
<td>Metatarsal phalangeal</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>oxLDL</td>
<td>Oxidized low-density lipoproteins</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PIP</td>
<td>Proximal interphalangeal</td>
</tr>
<tr>
<td>PWA</td>
<td>Pulse wave analyses</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RF</td>
<td>Rheumatoid factor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>RR</td>
<td>Risk ratio</td>
</tr>
<tr>
<td>s</td>
<td>Soluble</td>
</tr>
<tr>
<td>SE</td>
<td>Shared epitope</td>
</tr>
<tr>
<td>SIR</td>
<td>Standardised incidence ratio</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>SMR</td>
<td>Standard mortality ratio</td>
</tr>
<tr>
<td>Th1</td>
<td>T helper 1 cells</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T-cells</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular adhesion molecule-1</td>
</tr>
<tr>
<td>VWF</td>
<td>Von Willebrand’s factor</td>
</tr>
</tbody>
</table>
INTRODUCTION

Rheumatoid arthritis

Epidemiology

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease leading to joint destruction, first described clinically in the 19th century. RA is prevalent in all populations around the world, however its prevalence is higher in northern Europe than southern Europe, and even lower in less developed rural parts of the world. The prevalence in northern Europe is 0.3-0.6% for men and 0.6-1.1% for women (Alamanos 2006). In studies from southern Sweden the prevalence was found to be 0.5% (Simonsson 1999) and the incidence was found to be 0.2/1000 individuals and year, being 0.3/1000 for women and 0.2/1000 for men (Söderlin 2002). These numbers are consistent with other recent European studies in which the incidence of RA has been found to be 0.1-1.3/1000 individuals and year (Watson 2003, Alamanos 2006).

Diagnosis and disease activity

Diagnosis of RA is based upon criteria (see Table 1) established by the American College of Rheumatology (ACR), where the diagnostic criteria are based on patients with established disease (Arnett 1988). One of these criteria is demonstration of abnormal amounts of rheumatoid factor (RF), however, more recently antibodies against cyclic citrullinated peptides/proteins (ACPA) have been demonstrated to have a greater specificity at the equivalent sensitivity for diagnosis of early RA compared with RF (Rantapää-Dahlqvist 2005). At present the presence of ACPA is not included in the diagnostic criteria.

Table 1: Criteria for the classification of Rheumatoid Arthritis. A patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. The first four criteria must have been present for at least 6 weeks. Adapted from Arnett 1988.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning stiffness</td>
<td>Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement.</td>
</tr>
<tr>
<td>Arthritis of 3 or more joint areas</td>
<td>At least 3 joint areas simultaneously have had soft tissue swelling or fluid.*</td>
</tr>
<tr>
<td>Arthritis of hand joints</td>
<td>At least 1 area swollen in a wrist, MCP, or PIP joint</td>
</tr>
<tr>
<td>Symmetric arthritis</td>
<td>Simultaneous involvement of the same joint areas on both sides of the body.</td>
</tr>
<tr>
<td>Rheumatoid nodules</td>
<td>Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions.</td>
</tr>
<tr>
<td>Serum rheumatoid factor</td>
<td>Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in &lt;5% of normal control subjects.</td>
</tr>
<tr>
<td>Radiographic changes</td>
<td>Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs.</td>
</tr>
</tbody>
</table>

* The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints

In patients with RA in order to evaluate the need for, and the effect of, treatment it is valuable to measure the disease activity at diagnosis and during any follow-up in a standardized
manner. For this purpose a Disease Activity Score (DAS) has been developed (Prevoo 1995). This score takes into account the patients general health, number of swollen and tender joints as well as the erythrocyte sedimentation rate (ESR). The DAS28 takes into account 28 different joints and a value >5.1 is regarded as high disease activity, <3.2 is regarded as low activity and <2.6 is regarded as remission. Another measurement of treatment response, defined by the ACR, i.e., ACR20 requires at least a 20% improvement in both the number of swollen and tender joints, as well as a 20% improvement in three of the following five parameters: ESR, health assessment questionnaire (HAQ), physicians global assessment of disease activity, patients global assessment and patients pain (Felson 1995). Correspondingly, the ACR50 requires a 50% improvement and the ACR70 a 70% improvement. Furthermore, the ACR has defined remission criteria based on the same variables (Pinals 1981).

Etiology

The etiology of RA is not clear and an interaction between environmental and genetic factors is suggested. Several genetic markers have been identified; the best known being denoted as shared epitope (SE) (Gregersen 1987). SE consists of alleles in the human leukocyte antigen (HLA) – DRB1 region that share a similar amino acid sequence. However, several other genetic markers have also been identified in recent years, e.g., PTPN22 (Begovich 2004, Johansson 2006). Moreover, in a genome wide scan, including the Swedish population, several additional loci have been identified as being of potential relevance to RA (Plenge 2008). Smoking is one of the environmental factors suggested to increase the susceptibility for developing RA, where smoking increases the risk of developing RA (Symmons 1997, Hutchinson 2001), especially seropositive RA (Symmons 2002), and increase the risk for developing ACPAs in individuals carrying the SE allele (Klareskog 2006). Gender is also important, RA being more common among women than men in all age groups (Symmons 2002) and hormonal factors seem to play a role since the difference between men and women becomes less obvious in post-menopausal women. Moreover, infection, trauma, obesity and social deprivation, among other factors, might increase the risk of developing RA (Symmons 2002).

Treatment

Since RA is a chronic disease it leads to long-term suffering for the patients. Early and effective treatment to reduce joint destruction, minimise pain and disability is important. Non-steroidal anti-inflammatory drugs (NSAID) are used to reduce the patient’s pain and stiffness, but have no effect on the joint destruction. The main goal for the treatment of RA is remission, i.e., no symptoms of RA. Currently patients with RA in Sweden are treated in accordance with thoroughly evaluated national recommendations, based on recommendations from the European League Against Rheumatism (Combe 2007). Almost all patients are treated with one or more disease-modifying anti-rheumatic drugs (DMARDs), the most common being methotrexate. Methotrexate is a folic acid antagonist with cytostatic and immunosuppressive effects. The disease modifying effects of methotrexate in RA are complex, however inhibitory effects of T-cells have been proposed (Tian 2007). It has recently been demonstrated that methotrexate increases the extra-cellular levels of adenosine, an anti-inflammatory signalling molecule, and thereby exerts some of the drugs disease modifying effects (Tian 2007). To minimise unpleasant side effects of folic acid depletion due to treatment with methotrexate almost all patients are prescribed supplementary folic acid at a dosage of 5mg between 2 and 6 days per week. Besides treatment with other DMARDs effective treatments with tumour necrosis factor (TNF) blocking agents and other biologic
agents, e.g., interleukin-1 (IL-1) blocking agent, CD20 antagonists and anti-cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) immunoglobulin, have been developed, increasing the opportunities to diminish inflammatory activity, joint destruction and disability (Furst 2007).

**Mortality**

Until the middle of the twentieth century it was believed that RA was not a fatal disease. However, in the 1950’s indications of a decreased life expectancy in patients with arthritis were found (Cobb 1953). Since then many studies have confirmed an increased mortality in patients with RA, with a few reports showing contradicting results (Table 2).

**Table 2:** Standard mortality ratio (SMR) in some of the epidemiological studies on patients with RA (statistically significant when not otherwise given).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Men</th>
<th>SMR Women</th>
<th>Total</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allebeck 1982</td>
<td>2.31</td>
<td>2.53</td>
<td>2.48</td>
<td>Sweden</td>
</tr>
<tr>
<td>Wolfe 1994</td>
<td>2.14</td>
<td>2.36</td>
<td>2.26</td>
<td>USA (California, Kansas) / Canada</td>
</tr>
<tr>
<td>Myllykangas-Luosujärvi 1995a</td>
<td></td>
<td></td>
<td>1.37</td>
<td>Finland</td>
</tr>
<tr>
<td>Wållberg-Jonsson 1997</td>
<td>1.47</td>
<td>1.64</td>
<td>1.57</td>
<td>Sweden</td>
</tr>
<tr>
<td>Sokka 1999</td>
<td>0.98&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.28&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>Finland</td>
</tr>
<tr>
<td>Riise 2001&lt;sup&gt;†&lt;/sup&gt;</td>
<td>2.2</td>
<td>1.9</td>
<td>2.0</td>
<td>Norway</td>
</tr>
<tr>
<td>Björnådal 2002</td>
<td></td>
<td></td>
<td>2.03</td>
<td>Sweden</td>
</tr>
<tr>
<td>Goodson 2002</td>
<td>1.08&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.99&lt;sup&gt;ns&lt;/sup&gt;</td>
<td></td>
<td>UK</td>
</tr>
<tr>
<td>Gabriel 2003</td>
<td>1.08&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.41</td>
<td>1.27</td>
<td>USA (Minnesota)</td>
</tr>
<tr>
<td>Thomas 2003</td>
<td>2.07</td>
<td>1.97</td>
<td></td>
<td>UK</td>
</tr>
<tr>
<td>Pincus 2004</td>
<td></td>
<td></td>
<td>1.60</td>
<td>USA</td>
</tr>
<tr>
<td>Goodson 2005</td>
<td>1.45</td>
<td>1.84</td>
<td></td>
<td>UK</td>
</tr>
<tr>
<td>Young 2007</td>
<td></td>
<td></td>
<td>1.27</td>
<td>UK</td>
</tr>
<tr>
<td>Gonzalez 2007</td>
<td>1.12&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.49</td>
<td>1.35</td>
<td>USA (Minnesota)</td>
</tr>
<tr>
<td>Jacobsson 2007</td>
<td></td>
<td></td>
<td>1.56</td>
<td>Sweden</td>
</tr>
<tr>
<td>Carmona 2007</td>
<td>1.55</td>
<td>1.46</td>
<td>1.49</td>
<td>Spain</td>
</tr>
</tbody>
</table>

<sup>ns</sup> Not significant, i.e., 95% confidence interval includes 1.0

<sup>†</sup> Results given as mortality rate ratios adjusted for age

Table 3: Standard mortality ratio (SMR) for cardiovascular disease in some of the studies on patients with RA (statistically significant when not otherwise given).

<table>
<thead>
<tr>
<th>Reference</th>
<th>SMR</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Wållberg-Jonsson 1997</td>
<td>1.36</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>1.41</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>0.57&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.39&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Riise 2001&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Björmådal 2002</td>
<td>total</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>1.79</td>
<td>1.50</td>
</tr>
<tr>
<td>Goodson 2002</td>
<td>total</td>
<td>0.70&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thomas 2003</td>
<td>total</td>
<td>2.03</td>
</tr>
<tr>
<td>Krishnan 2004</td>
<td>cardiovascular</td>
<td>1.59</td>
</tr>
<tr>
<td>Goodson 2005</td>
<td>total</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>cardiovascular</td>
<td>1.46</td>
</tr>
<tr>
<td>Young 2007</td>
<td>cardiovascular</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>cerebrovascular</td>
<td>1.10&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carmona 2007</td>
<td>total</td>
<td>1.23&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ns</sup> Not significant, i.e., 95% confidence interval includes 1.0

<sup>†</sup> Results given as mortality rate ratios adjusted for age

Among other causes of death the RA disease itself is reported to contribute. Infectious disease has been demonstrated to be one of the other main causes of death, especially respiratory and renal infections. Another main cause of death is malignancy, in particular haematopoietic malignancy. Furthermore gastrointestinal diseases, renal diseases and accidents may contribute to the excess mortality.

Table 4: Incidence of cardiovascular disease in some of the studies on patients with RA (statistically significant when not otherwise given).

<table>
<thead>
<tr>
<th>Reference</th>
<th>AMI Type of statistics</th>
<th>Stroke Type of statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACM Men</td>
<td>Women</td>
</tr>
<tr>
<td>del Rincón 2001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Solomon 2003</td>
<td>RR</td>
<td>2.07</td>
</tr>
<tr>
<td>Turesson 2004</td>
<td>SIR</td>
<td>1.72&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Watson 2003</td>
<td>IRR</td>
<td>1.4</td>
</tr>
<tr>
<td>Fisher 2004</td>
<td>OR&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.22&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maradit-Kremers 2005b</td>
<td>OR</td>
<td>—</td>
</tr>
<tr>
<td>Solomon 2006</td>
<td>RR</td>
<td>—</td>
</tr>
<tr>
<td>Han 2006</td>
<td>RR</td>
<td>—</td>
</tr>
<tr>
<td>Nadareishvili 2008</td>
<td>OR</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>IRR</sup>=incidence rate ratio; <sup>RR</sup> = risk ratio; <sup>SIR</sup> = standardized incidence ratio; <sup>OR</sup> = odds ratio

<sup>ns</sup> Not significant, i.e., 95% confidence interval includes 1.0

<sup>*</sup> Adjusted for traditional CVD risk factors and anti-inflammatory drugs
**Introduction**

**Co-morbidity**

A co-morbid disease is a disease, other than the RA, which is present along with the rheumatic condition. In recent years it has become obvious that there are several co-morbid conditions that are important for the disability and mortality in the RA patients. There are some fine reviews in the area indicating lymphoma as one of the most common co-morbidities (Mikuls 2003, Michaud 2007), the data on other malignancies are inconsistent. Infections have been demonstrated as a common co-morbid disease and furthermore, cataract, gastrointestinal disease and osteoporosis have been mentioned. However, again CVD has been referred to as the most common cause of co-morbidity (Table 4).

**Pathogenesis**

Rheumatoid arthritis is an autoimmune disease, hence the immune system is directed towards the patient’s own body, however no specific auto-antigen has yet been identified. In the joint, the primary organ involved in RA, there is a chronic inflammation in the synovium. This causes a hyperplasia of the synovial membrane as well as a neoangiogenesis. There is a cellular infiltration into the synovium of lymphocytes, macrophages, synovial fibroblasts, dendritic cells, mast cells, plasma cells and polymorpho nuclear leucocytes (Feldmann 1996, Goronzy 2005, Karouzakis 2006). The synovial membrane then extends to the cartilage and bone and actively destroys the underlying tissue. It has become clear that RA is driven by the adaptive immune response and that both T- and B-cells are part of the pathology (Goronzy 2005). The humoral response has long been known since RF, as well as other auto antibodies, is prevalent among patients with RA. RF is an autoantibody directed towards the Fc portion of IgG. In the cell-mediated immune response mostly CD4+ T-cells have presented as important for the pathogenesis in RA. Depending on the cytokine environment, activated CD4+ T-cells can differentiate into T-helper 1 (Th1)-cells, that are mostly pro-inflammatory, or Th2-cells, that are mostly anti-inflammatory. Upon activation Th1-cells, the predominant category in the RA synovium, produce TNF among other cytokines (Feldmann 1996, Goronzy 2005, McInnes 2007). TNF in turn triggers a cascade of other cytokines, and together with IL-1 gives rise to production of acute phase reactants in the liver. Interleukin-6 (IL-6), secreted by many cell types, also stimulates this production. TNF and IL-1, together with interleukin 17 (IL-17) from Th17-cells, induce the production of metalloproteinases, the enzymes capable of degrading matrix proteins such as collagen and, thereby, contribute to the pathogenesis of RA (Karouzakis 2006, McInnes 2007). Th1-cells produce IFNγ, in turn activating macrophages, whilst macrophages, on the other hand, produce interleukin 12 (IL-12), which stimulates T-cells, thus giving rise to a positive feedback loop. T-cells also produce interleukin 2 (IL-2), which acts in an autocrine manner stimulating further T-cell proliferation. The receptor for IL-2 is expressed mainly on T-cells. Following antigen recognition, the T-cell expresses the receptor α-chain, making the receptor capable of binding IL-2. Upon binding, this α-chain is cleaved off and becomes soluble in the extra-cellular compartment giving rise to the IL2-soluble receptor α (IL-2 sRα) which has been linked to disease severity in RA patients, and, in some studies, to other measures of inflammation in RA (Wållberg-Jonsson 2002, Witkowska 2005). It is becoming apparent that there is a disequilibrium in the rheumatoid synovium, with an activation of pro-inflammatory cytokines (TNF, IL-1, IL-17 and IFNγ), as well as of the anti-inflammatory cytokines and receptors (IL-10 and IL-1 receptor antagonist (IL-1Ra), but the balance is tilted towards the pro-inflammatory state (Feldmann 1996). Recently, regulatory T-cells (Treg) have been demonstrated being important in maintenance of self-tolerance. This type of T-cell modulates the function of other inflammatory cells, and in RA it is postulated that regulatory T-cells have an impaired regulatory function (McInnes 2007).
The cytokines do not only have local effects, for example, TNF has the capacity to stimulate vascular endothelium inducing the expression of selectins as well as intercellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1), in turn causing lymphocytes to adhere to the endothelium. ICAM-1, VCAM-1 and E-selectin have been related to RA in studies showing the levels of these biomarkers to be higher in the RA patients than in the controls (Wållberg-Jonsson 2001, Dessein 2005a, Pahor 2006, Klimiuk 2007). IL-6 and other proinflammatory cytokines are involved in adhesion molecule expression and in the secretion of monocyte chemoattractant protein-1 (MCP-1) by macrophages and synovial fibroblasts (McInnes 2007). MCP-1 has been linked to the RA disease in one study which reported elevated levels of MCP-1 in the synovial fluid of RA patients, compared with the levels in plasma of the same individuals (Ellingsen 2001). Also, elevated MCP-1 has been shown to precede the diagnosis of RA in ACPA and RF positive individuals (Rantapää-Dahlqvist 2007). When measuring inflammatory activity in RA patients in clinical practice, however, the more common measurement is ESR or any of the acute phase reactants, e.g., C-reactive protein (CRP), orosomucoid or haptoglobin. The sedimentation rate depends mainly upon the level of fibrinogen, another acute phase reactant.

In long-standing RA, with a prolonged immune activation and probably mediated by a systemic effect of cytokines, there is loss of lean muscle weight, along with severe fatigue, denoted as cachexia (Rall 2004). Other systemic disease manifestations apparently mediated by the immune response in RA are exudative serositis (mainly of the hearts and lungs), neuropathy, scleritis, glomerulonephritis and vasculitis, together denoted as extra-articular RA.

**Cardiovascular disease**

**Epidemiology**

The main cause of death in the general population in the western world is CVD. Mortality due to CVD in northern Sweden is among the highest in the world, with a mortality due to acute myocardial infarction (AMI) shown to be about 3.0/1000 persons and year and a mortality due to stroke about 1.7/1000 persons and year (Socialstyrelsen). In patients with RA the mortality following a CVD event is approximately 1.5 times that in the general population according to recent studies (Table 3). Accordingly, the incidence of CVD is high in the general population as well as in RA. In the general population of northern Sweden the incidence of AMI has been found to be about 5.6/1000 persons and year (Socialstyrelsen), and the incidence of stroke to be 2.64/1000 and year (Stegmayr 2003a). In patients with RA the incidence of AMI has been shown to be 1.5-4.0 times that in the general population (Table 4). The results regarding stroke in RA are more contradictory, although there are several studies showing about 1.5 times higher risk of stroke in patients with RA compared with the general population (Table 4).

**Diagnosis**

The diagnosis of an AMI is based on symptoms, electrocardiographic (ECG) changes, elevation of biomarkers, and, in fatal cases, autopsy findings (Thygesen 2007). Chest pain is the most common symptom of an AMI and most often the reason for the patient to seek medical care. The presentation of an AMI can also be as sudden death or acute heart failure. ECG changes are present in the majority of AMI cases, most typically with ST elevations and/or a development of a pathologic Q-wave. The biomarker measured until a few years ago was, in most cases, creatine kinase-MB (CK-MB) when it was replaced with either the more cardiac specific Troponin-T or Troponin-I. Regardless of which biomarker is measured, a
significant elevation is defined as a peak value exceeding the 99th percentile of a reference population (Thygesen 2007).

The diagnosis of stroke is defined by the World Health Organization as symptoms of a disturbance of the blood supply to the brain lasting more than 24 hours (Stegmayr 2003b). The symptoms depend on which part of the brain that is affected, however the most common symptoms are weakness or numbness of a part of the body, confusion, disturbances in vision or in the ability to speak.

Pathogenesis

The development of atherosclerosis is a chronic process in the vessel wall ultimately leading to plaque formation and CVD (Figure 1).

The first step in the atherosclerotic process is endothelial activation, which is an increase in endothelial adhesiveness to leucocytes and platelets, increase in endothelial permeability, change in phenotype from antithrombotic to thrombotic, production of several cytokines and increase in inflammatory gene expression (Hansson 1993, Ross 1999). Endothelial activation can be caused by a variety of stimuli, e.g., smoking, hypertension or diabetes (Ross 1999, Hansson 2006). In addition, reactive substances such as oxidized low-density lipoproteins (oxLDL), elevated plasma homocysteine and microbes might activate the endothelium (Ross 1999, Hansson 2006). This activation is associated with a decreased bioavailability of nitric oxide (NO), a potent vasodilator produced from arginine by nitric oxide synthase (NOS) in response to stimuli like shear stress (Moncada 1991). The endothelial activation causes increased permeability, vasoconstriction, coagulation and triggers inflammatory reactions causing T-cells, dendritic cells, mast cells and possibly also certain types of regulatory T-cells to adhere to the endothelium and migrate towards the intima (Ross 1999, Hansson 2006). In the intima, the macrophages express scavenger receptors that bind to low-density lipoproteins (LDL) and other lipoproteins. Ultimately the macrophages differentiate into foam cells after which the lesion is macroscopically visible as a fatty streak (Ross 1999, Hansson 2006) (Figure 1a). In the fatty streak, T-cells (mainly Th1-cells) are activated and, when the inflammation is allowed to proceed, smooth muscle cells in the vessel wall are stimulated to migrate to and proliferate in the intima. Moreover, extra-cellular matrix production is stimulated, forming a fibrous cap around the cells (Ross 1999, Hansson 2006). When this process is allowed to continue it ultimately leads to thickening of the vessel wall and the formation of an atheroma. Thus, an advanced atherosclerotic lesion is characterized by a core of lipids and necrotic tissue, constituted mainly of necrotic macrophages that have emptied their lipid content into the core, covered by a fibrous cap, comprising smooth muscle cells and collagen (Ross 1999, Hansson 2006) (Figure 1b). Activated macrophages express metalloproteinases that degrade the matrix in the fibrous cap triggering a thrombotic event and subsequently an infarction (Ross 1999, Hansson 2006) (Figure 1c). However, in about 30% of the cases there is no rupture, instead the endothelium is replaced by pro-thrombotic inflammatory cells, causing the thrombus and subsequently an infarction (Hansson 2006).
**Figure 1**: Activation and rupture of an atherosclerotic plaque: a) Atheroma with a smooth muscle cap covering the lipid-rich core. Occasional inflammatory cells are present throughout the atheroma. b) T-cells and monocytes adhere to the endothelium by up-regulated adhesion molecules. Macrophages, T-cells, and mast cells respond to pro-inflammatory stimuli by producing enzymes (proteases), cytokines, and pro-thrombotic factors. Proteolytic destruction of collagen and inhibition of smooth muscle growth leads to reduced thickness and strength of the cap. c) The weakened cap, which cannot withstand the haemodynamic forces, fissures and, consequently, exposes thrombogenic plaque material. The subsequent precipitation of platelets and coagulation factors forms a thrombus. Adapted from Hansson 2006. Reprinted, with permission, from the Annual Review of Pathology: Mechanisms of Disease, Volume 1 ©2006 by Annual Reviews www.annualreviews.org

**Traditional cardiovascular risk factors**

There are several so-called traditional risk factors for CVD of importance in the general population. In RA these traditional CV risk factors cannot fully explain the increased risk of CVD among patients (*del Rincón* 2001, *Gonzalez* 2008). The best documented risk factors are male sex and greater age in the general population (*Wilson* 1998), as well as in RA (*Wällberg-Jonsson* 1999, *del Rincón* 2001, *Warrington* 2005, *van Halm* 2006, *Naranjo* 2008). The use of tobacco, and especially smoking, has also been well studied as a risk factor for CVD (*Wilson* 1998). In Sweden the use of oral moist snuff is relatively widespread, and there have been
some studies identifying this source of tobacco as an additional risk factor for mortality in CVD (Hergens 2007). Several studies have suggested a relationship between smoking and CVD in RA (del Rincón 2001, Maradit-Kremers 2005a, Naranjo 2008), however others have not found that relationship (Wällberg-Jonsson 1999, Goodson 2002, Nadareishvili 2008, Banerjee 2008). Overweight, defined as a body mass index (BMI) ≥25 kg/m², has been associated to an increased risk for CVD in the general population (Yusuf 2005). The results regarding BMI and CVD in patients with RA have been contradictory when most studies found no such association (del Rincón 2001, Maradit-Kremers 2005a, Gonzalez 2008, Nadareishvili 2008). However, in recent years it has become more apparent that a low BMI (<20 kg/m²) is a greater risk factor for patients with RA than a high BMI (Maradit-Kremers 2004, Gonzalez 2008). In the general population high cholesterol, as well as high LDL and low high-density lipoproteins (HDL), together denoted as atherogenic dyslipidemia, have been associated with an increased risk of CVD (Wilson 1998). In RA the lipid profile differs between studies in that some studies have revealed a profile with low cholesterol and low triglycerides, as a part of the acute phase response (Rantapää-Dahlqvist 1991, McIntegart 2001, del Rincón 2003, Daza 2007), whilst others report a more pro-atherogenic profile in patients with RA (Park 1999, Dessein 2002, Han 2006, Georgiadis 2006, van Halm 2007).

Still there are some studies demonstrating a positive relationship between the cholesterol level and presence of atherosclerosis in RA (Wällberg-Jonsson 2001, del Rincón 2001, del Rincón 2005, Roman 2006, Banerjee 2008, Naranjo 2008). Studies in the general population have shown that oxLDL, in particular, is harmful to the endothelium, and oxLDL can induce endothelial damage and inflammation by stimulating additional migration of macrophages and up-regulation of vascular adhesion molecules (Ross 1999). Recently antibodies against phosphorylcholine, a component in the oxLDL particle, have been demonstrated to be protective against atherosclerosis in mice (Binder 2003) and that low levels of these antibodies are a novel risk factor for CVD, e.g., stroke in men (Sjöberg 2008).


**Endothelial activation and inflammation**

Inflammation appears to be of importance for the development of atherosclerosis in the general population (Ross 1999, Hansson 2006). There are a few studies of patients with RA revealing an association between the systemic inflammation and CVD (Wällberg-Jonsson 1999, Maradit-Kremers 2005a, Banerjee 2008). Moreover, a relationship between CVD and extra-articular RA, regarded as a result of high inflammatory load, has been shown
Studies on patients with long-standing RA show a relationship between CVD and disease duration (Solomon 2003, Wållberg-Jonsson 2004, van Halm 2006), however, other found no such relation (Maradit-Kremers 2005a, Nadareishvili 2008). One report suggested that the inflammation in RA interacts with the traditional CV risk factors when increasing the risk for CVD in RA (del Rincón 2005), and there are studies describing RA as an independent risk factor for CVD (Warrington 2005). In one study RF has been independently associated with CVD in males in the general population (Heliövaara 1995) as well as in RA (Maradit-Kremers 2005a, van Halm 2006). Moreover, there is an increasing opinion that the systemic inflammation in different diseases has an important influence on the progression of atherosclerosis (van Leuven 2008), and studies have demonstrated an increased CVD in several other diseases with chronic inflammation: in systemic lupus erythematosus (SLE) (Manzi 1999, Urowitz 2000); in Behçet’s disease (Keser 2005); in Sjögren’s syndrome (Vaudo 2005); in psoriatic arthritis (Han 2006); in ankylosing spondylitis (Lehtinen 1993); in systemic sclerosis (Khurma 2008); and in periodontal disease (Demmer 2006). In patients with RA there is an indication of a decrease in the incidence of CVD, and an improved prognosis after a CVD event, following initiation of treatment with DMARDs (Wållberg-Jonsson 1999, Choi 2002, van Halm 2006, Suissa 2006, Banerjee 2008, Naranjo 2008).

Activated endothelial cells produce IL-6 and numerous studies in the general population have shown that the level of an acute phase reactant, like high sensitivity C-reactive protein (hsCRP), is a strong and independent predictor of future CVD, and also predicts the outcome after a CVD event (Lindahl 2000, Ridker 2000, Ruef 2006). Activated endothelial cells, as well as activated T-cells and macrophages, produce TNF, in turn adhering leucocytes to the endothelium by adhesion molecules like E-selectin, P-selectin, ICAM-1 and VCAM-1. These adhesion molecules have been demonstrated to be elevated in CVD in the general population (Haught 1996, Martinovic 2005, Ruef 2006, Tousoulis 2007), as well as in RA (Dessein 2005a). Prospective studies on these biomarkers have demonstrated that it is possible to reduce the levels of sICAM-1 and sE-selectin by anti-rheumatic treatments (Dessein 2006, Weisman 2006, Klimiuk 2007, Visvanathan 2007). Recently, L-selectin was shown to be important for directing lymphocytes to sites of inflammation. However, L-selectin is shed from the activated leukocyte becoming soluble (sL-selectin), and in this form inhibits the attachment of lymphocytes to the endothelium (Rainer 2002). Soluble L-selectin has been shown to be decreased in patients with CVD, due to the binding of sL-selectin to activated endothelial cells by increased expression of L-selectin ligands (Haught 1996, Rainer 2002, Ruef 2006). Moreover L-selectin is down-regulated on circulating leucocytes during chronic inflammation (Rainer 2002). Activated smooth muscle cells in the arterial wall secrete MCP-1, a potent chemo-attractant for monocytes causing them to migrate into the intima. Furthermore MCP-1, shown to be elevated in CVD in the general population (Martinovic 2005), promotes macrophage accumulation, lipid accumulation and plaque growth, thereby augmenting the progression of atherosclerosis (Shin 2002). Patients with RA have also been shown more often to have chronic inflammatory infiltration in the media and adventitia of coronary arteries than controls (Hollan 2007).

Atherothrombosis

The coagulation cascade can be activated by inflammation leading to atherothrombosis. Upon activation, the thrombocytes release fibrinogen that is readily degraded to fibrin, making up the skeleton of the thrombus. Fibrinogen is an acute phase reactant, and thus increases during inflammatory reactions. Elevated fibrinogen has been identified as a risk factor for CVD (Danesh 2005) being increased in individuals with traditional CV risk factors like smoking,
hypertension, and diabetes mellitus (Danesh 2005). Levels of fibrinogen have been demonstrated to be elevated in RA patients compared with controls (McEntegart 2001), however data on a relationship between the level of fibrinogen and CVD in RA are scarce (Wällberg-Jonsson 2000). Von Willebrand’s factor (VWF) is another factor up-regulated by inflammation and involved in coagulation. This biomarker is synthesized by endothelial cells and stored in thrombocytes. Upon release it interacts with receptors on thrombocytes, adhering these cells to sub-endothelial structures. Recently it has become clear that VWF is important in the pathogenesis of CVD and VWF has been established to be related to CVD in the general population (Jansson 1998, Thögersen 1998, Ruef 2006, Tousoulis 2007). Patients with RA have been found to have higher levels of VWF than controls (McEntegart 2001, Daza 2007, Bhaita 2008) and high levels in patients with RA predict increased incidence of CVD (Wällberg-Jonsson 2000, Bhaita 2008). Degradation of the thrombus is regulated by fibrinolysis through the action of plasmin on fibrin. Plasminogen, in turn, is activated mainly by tissue plasminogen activator (tPA), produced by endothelial cells and released upon activation. Increased levels of tPA-antigen have been linked to an increased incidence of, and mortality after, a CVD event in the general population (Salomaa 1995, Jansson 1998, Thögersen 1998, Tousoulis 2007). In established RA a low tPA capacity, a measurement of the biologically active portion of tPA, has been related to CVD (Wällberg-Jonsson 2000). Plasminogen activator inhibitor-1 (PAI-1), an acute phase reactant, is involved in the coagulation pathway, however its function is to inhibit fibrinolysis by formation of an inactive complex with tPA. PAI-1, synthesised by endothelial cells and hepatocytes, and stored in thrombocytes, has been shown to be associated with CVD and to be increased in parallel with BMI, lipid levels, blood pressure and smoking (Salomaa 1995, Thögersen 1998). Patients with RA have been demonstrated to have higher levels of PAI-1 than controls (Wällberg-Jonsson 2002), and patients with RA and CVD have higher levels of PAI-1 compared with those without CVD (Wällberg-Jonsson 2000). Moreover, both tPA and PAI-1 predicted CVD progression independently of traditional CVD risk factors in patients with RA (Wällberg-Jonsson 2000).

**Homocysteine and asymmetric dimethylarginine (ADMA)**

Homocysteine is a sulphur containing amino acid involved in the metabolism of methionine to cysteine (Figure 2). This process is dependent on vitamin B12, vitamin B6 and folic acid.

**Figure 2:** Homocysteine metabolism. Adapted from Dayal 2005.
High levels of homocysteine have been linked to an increased risk of CVD (Homocysteine Studies Collaboration 2002) and recent studies have found that homocysteine might cause oxidative stress by oxidation of LDL, generation of free radicals, inhibition of DNA synthesis, damage of endothelium by smooth muscle cell proliferation and intimal thickening as well as enhanced thrombogenicity (Ross 1999, Mallika 2007). Plasma homocysteine levels are increased by a wide range of variables, for example, greater age, male sex, menopause, smoking, coffee intake, diet poor in folic acid, hepatic or renal dysfunction, MTHFR 677C→T polymorphism, diabetes mellitus, malignancies, folate antagonists, anti-convulsant drugs, metformin, thiazide diuretics, some glitazones and some lipid lowering drugs (Refsum 1998, Wierzbicki 2007). On the other hand, total homocysteine in the plasma can be lowered by supplementation with vitamins B6, B12 and folic acid (Refsum 1998, Wierzbicki 2007). However, recent studies showed no reduced risk of CVD in patients supplemented with these vitamins (Toole 2004, Lonn 2006, Bonaa 2006, Albert 2008) although randomized trials still are ongoing (B-vitamins Treatment Trialist’s collaboration 2006). Higher levels of homocysteine have also been associated with increased levels of PAI-1 antigen, tPA antigen, fibrinogen and VWF, suggesting that homocysteine is associated with an impaired fibrinolytic activity (Tofler 2002). One study has found an association between elevated homocysteine levels and inflammation variables in RA (Wållberg-Jonsson 2002) and homocysteine has been related to an increased risk of CVD in RA patients (Cisternas 2002). Moreover, homocysteine has been revealed to be able to induce other inflammatory biomarkers like MCP-1 (Desai 2001), IL-6, TNF (Su 2005) and VCAM-1 (Carluccio 2007).

High levels of homocysteine have been demonstrated to decrease the bioavailability of NO (Ungvari 2002), although the reason for this is not clear. However, asymmetric dimethylarginine (ADMA) is a competitive inhibitor of endothelial NOS (eNOS), thereby inhibiting the production of NO. There is a metabolic connection between homocysteine and ADMA (Figure 3) (Dayal 2005) and ADMA was found to be increased in hyper-homocysteinaemia (Böger 2001).

**Figure 3**: Metabolic interactions between homocysteine and ADMA. Adapted from Dayal 2005.

Therefore, it is postulated that ADMA contributes to the impaired NO bioavailability in hyper-homocysteinaemia (Dayal 2005). Studies have shown an association between ADMA and CVD in the general population (Böger 2001, Valkonen 2001, Schulze 2006, Furuki 2007). There are a few studies (as yet only published in abstract form) on ADMA in which the levels of ADMA were shown to be increased in patients with RA compared with controls and to be
related to parameters of inflammation parameters, e.g., the number of swollen or tender joints, DAS28, fibrinogen, ESR and CRP, in the RA patients (Kwasny-Krochin 2008, Atzeni 2008). However, one study found no increased levels of ADMA in patients with RA and CVD compared with patients with RA but no CVD (van Halm 2005).

**Measurements of atherosclerosis**

*Physiological measures*

During the atherosclerotic process there are several methods of measuring the sub-clinical progress of the atherosclerosis physiologically. Once the endothelium is activated a dysfunction of the endothelium develops, and there are several ways to identify this dysfunction. The most widely established method is to invasively measure the arterial dilation when stimulating the vessel with the endothelial dependent vasodilator acetylcholine (Panza 1990). The arterial dilation can more easily be measured non-invasively by means of flow mediated dilation (FMD) whereby the arterial diameter is measured before and after an occlusion of the artery, hyperaemic arterial dilation being endothelium dependent (Celermajer 1992, Corretti 2002) (Figure 4). The FMD can be further attenuated by adding ischemic handgrip exercise (Agewall 2002).

**Figure 4**: Flow mediated dilatation (FMD) of brachial artery. Diameter A) at baseline B) post occlusion (endothelial dependent dilatation) and C) after nitroglycerine (endothelial independent dilatation). Distance between tick marks: 5mm.

Arterial stiffness can also be measured by aortic pulse wave analyses (PWA), i.e., the time between two pulse measurements at different sites of the body is registered and the velocity of the pulse wave between these sites calculated. This is most often Doppler measured. Using the same recording, the Augmentation Index (Alx), defined as the augmentation the arterial wave reflections have on the pulse wave in the artery, can be calculated (Murgo 1980, Cohn 1995). When the atherosclerotic process has been allowed to continue for some time the intima of the vessel wall is thicker and the intima media thickness (IMT) can be measured ultrasonically at different sites, but usually in the common carotid artery (CCA) and/or the femoral artery (Wendelhag 1993) (Figure 5). It is customary to also register the presence of established plaques.
**Figure 5**: Intima media thickness (IMT) of the right common carotid artery measured in a 10 mm long segment proximal to the carotid bulb

![Image of carotid bulb and common carotid artery (cca)](image)

**Table 5**: Intima media thickness (IMT) of a. carotis communis in patients with RA compared with controls.

<table>
<thead>
<tr>
<th>Reference</th>
<th>RA disease duration (m)</th>
<th>Mean age (years)</th>
<th>Number of RA patients</th>
<th>IMT in RA (mm) (SEM)</th>
<th>IMT in controls (mm) (SEM)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wållberg-Jonsson 2001</td>
<td>228-276</td>
<td>52 (38-65)</td>
<td>39</td>
<td>0.79±0.04</td>
<td>0.70±0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Park 2002</td>
<td>49.9±45.0</td>
<td>55±3</td>
<td>53</td>
<td>0.77±0.09</td>
<td>0.68±0.14</td>
<td>&lt;0.001</td>
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<tr>
<td>Kumeda 2002</td>
<td>unknown</td>
<td>55±17</td>
<td>138</td>
<td>0.64±1.127</td>
<td>0.57±0.115</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>del Rincón 2003</td>
<td>114 (6-600)</td>
<td>60 (40-83)</td>
<td>204</td>
<td>1.03±0.434</td>
<td>0.97±0.449</td>
<td>ns</td>
</tr>
<tr>
<td>Alkaabi 2003</td>
<td>72-384</td>
<td>56 (37-77)</td>
<td>40</td>
<td>0.73±0.03</td>
<td>0.62±0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Gonzalez-Juanatey 2003b</td>
<td>186±102</td>
<td>59±13</td>
<td>47</td>
<td>0.77±0.164</td>
<td>0.699±0.129</td>
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<td>Gerli 2005</td>
<td>132±108</td>
<td>63±11</td>
<td>101</td>
<td>0.85±0.22</td>
<td>0.85±0.26</td>
<td>ns</td>
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<td>Grover 2006</td>
<td>96±66</td>
<td>42±8</td>
<td>57</td>
<td>0.55±0.137</td>
<td>0.416±0.002</td>
<td>&lt;0.0001</td>
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<td>Roman 2006</td>
<td>144</td>
<td>48±13</td>
<td>98</td>
<td>0.64±0.17</td>
<td>0.70±0.17</td>
<td>0.011</td>
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<td>Pahor 2006</td>
<td>114.64±75.08</td>
<td>42±5</td>
<td>70</td>
<td>0.586±0.097</td>
<td>0.479±0.007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Daza 2007</td>
<td>138 (60-384)</td>
<td>44±8†</td>
<td>55</td>
<td>0.67±0.18</td>
<td>0.58±0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Hannawi 2007</td>
<td>7 (1.0-12)</td>
<td>53 (22-78)</td>
<td>40</td>
<td>0.64±0.13</td>
<td>0.58±0.09</td>
<td>0.03</td>
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<tr>
<td>Kerekes 2008</td>
<td>126±102</td>
<td>51±12</td>
<td>52</td>
<td>0.63±0.14</td>
<td>0.54±0.15</td>
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<td>Georgiadis 2008</td>
<td>&lt; 12</td>
<td>53±13</td>
<td>49</td>
<td>0.82±0.29</td>
<td>0.57±0.11</td>
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<td>Ciftci 2008</td>
<td>113.5±13.2</td>
<td>44±9</td>
<td>30</td>
<td>0.57±0.09</td>
<td>0.49±0.11</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data given as mean ±SD or mean (range)

* patients with RA compared with controls
m=months; ns= not significant
† No matching for age between patients with RA and controls

Calcification and plaques can also be measured in the coronary arteries or aorta directly by cardiovascular magnetic resonance (Jaffer 2002) and the anatomy of arteries, including stenosis, may be visualised by angiography. All of these methods have been demonstrated to predict a future CV event as well as mortality due to CVD in the general population (Bots 1997, O’Leary 1999, Schächinger 2000, Suwaidi 2000, Meaume 2001, Iglesias del Sol 2002, Lorenz 2006, Simon 2007, Shechter 2008). Moreover, several studies have demonstrated that reduction of traditional CV risk factors and/or treatment of CVD can improve measurements...


Table 6: Endothelial dependent flow mediated dilation (ED-FMD) of a. brachialis in patients with RA compared with controls.

<table>
<thead>
<tr>
<th>Reference</th>
<th>RA disease duration (m)</th>
<th>Mean age (years)</th>
<th>Number of RA patients</th>
<th>ED-FMD in RA (%)</th>
<th>ED-FMD in controls (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hürlimann 2002</td>
<td>108±24</td>
<td>46±5</td>
<td>11</td>
<td>103.2±0.4</td>
<td>105.0±0.5</td>
<td>0.023</td>
</tr>
<tr>
<td>Gonzalez-Juanatey 2003a</td>
<td>&gt; 60</td>
<td>59±12</td>
<td>55</td>
<td>103.8±4.9</td>
<td>108.0±4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>van Doornum 2003</td>
<td>164.4±108</td>
<td>45±8</td>
<td>25</td>
<td>107.6±4.6</td>
<td>108.5±4.1</td>
<td>ns</td>
</tr>
<tr>
<td>Vaudo 2004</td>
<td>132±96</td>
<td>50±7</td>
<td>32</td>
<td>103.2±1.3</td>
<td>105.7±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Irace 2004</td>
<td>84±24</td>
<td>45±4</td>
<td>10</td>
<td>103.7±1.9</td>
<td>111.6±2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Arosio 2007</td>
<td>72±36</td>
<td>41-52</td>
<td>65</td>
<td>108.2±2</td>
<td>111.5±3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mäki-Petäjä 2007</td>
<td>156±120</td>
<td>58±12</td>
<td>20</td>
<td>103.70±2.32</td>
<td>106.74±3.78</td>
<td>0.01</td>
</tr>
<tr>
<td>Bosello 2008</td>
<td>154±108</td>
<td>53±7</td>
<td>10</td>
<td>107.71±2.78</td>
<td>114.91±6.41</td>
<td>0.008</td>
</tr>
<tr>
<td>Kerekes 2008</td>
<td>126±102</td>
<td>51±12</td>
<td>52</td>
<td>105.32±4.66</td>
<td>108.30±3.96</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data given as mean ±SD or range
* patients with RA compared with controls
m=months; ns= not significant

Furthermore, electron beam tomography has been used to demonstrate an increased prevalence and severity of coronary calcification in patients with RA compared with controls (Chung 2005) and in one study the skin microcirculation was found to be impaired in active RA patients compared with controls indicating a vascular dysfunction in RA (Datta 2007).

Measurements by biomarkers

Apart from measuring the atherosclerosis physiologically it is apparent that there are several serological biomarkers that may be used to measure sub-clinical atherosclerosis and to predict a future CVD event and mortality. In the general population the most established is hsCRP (Lindahl 2000, Ridker 2000, Ruef 2006). Additionally, adhesion molecules and chemokines like sL-selectin, sE-selectin, sP-selectin, sICAM-1, sVCAM-1 and sMCP-1 (Haught 1996, Rainer 2002, Martinovic 2005, Ruef 2006, Tousoulis 2007), atherothrombotic markers like fibrinogen, PAI-1, tPA and VWF (Salomaa 1995, Jansson 1998, Thögersen 1998, Danesh 2005, Tousoulis 2007) as well as other biomarkers, e.g., homocysteine (Homocysteine Studies...
Introduction

\textit{Collaboration 2002} and ADMA (Böger 2001, Valkonen 2001, Schulze 2006, Furuki 2007) have been shown to be associated with presence of atherosclerosis in the general population. The importance and function of these biomarkers in CVD and RA have been discussed previously (see above).
Aims

AIMS

Rheumatoid arthritis (RA) is a chronic disabling disease that is associated with a shortening of life span in affected individuals. Cardiovascular disease (CVD) contributes to this increased mortality, and, to a great extent, the co-morbidity in RA. In this thesis I have attempted to clarify these relationships with special attention to:

› the incidence of, and prognosis after, acute myocardial infarction (AMI) and stroke in patients with RA compared with the general population of Västerbotten County

› whether it is possible to decrease the homocysteine level in patients with RA by treatment with vitamins B6, B12 and folic acid, and whether there is any impact of inflammation and/or methotrexate treatment on the homocysteine level in patients with RA

› the progression of sub-clinical atherosclerosis in patients with very early RA compared with controls

› whether there are biomarkers of endothelial activation that could reflect early atherosclerosis in RA, and the potential impact of inflammation, traditional cardiovascular risk factors and anti-rheumatic treatment on the progression of atherosclerosis
SUBJECTS AND METHODS

Subjects

The relationships between the different cohorts in this thesis are presented in Figure 6 and the demographics of the different cohorts are presented in Table 7.

Figure 6: Distribution of the different cohorts in the papers included in this thesis.

Papers I and II

The original cohort of patients with RA (Ropes 1959) consisted of all individuals registered with seropositive RA at the Department of Rheumatology, University Hospital, Umeå in 1979 (n=640; 435 females, 205 males). This patient cohort has been evaluated in earlier epidemiological studies (Wållberg-Jonsson 1997, Wållberg-Jonsson 1999) and included inpatients, outpatients and consultation patients. At that time the Department of Rheumatology was the only reference centre for patients with rheumatoid arthritis in Västerbotten County with a reference population of 250 000. Since 1985 all cases of AMI and stroke in Västerbotten and Norrbotten counties are recorded in the northern Sweden World Health Organization Multinational Monitoring of Trends and Determinants of Cardiovascular Disease (MONICA) project (Stegmayr 2003b). Only patients from Västerbotten County were included in the present studies, resulting in a total of 5368 validated AMI events and 17180 validated stroke events between 1985 and 2003. From the original RA cohort 35 patients with AMI and 41 with stroke were identified within the MONICA project. For each RA patient three controls with AMI or stroke, but without RA, were randomly assembled from the MONICA register. The controls were matched for sex, age (± 4 years) and year of event (± 3 years for AMI and ± 4 years for stroke).
Table 7: Demographic data, some of the traditional CVD risk factors and some of the measurements of disease activity in the different cohorts in this thesis.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>Controls</td>
<td>RA</td>
<td>Controls</td>
<td>B-vitamin group</td>
<td>RA</td>
</tr>
<tr>
<td>Number of individuals</td>
<td>35</td>
<td>105</td>
<td>40</td>
<td>120</td>
<td>31</td>
</tr>
<tr>
<td>Age, years</td>
<td>61 (58-63)</td>
<td>62 (59-64)</td>
<td>65.2 ±7.9</td>
<td>65.2 ±7.9</td>
<td>62 ±16</td>
</tr>
<tr>
<td>Females</td>
<td>17(49)</td>
<td>51(49)</td>
<td>25(63)</td>
<td>75(63)</td>
<td>20(65)</td>
</tr>
<tr>
<td>Disease duration, m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>168 ±156</td>
</tr>
<tr>
<td>DAS28</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.89 (3.39-4.45)</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>24 (12-32)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>14 (10-37)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Previous CVD event</td>
<td>8(23)</td>
<td>12(12)</td>
<td>10(25)</td>
<td>25(21)</td>
<td>9(29)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6(21)</td>
<td>16(20)</td>
<td>6(15)</td>
<td>17(14)</td>
<td>—</td>
</tr>
</tbody>
</table>

Data given as mean±SD/median (IQR) or number of individuals and percent within parentheses

Paper III

Two-hundred and thirty-five consecutive patients with RA (Arnett 1988) visiting the Department of Rheumatology, University Hospital, Umeå during April and May of 2000 were asked to donate a blood sample for measurement of the homocysteine level. Of these, 103 patients were found to have a homocysteine level ≥ 12 µmol/L (i.e., the mean level established for control subjects in a previous study +2SD (Wållberg-Jonsson 2002)). Four of these patients were excluded because they were living very distant from the hospital, one was already receiving treatment with the study drug, one because of daily substitution treatment with folic acid and another because of vitamin B12 deficiency (<142 pmol/L) requiring treatment. Eighteen patients declined to participate in the study. Of the remaining patients, all who were not on methotrexate, (n=42) were included in the study. The patients were not randomised for methotrexate treatment but the subsequent 20 patients already taking methotrexate were included. The 62 patients included were randomised to receive either a placebo or a combination of 3 mg vitamin B6, 0.5 mg vitamin B12 and 0.8 mg folic acid (Recip AB, Årsta, Sweden)

Papers IV and V

This study is part of an ongoing structured follow-up programme on early RA (Arnett 1988) (≤12 months of symptoms of disease) at the Department of Rheumatology, Umeå University Hospital, using the nationwide Swedish Rheumatoid Arthritis Registry. Between 2000 and 2004 all patients under 60 years of age from the counties of Västerbotten, Norrbotten and Jämtland (n=87) were consecutively invited to participate in an extended survey on CV morbidity; 79 patients agreed to participate, and were enrolled into the study presented in paper IV (T0). Six patients declined participation, one was pregnant and another was excluded due to advanced breast cancer. Forty-four controls without RA were assembled, of whom 39 were randomly selected from the population register of the same region. In order to include appropriate very young controls, three hospital staff and two students were also selected randomly. The controls were matched for sex and age (±5 years). In the follow-up study
presented in paper V (T18) 27 patients with RA and their 27 age and sex matched controls were asked to participate. One patient with RA declined to participate in this follow-up.

Methods

Clinical examination and surveys
The MONICA protocol requires the inclusion of all incident AMIs or strokes and recording of variables reflecting risk factors, care and treatment during the event and at discharge from hospital. Thus, recording of an event, together with all other designated variables, was undertaken in exactly the same way for all individuals in paper I and II, irrespective of having RA. For paper III, IV and V the number of swollen and tender joints (28 joint count), both the patient’s and physician’s global assessment, patient’s pain assessment and HAQ (Fries 1980) were registered. For the patients with RA in paper III DAS28 (Prevoo 1995) was calculated at inclusion and after 3, 6 and 12 months, and for the patients with RA in papers IV and V, DAS28 was calculated at diagnosis and after 3, 6, 12, 18, 24, 36 and 60 months. All patients in paper IV were also asked to answer a survey on co-morbidity, including weight and height for calculation of BMI, as well as a survey on CVD risk factors and life style (Rose 1992). For paper V the surveys were retaken at a re-examination 18 months after inclusion.

Blood sampling and analytic procedures
For paper III blood was drawn at inclusion and after 3, 6, 9 and 12 months, whilst for papers IV and V all patients and controls donated a blood sample at the time of the ultrasound measurements; blood was also drawn after an overnight fast for analysis of blood lipids (cholesterol (mmol/L), HDL (mmol/L) and triglycerides (mmol/L)) measured by routine methods at each of the collaborating hospitals. For the RA patients in papers III, IV and V the ESR, as well as CRP, was measured at all clinical examination time points. All blood samples were separated into plasma, serum and buffy coat cells by centrifugation at 4400g for 15 minutes and stored at -80°C. After thawing, orosomucoid (g/L) and haptoglobin (g/L) were determined by immunoturbidimetry methods on a Hitachi 911 multianalyser (Roche Diagnostics, Basel, Switzerland). Folate (nmol/L) and vitamin B12 (pmol/L) were measured using Radioassay (BioRad Diagnostic group, CA, USA). Homocysteine (μmol/L) was measured using a Fluorescence Polarisation Immunoassay (IMX system, Abbot, Oslo, Norway) for paper III and by a routine spectrophotometric method (Vitros, Ortho-Clinical Diagnostics, Johnson & Johnson AB, Sweden) for paper V. The levels of IL-2sRα (ng/L), IL-6 (ng/L), sVCAM-1 (ng/mL), sICAM-1 (ng/mL), sL-Selectin (ng/mL) and sE-Selectin (ng/mL) were measured in serum using ELISA (R&D Systems, Abingdon, UK) and MCP-1 (pg/mL) using ELISA (HyCult Biotechnology, Uden, The Netherlands). Mass concentrations of PAI-1 (μg/L) and tPA (μg/L) were measured in plasma using ELISA (Trinity Biotech Inc, Bray, Ireland) and VWF (%) using ELISA as previously described (Nilsson 2005). ADMA (μmol/L) was measured by high performance liquid chromatography (HPLC) (Dionex Corporation, Sunnyvale, CA). The presence of ACPA in serum was detected using the Diastat kit (Axis-Shield Diagnostics, Dundee, UK), with a cut off value of 5 units/mL, which uses the CCP2 technique that is more sensitive then the first developed technique for ACPA detection (Rantapää-Dahlqvist 2005). The presence of rheumatoid factor (RF) was analysed routinely by Waaler Rose hemagglutination test. Soluble CRP (mg/L) and creatinine (μmol/L) were measured according to routine methods at each of the hospitals and ESR (mm/h) was measured according to the Westergren method.
Subjects and methods

Ultrasound investigations
Ultrasound examinations for papers IV were performed as soon as possible after the first symptoms of RA, and no longer than 12 months after diagnosis. The examinations were repeated after 18 months in paper IV. All examinations were performed by the same experienced investigator (EL) with the patients in a supine position in a quiet temperature controlled room. A Sequoia 512 ultrasound system (Siemens (Acuson) Corp) was used with a 15L8 transducer for brachial artery and a 8L5 transducer for carotid artery studies. All investigations were digitally stored for analyses, which were performed by a single observer (EL with an intrapersonal variability r=0.988) on a Sequoia 512 ultrasound system.

FMD
R-wave triggered end diastolic right brachial artery longitudinal images proximal to the antecubital fossa were recorded at baseline after 15 minutes of supine rest. Transducer position was carefully noted for subsequent investigations. For endothelial dependent flow mediated dilatation (ED-FMD) R-wave triggered images were stored for 90 seconds following ischemia. The ischemia was induced using a cuff on the forearm inflated to 20mm Hg above the systolic blood pressure for 5 minutes with additional lower arm muscular work by repetitively squeezing a small ball during the last minute of ischemia. Maximal brachial artery diameter was calculated as a mean of three measurements. FMD was calculated as a percentage of the baseline diameter.

IMT
Carotid artery studies were performed with the patient in supine position with the neck extended and the chin turned away from the side being examined. The right common carotid artery proximal to the bulb was imaged in multiple longitudinal planes for the best resolution of the IMT of the far wall. The IMT was obtained by manually tracing the intima-media in the far wall of the artery for a distance of approximately 10 mm. Measurements were performed on three end diastolic images and averaged.

Ethical aspects
All studies were approved by the Regional Ethics Committee of Umeå University, Umeå, Sweden. For the studies presented in papers III, IV and V all patients and controls gave their informed consent before inclusion.

Statistics
In paper III the data were analysed according to intention-to-treat, and data not normally distributed were transformed to their logarithm. In papers I and II the SIR, i.e., the ratio of the observed incidence of AMI or stroke in the RA cohort to that expected according to the crude AMI or stroke incidence in the whole MONICA register, based on age, sex and year adjusted rates in the general population, was calculated by means of the Poisson distribution. Survivorship functions in paper I and II were estimated by the product limit (Kaplan-Meier) estimator, followed by the log-rank test in paper I and also by the Breslow and Tarone-Ware tests in paper II. In paper III comparison between groups over time was made by using repeated measurements analysis of variance (ANOVA) and the Friedman test was used for analysis of significance of changes in data over time. Differences in variables between paired groups were analysed by Wilcoxon matched-pairs signed-ranks test in paper III and by conditional logistic regression in papers IV and V. Chi-square test was used for category data in paper III. In papers III, IV and V differences in continuous data between two groups were
analysed with the Mann-Whitney U test and between three groups with the Kruskal-Wallis test. Predictors for dependent variables were identified by simple and multiple stratified (i.e., with and without RA) Cox proportional hazards regression models in paper I, by simple and multiple Cox proportional hazards regression models in paper II, and by simple and multiple linear regression analysis in papers III-V. Simple regression together with clinical assumptions, determined which covariates were included in the multiple regression models. In all papers p-values ≤0.05 were considered as significant and two-sided tests were applied. In all papers the SPSS package 10.0 or 15.0 (SPSS Inc, Chicago, US) was used for the statistical analyses, in paper III also StatView 4.51 (BrainPower, Calabasas, CA, USA) was used for some of the analyses. Power calculations were performed in paper III and showed that a sample size of 12 in each group would have 80% power to detect a difference in homocysteine with means of 3.0(μmol/L), assuming a SD of 2.5(μmol/L), based on previous publication (Clarke 1998). Concerning papers IV and V, power calculations showed that a sample size of 26 in each group would have 95% power to detect a difference in IMT of 0.1±0.1 mm between the two groups, based on previous publications (Table 5, Introduction)
RESULTS AND DISCUSSION

Epidemiology

Standardized incidence ratio of AMI and stroke (Paper I and II)
The overall standardized incidence ratios (SIR) of AMI was found to be 2.9 (95% confidence interval [CI] 1.9 to 4.1), being 3.2 (95% CI 1.7 to 5.4) for women and 2.67 (95% CI 1.6 to 4.3) for men. The SIR for stroke was found to be 2.7 (95% CI 1.9 to 3.8), being 2.1 (95 %CI 1.2 to 3.2) for women and 4.8 (95% CI 2.6 to 8.0) for men. These figures are essentially the same as those found by others (Table 4, Introduction) showing an increased risk of an AMI among patients with RA compared with the general population. The published results regarding stroke are more contradictory and the results of the study presented in paper II showed a somewhat higher risk for stroke among patients with RA compared with the general population than most other recent studies. This difference in results may, at least in part, be explained by different inclusion and/or exclusion criteria, follow-up times, and type of cohorts, sex ratios and size of the study cohorts. Another possible explanation is the fact that the different populations studied have different genetic backgrounds. All CVD events in this study were validated in accordance with strict WHO-criteria (Stegmayr 2003b), and the data recording was undertaken in the exactly same way in patients with RA and the general population. Moreover, the initial cohort of RA patients in this study was very close to the prevalent seropositive adult RA population in Västerbotten County at that time, providing a unique opportunity to compare the true incidence of CVD events for RA patients and the general population in a community-based manner.

Prognosis after an AMI or stroke (Papers I and II)
An impaired prognosis after either an AMI or stroke in patients with RA compared with matched controls was shown. During the whole follow-up period after an AMI the hazard ratio (HR) was 1.7 (95% CI 1.1 to 2.7) for patients with RA compared with matched controls (Figure 7). When only individuals without an AMI prior to the study period were taken into account, the same decreased estimated survival for patients with RA was observed (Figure 7).

In recent studies the survival after an AMI was correspondingly significantly decreased in patients with RA compared with controls (Douglas 2006, van Doornum 2006). Likewise, the HR was 1.7 (95% CI 1.1 to 2.7) during the whole follow-up after a stroke for patients with RA compared with matched controls (Figure 8).

With regard to stroke there is, to my knowledge, only one previous study on the prognosis in patients with RA (van Doornum 2006). That study showed no impaired prognosis after the event for patients compared with controls, however, in that study controls and patients were not matched for age and/or sex, which was the case in this study.
Results and discussion

Figure 7: Estimated survival after onset of an AMI

(+): All individuals taken into account
(++) Only individuals without AMI before 1985 taken into account
Md = Median survival time

Figure 8: Estimated survival after onset of a stroke.

Md = Median survival time

When considering the case fatality after an AMI, variables reflecting care and treatment adjacent to the acute event were taken into account. There was a difference, albeit not statistically significant, regarding treatment with cardio-protective drugs in that numerically fewer RA patients than matched controls received treatment with cardio-protective drugs during and after hospitalisation (data not shown). Furthermore, numerically fewer RA patients (69.7%) were admitted to an intensive care unit (ICU) compared with matched controls (71.9%). It can only be speculated as to whether the patients with RA received a less optimal care.
than the controls. It is known that patients with one chronic illness, like RA, are less likely to
be prescribed the best medication for co-morbid conditions, such as an AMI (Redelmeier
1998, MacLean 2000). Patients with RA, and their caretakers, may mistakenly consider the
pain symptoms of the AMI to be part of their rheumatic condition. It is also speculated as to
whether patients with RA have infarctions with atypical manifestations. Recent studies have
found several similarities between an AMI in patients with RA and those in patients with DM
(Maradit-Kremers 2005b, Douglas 2006) where the infarction is often silent or manifests as

Intima media thickness (Papers IV and V)
The measurements of IMT are presented in Table 8. There was no significant difference
between the patients with RA and controls at T0.

Table 8: Intima media thickness (IMT) of a. carotis communis in patients with early RA
compared with controls.

<table>
<thead>
<tr>
<th>Time</th>
<th>RA duration of symptoms (m)</th>
<th>Mean age (y)</th>
<th>Number of RA patients</th>
<th>IMT in RA (mm)</th>
<th>IMT in controls (mm)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>16.2±6.6</td>
<td>46.4±10.7</td>
<td>79</td>
<td>0.52±0.13</td>
<td>0.55±0.15</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>(Paper IV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T18</td>
<td>35.9±6.1</td>
<td>47.6±10.9</td>
<td>26</td>
<td>0.57±0.15</td>
<td>0.54±0.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>(Paper V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data given as mean ±SD or mean (range)
* patients with RA compared with matched controls
m=months; y= years; ns= not significant

There are only two other studies on IMT in patients with early RA, both of which reported an
increased IMT among the patients compared with controls (Hannawi 2007, Georgiadis 2008).
In the study by Hannawi et al. the patients and controls were matched for hypertension,
hyperlipidaemia and smoking status, in addition to age and sex (Hannawi 2007), whilst in the
study by Georgiadis et al. all patients and controls with CVD or any traditional CV risk factor
were excluded (Georgiadis 2008). In the present study all patients ≤60 years of age were
included, and the controls were only matched for sex and age. These methodological
differences may partly explain the contradiction in results. The lack of difference in
traditional CVD risk factors between patients with early RA and controls might have revealed
a difference in atherosclerosis possibly caused by an additive effect of the inflammatory load
on the traditional CVD risk factors in the patients with RA that was concealed in the other
studies. In patients with established RA, the IMT has been found to be increased compared
with controls in many studies (Table 5, Introduction), with only a few reporting contradicting
results (del Rincón 2003, Gerli 2005, Roman 2006). A significant increase in the IMT was
found after 18 months for the patients with newly diagnosed RA. There are, to date, no other
epidemiological prospective studies on IMT in patients with early RA. However, in a study by
Nagata-Sakurai et al. there was a significant increase in IMT during 18-76 months in patients
with established RA (Nagata-Sakurai 2003). Moreover, that it is at all possible to follow IMT
over time is also shown by a few prospective studies in RA that have revealed the possibility
to lower the IMT by anti-rheumatic treatment (Wållberg-Jonsson 2004, Georgiadis 2008).

In studies on the general population, as well as on patients with established RA, an increased
CCA IMT has been demonstrated to independently predict a future cardiovascular event
(O’Leary 1999, Lorenz 2006) and to be associated with traditional CVD risk factors (O’Leary
Results and discussion

1992, Campuzano 2006). IMT is now regarded as an established measurement of sub-clinical atherosclerosis. It is difficult to estimate a “normal value” since the IMT varies with sex, age and the population studied however one study found a median CCA IMT of 0.48 and 0.50 among women and men aged between 41 and 50 years, respectively. (Denarié 2000). Furthermore, another study defines a normal progression rate of CCA IMT as 0.010 mm per year (Howard 1993). These values can be compared with the current results (Table 8) which agree with these “normal values” at T0. The progression rate of the IMT among patients with early RA was also increased in comparison with these data for the general population.

Endothelium dependent flow mediated dilation (Papers IV and V)
The measurements of ED-FMD are presented in Table 9. The difference was not statistically significant between the patients with RA and controls, neither at T0 nor at T18.

Table 9: Endothelial dependent flow mediated dilation (ED-FMD) of a. brachialis in patients with early RA compared with controls.

<table>
<thead>
<tr>
<th>Time</th>
<th>RA duration of symptoms (m)</th>
<th>Mean age (y)</th>
<th>Number of RA patients</th>
<th>ED-FMD in RA (%)</th>
<th>ED-FMD in controls (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>16.2±6.6</td>
<td>46.4±10.7</td>
<td>79</td>
<td>108.9±4.7</td>
<td>107.2±4.6</td>
<td>ns</td>
</tr>
<tr>
<td>(Paper IV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T18</td>
<td>35.9±6.1</td>
<td>47.6±10.9</td>
<td>26</td>
<td>106.9±5.2</td>
<td>107.5±4.4</td>
<td>ns</td>
</tr>
<tr>
<td>(Paper V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data given as mean ±SD or mean (range)
* patients with RA compared with matched controls
m=months; y= years; ns= not significant

There is no published study on ED-FMD comparing patients with early RA to controls whilst there are several studies of patients with established RA showing an impaired ED-FMD in patients compared with controls (Table 6, Introduction). Moreover, there are two studies on patients with early RA using an alternative method, i.e., infusion of the endothelium dependent vasodilator acetylcholine, in which an impaired endothelial function was found (Bergholm 2002, Cardillo 2006). There are a number of small studies on the endothelial function in patients with established RA using different methods, i.e. ED-FMD, PWA or infusion with acetylcholine, all revealing an impaired endothelial function in RA (Hürlimann 2002, Gonzalez-Juanatey 2003a, Klocke 2003, Wong 2003, Vaudo 2003, Yki-Järvinen 2003, Hänsel 2003, van Doornum 2003, Irace 2004, Herbrig 2006, Mäki-Petäjä 2006, Tanaka 2006, Avalos 2007, Arosio 2007, Mäki-Petäjä 2007, Bosello 2008, Kerekes 2008, Wällberg-Jonsson 2008).

Altough there was a slight decrease in patients with RA, the change in ED-FMD after18 months was not statistically significant for the patients with newly diagnosed RA. There are no comparable prospective studies on ED-FMD comparing patients with early RA and controls. However, there are several prospective studies, mostly on patients with established RA, demonstrating an improvement in the endothelial function by different anti-rheumatic treatments (Hürlimann 2002, Bergholm 2002, Irace 2004, Mäki-Petäjä 2006, Bilsborough 2006, Cardillo 2006, Datta 2007, Komai 2007, Bosello 2008, Ikonomidis 2008), and by statin treatment (van Doornum 2004, Hermann 2005, Tikiz 2005, Mäki-Petäjä 2007). Studies on other treatments, e.g., ACE-inhibitor (Tikiz 2005) and prednisolone (Hafström 2007), showed no effect on ED-FMD. All of these studies are, however, of short duration and comparatively small in terms of cohort size. For this study all eligible patients ≤60 years of age were
Results and discussion

included consecutively over a four year period. Thus, the basis for the results are relatively large, although only 33% (n=26) of the patients were available for follow-up measurements after 18 months.

Pathogenesis

Traditional cardiovascular risk factors (Paper IV)

Among traditional CVD risk factors, a higher systolic blood pressure, higher levels of triglycerides and more smoking years were identified among the patients with early RA compared with age- and sex-matched controls (Table 10). In patients with established RA data on the presence of traditional CVD risk factors are divergent (see Introduction). However, in recent years it has become apparent that the inflammatory load in patients with RA has an additive effect on the traditional CVD risk factors for the development of atherosclerosis (del Rincón 2005).

In the study presented in paper IV relationships between traditional CVD risk factors and the extent of CVD seen in the general population or in patients with established RA were evaluated in newly diagnosed patients with RA. Among the patients with RA there were relationships between the IMT and sex ($\beta=-1.02$/female, $p=0.005$), age ($\beta=0.001$/year, $p<0.001$), systolic BP ($\beta=0.030$/mmHg, $p=0.001$), cholesterol ($\beta=0.004$/mmol L$^{-1}$, $p=0.027$), smoking years ($\beta=0.029$/year, $p=0.005$) and snuff years ($\beta=0.043$/year, $p<0.027$). These results are analogous with studies on the general population (O’Leary 1992, Campuzano 2006) and on patients with established RA (del Rincón 2005, Dessein 2005b, Daza 2007). Likewise, the relationship of ED-FMD to sex ($\beta=3.82$/female, $p=0.002$) and age ($\beta=-0.178$/year, $p<0.001$) among the patients with early RA is comparable with studies on the general population (Benjamin 2004, Campuzano 2006) and patients with established RA (Kerekes 2008). Nevertheless, there are several studies on patients with RA that do not confirm any relationships between ED-FMD and traditional CVD risk factors (Gonzalez-Juanatey 2003a, Hafström 2007). Taken together, both this study and others more readily reveal a relationship between traditional CVD risk factors and IMT than with ED-FMD. A possible explanation is that these risk factors have gradual effects over time and changes of ED-FMD can be rapid whilst the variability of IMT is more complex and, therefore, more inert to temporary changes. Moreover, in RA the traditional CVD risk factors are suggested to be diluted by effects of the disease related factors on the endothelium (Gonzalez 2008). The relationships between the traditional risk factors and IMT or ED-FMD would then be easier to reveal among the general population than patients with RA.
Table 10: Traditional CVD risk factors and endothelial biomarkers at T0 in patients with early RA and age- and sex-matched controls. Data are expressed either as mean value ± SD or number of individuals (percentage for whom data are given).

<table>
<thead>
<tr>
<th></th>
<th>RA (n=79)</th>
<th>Controls (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>64 (80%)</td>
<td>34 (77%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>46.4 ± 10.7</td>
<td>47.7 ± 11.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>123.6 ± 14.3*</td>
<td>117.4 ± 11.0</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77.3 ± 8.6</td>
<td>76.3 ± 8.3</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>72.3 ± 10.5</td>
<td>67.9 ± 9.4</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.4 ± 1.0</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3 ± 0.5 *</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.5 ± 0.5</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>BMI, kg/cm²</td>
<td>25.7± 4.0</td>
<td>25.1 ± 4.9</td>
</tr>
<tr>
<td>Smoking, years</td>
<td>13.8 ± 14.3 *</td>
<td>7.0 ± 10.3</td>
</tr>
<tr>
<td>Oral snuff, years</td>
<td>3.1 ± 8.0</td>
<td>2.6 ± 7.4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Previous CVD event</td>
<td>5 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>25 (42%)</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>University education</td>
<td>23 (30%)</td>
<td>20 (40%)</td>
</tr>
<tr>
<td>Familial history of CVD</td>
<td>17 (24%)</td>
<td>7 (18%)</td>
</tr>
<tr>
<td>sVCAM-1, ng/mL</td>
<td>742.7 ± 237.9</td>
<td>631.4 ± 173.2</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>354.1 ± 131.8*</td>
<td>290.6 ± 66.9</td>
</tr>
<tr>
<td>sE-selectin, ng/mL</td>
<td>51.8 ± 19.2</td>
<td>53.8 ± 16.7</td>
</tr>
<tr>
<td>sL-selectin, ng/mL</td>
<td>1252.0 ± 306.2</td>
<td>1279.7 ± 273.2</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>1945.6 ± 936.9***</td>
<td>1125.1 ± 475.5</td>
</tr>
<tr>
<td>PAI-1, µg/L</td>
<td>64.1 ± 37.3</td>
<td>59.1 ± 28.7</td>
</tr>
<tr>
<td>tPA-mass, µg/L</td>
<td>7.17 ± 3.0</td>
<td>8.06 ± 4.3</td>
</tr>
<tr>
<td>VWF, %</td>
<td>203.1 ± 74.1*</td>
<td>161.8 ± 61.2</td>
</tr>
<tr>
<td>Homocysteine, µmol/L</td>
<td>10.5 ± 4.1</td>
<td>8.8 ± 2.4</td>
</tr>
<tr>
<td>ADMA, µmol/L†</td>
<td>0.62 ± 0.15</td>
<td>0.53 ± 0.13</td>
</tr>
</tbody>
</table>

*p-value <0.05 ** p-value<0.01 *** p-value<0.001
† Measured in the 26 patients with RA included in paper V

Endothelial activation and inflammation (Papers I, II, IV and V)

Atherosclerosis is regarded as an inflammatory process (Hansson 1993, Ross 1999) and in my studies inflammatory markers and biomarkers of endothelial activation were used in an effort to reflect the atherosclerotic process.

The patients with very early RA had significantly higher levels of sICAM-1 and MCP-1, endothelial biomarkers with predictive value for CVD, than the controls (Table 10). Other studies have found similar results in that the levels of sICAM-1 (Wällberg-Jonsson 2002, Dessein 2005a, Pahor 2006) and MCP-1 (Ellingsen 2001) were shown to be higher among patients with long standing RA than controls.
In simple linear regression models on the patients with early RA, MCP-1, as well as the inverse levels of sL-selectin, were significantly related to IMT, with sL-selectin also being significantly related to ED-FMD (Table 11).

Table 11: Simple linear regression models of relations between some of endothelial biomarkers analyzed at T0 and IMT or ED-FMD in 79 patients with early RA.

<table>
<thead>
<tr>
<th></th>
<th>IMT T0</th>
<th></th>
<th></th>
<th></th>
<th>ED-FMD T0</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95%CI</td>
<td>p-value</td>
<td>β</td>
<td>95%CI</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>4.65/pg mL^{-1}</td>
<td>1.70; 7.60</td>
<td>0.002</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sL-selectin</td>
<td>-1.06/ng mL^{-1}</td>
<td>-1.99; -0.13</td>
<td>0.026</td>
<td>4.961/ng mL^{-1}</td>
<td>1.61; 8.31</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>VWF</td>
<td>5.91/%</td>
<td>2.12; 9.71</td>
<td>0.003</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.026/μg L^{-1}</td>
<td>-0.06-0.002</td>
<td>0.064</td>
</tr>
<tr>
<td>tPA-mass</td>
<td>0.14/μg L^{-1}</td>
<td>0.04; 0.23</td>
<td>0.005</td>
<td>Ns</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns = Not significant

Additionally, patients with RA and high (i.e., >median value) IMT at T18 had higher levels of sICAM-1 at T0 than patients with RA and low IMT (360.8 ng/mL vs. 279.3 ng/mL, p<0.05). In multiple linear regression models, adjusting for atherothrombotic factors and inflammation, MCP-1 was independently related with IMT at T0, with a tendency for an inverse relationship with sL-selectin (Table 12).

Table 12: Multiple linear regression models of some of the inflammatory markers and endothelial biomarkers and IMT or ED-FMD in 79 patients with early RA.

<table>
<thead>
<tr>
<th></th>
<th>IMT T0</th>
<th></th>
<th></th>
<th></th>
<th>ED-FMD T0</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95%CI</td>
<td>p-value</td>
<td>β</td>
<td>95%CI</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>VWF</td>
<td>3.95/%</td>
<td>0.33; 7.56</td>
<td>0.033</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tPA-mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.37/μg L^{-1}</td>
<td>-0.77; 0.03</td>
<td>0.072</td>
</tr>
<tr>
<td>sICAM-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sL-selectin</td>
<td>-0.76/ng mL^{-1}</td>
<td>-1.63; 0.11</td>
<td>0.087</td>
<td>6.41/ ng mL^{-1}</td>
<td>2.78; 10.03</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>3.41/pg mL^{-1}</td>
<td>0.41; 6.41</td>
<td>0.027</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns = Not significant

In other studies on patients with RA, sICAM-1 and sE-selectin were associated with the IMT or the presence of plaque (Wållberg-Jonsson 2002, Wållberg-Jonsson 2004, Dessein 2005a). There are to my knowledge no other studies on MCP-1 and CVD in RA, however, MCP-1 was shown to be associated with the IMT in chronic haemodialysis patients, a category of patients known to have a high risk for CVD (Kusano 2004). In a multiple linear model adjusting for other biomarkers of endothelial activation, atherothrombotic markers and inflammation, there was an independent relation also between sL-selectin and ED-FMD at T0 (Table 12). Levels of sL-selectin have been found to be decreased in patients with CVD, causing a reduced inhibition of attachment of lymphocytes to the endothelium (Haught 1996, Rainer 2002, Ruef 2006). Taken together, these results indicate an endothelial activation in patients with RA. This endothelial activation was apparent in the patients with newly diagnosed RA when judged by an up-regulation of the biomarkers, albeit the vascular changes apparent using physiological measurements have not yet evolved.
There was no obvious direct relationship between acute phase reactants and the physiological measurements at either T0 or T18. It is, however, tempting to propose that endothelial activation, leading to an accelerated atherosclerosis, is driven by the systemic inflammation in patients with RA. The data presented in paper IV offers some support for this proposition in that several adhesion molecules, i.e., sICAM-1, sE-selectin and inversely sL-selectin, were related to several measurements of inflammation, i.e., ESR, CRP and DAS28 (Table 13). This is in spite that the patients with RA were well treated and low in inflammatory activity (Table 7, Subjects and methods). In other studies on patients with long-standing RA measurements of inflammation have been associated with sE-selectin and sICAM-1 (Kamper 2000, Wållberg-Jonsson 2002, Pamuk 2008). In one study MCP-1 was shown to have a stronger association with the number of swollen joints than either ESR or CRP were (Ellingsen 2001). Moreover, patients with RA had an increased mortality after an AMI (Table 14) or stroke (Table 15) compared with matched controls, even after adjustment for traditional CVD risk factors.

**Table 13:** Simple linear regression models of relations between some of endothelial biomarkers analysed at T0 and markers of inflammation in 79 patients with early RA.

<table>
<thead>
<tr>
<th></th>
<th>ESR</th>
<th>CRP</th>
<th>DAS28</th>
<th>IL-2sRα</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p-value</td>
<td>β</td>
<td>p-value</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>1.98/mm h⁻¹</td>
<td>0.029</td>
<td>ns</td>
<td>na</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>0.34/mm h⁻¹</td>
<td>0.010</td>
<td>0.31/mg L⁻¹</td>
<td>0.061</td>
</tr>
<tr>
<td>sL-selectin</td>
<td>-3.66/mm h⁻¹</td>
<td>0.072</td>
<td>ns</td>
<td>-63.8/unit</td>
</tr>
<tr>
<td>tPA mass</td>
<td>ns</td>
<td>ns</td>
<td>0.50/unit</td>
<td>0.045</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.55/mm h⁻¹</td>
<td>0.032</td>
<td>0.86/mg L⁻¹</td>
<td>0.006</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>ns</td>
<td>ns</td>
<td>9.41/ng L⁻¹</td>
<td>0.015</td>
</tr>
</tbody>
</table>

ns= Not significant; na= Not applicable

**Table 14:** Estimated hazard ratio (HR) and p-values for simple stratified (with or without RA) Cox regression analyses. Case fatality after the first AMI during the study period was the response variable in all analyses.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95 % CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypertensive treatment</td>
<td>1.8/+</td>
<td>1.1-3.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.95/+</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Previous AMI</td>
<td>1.88/+</td>
<td>1.05-3.30</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Table 15:** Estimated hazard ratio (HR) and p-values for a multiple Cox regression analysis of 40 patients with RA and 120 controls. Case fatality after stroke was response variable in all analyses.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95 % CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>1.79/+</td>
<td>1.11 – 2.86</td>
<td>0.016</td>
</tr>
<tr>
<td>Age</td>
<td>1.06/year</td>
<td>1.03 – 1.10</td>
<td>0.001</td>
</tr>
<tr>
<td>Year of event</td>
<td>0.93/year</td>
<td>0.87 – 0.99</td>
<td>0.016</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.93/+</td>
<td>1.09 – 3.42</td>
<td>0.024</td>
</tr>
</tbody>
</table>
The results from one study suggested that RA disease is an independent predictor for a more severe CVD (Warrington 2005) and, according to newly published recommendations, should be considered an independent risk factor for CVD (Pham 2006, Hippisley-Cox 2008). Furthermore it has been suggested that the systemic inflammation in patients with RA worsens the prognosis after an event such as an AMI (van Doornum 2006). Unfortunately data on inflammatory variables are not recorded in the MONICA register, thus further analysis on the influence of inflammation on case fatality was not feasible in the epidemiological studies undertaken here.

The fact that endothelial biomarkers were related to measures of subclinical atherosclerosis independent of ESR indicates that the endothelial up-regulation is not only regulated by the inflammation in early RA. Nevertheless, the studies in this thesis showed a link between systemic inflammation in RA and endothelial activation as well as atherosclerosis even though the measurements of inflammation were blunt and the inflammatory activity low among the patients with RA.

**Atherothrombosis (Paper IV)**

Patients with very early RA had higher levels of VWF than the controls (Table 10). Other studies on patients with established RA have also found elevated levels of fibrinogen, VWF and tPA-antigen compared with controls (McEntergart 2001, Bhaita 2008). In a multiple linear regression model, adjusting for inflammation and other endothelial biomarkers, VWF was independently related to IMT (Table 12). The level of VWF was also associated with IMT at T18. These findings are consistent with previous studies (Wällberg-Jonsson 2000, Daza 2007, Bhaita 2008) and implicate a pro-coagulant state among RA patients, possibly caused by endothelial activation leading to an increased synthesis of VWF. In my study the IMT at T0 was significantly related to tPA-mass (Table 11), which in turn was significantly related to MCP-1 ($\beta=13.813$/pg mL$^{-1}$, $p<0.001$) as well as to DAS28 (Table 13). High levels of tPA indicate a hyperfibrinolytic state and tPA-antigen has been associated with an increased risk of CVD in the general population (Salomaa 1995, Tousoulis 2007). Furthermore, since tPA, as well as MCP-1, is endothelially derived, the association between these variables is another indication of an endothelial activation related to increased IMT among these patients with very early RA, possibly driven by the inflammation as indicated by the association between tPA mass and DAS28. Moreover, there was a tendency for a relationship between PAI-1 and ED-FMD (Table 11), and PAI-1 was related to ESR, CRP as well as DAS28 (Table 13). The data give support to a scenario in which the activated endothelium causes an early alteration of haemostatic factors towards a pro-coagulant state that increases the risk of a thrombotic process as part of the atherosclerotic development in patients with RA.

**Homocysteine and asymmetric dimethylarginine (ADMA) (Papers III and V)**

There are studies showing elevated homocysteine levels among patients with long-standing RA, in part explained by altered metabolism of vitamin B6 (Woolf 2008), or of homocysteine, (Roubenhoff 1997) in this patient group. However, in my study homocysteine levels decreased significantly among the RA patients after only 3 months of supplementation with a combination of vitamin B6, vitamin B12 and folic acid (Figure 9).
Results and discussion

Figure 9: Homocysteine levels in 62 patients with RA, with and without methotrexate, and treated with B-vitamins or placebo. Data is presented as mean ± SEM. Patients treated with B-vitamin had a significant reduction of their homocysteine levels (P<0.0001).

From this data it was concluded that the homocysteine levels in RA patients with moderate disease activity are as easy to reduce as those in the general population (Refsum 1998). This study had a follow-up time of 12 months, which is too short for any further evaluation of CVD events. There are several earlier prospective studies that have confirmed a statistically significant positive association between elevated homocysteine levels and a future CV event, however there are also studies which have failed to confirm any such association (Homocysteine Studies Collaboration 2002, Wierzbicki 2007). Lowering the homocysteine levels by vitamin supplementation may have no effect on the risk for CVD events (Toole 2004, Lonn 2006, Bønaa 2006, Albert 2008) although, homocysteine is believed to be harmful to the endothelium (Ross 1999, Mallika 2007). Among those patients with early RA included in paper IV the level of homocysteine was significantly related to sICAM-1 (β=9.02/ng mL⁻¹, p=0.011) and a tendency for an association with sE-selectin (β=0.046/ng mL⁻¹, p=0.064) sVCAM-1 (β=3.49/ng mL⁻¹, p=0.08), and VWF (β=0.012%, p=0.059). Moreover, in another study it was found that in patients with long-standing RA a high homocysteine level (i.e., >11 μmol/L), together with age and male sex did predict CVD events during a follow-up lasting 6.5 years (p<0.001) (Berglund submitted).

In the study presented in paper III the homocysteine level at inclusion was significantly related to IL-2sRα, a marker of inflammatory activity (Table 13). Other studies have demonstrated an association between homocysteine and inflammatory markers (Lazzerini 2007).

Patients with newly diagnosed RA and high IMT at T18 tended to have higher levels of ADMA than those with low IMT (0.66 vs. 0.58μmol/L, p=0.087). Levels of ADMA, an inhibitor of eNOS, have been shown to be increased in patients with RA compared with controls, and to be related to variables of inflammation as well as to CVD in RA (Kwasny-
Krochin 2008, Atzeni 2008) and to CVD in the general population (Böger 2001, Valkonen 2001, Schulze 2006, Furuki 2007). Taken together, this implies that ADMA might contribute to the accelerated atherosclerotic process occurring in patients with RA, possibly made visible by an increased level of homocysteine in these patients.
General conclusions and future perspective

In this thesis I aimed to contribute to the clarification of the development of atherosclerosis in patients with RA. I have found that patients with an established RA had an increased incidence of AMI as well as stroke compared with the general population, and that the mortality after these events was increased compared with matched controls. These results strongly suggest that patients with RA have an increased and premature atherosclerosis. I subsequently aimed to identify if this premature atherosclerosis is an ongoing process over time and to identify early parameters indicative of the progression of atherosclerosis. Furthermore, I wished to follow this progression prospectively in patients with early RA. Using physiological measurements, e.g., IMT or ED-FMD, I did not find any increase of subclinical atherosclerosis in newly diagnosed patients with RA compared with matched controls. However, the atherosclerosis, measured by IMT, did increase significantly in patients with RA but not in controls during the subsequent 18 months. A proposed model for this accelerated atherosclerotic process in RA, supported by the findings in this thesis, could be as follows (Figure 10): The systemic inflammation in early RA, as measured by ESR, CRP, DAS28, cytokines (Il-2sRα) and chemokines (MCP-1) promote an activation of the endothelium. This is mirrored by up-regulation of adhesion molecules (sICAM-1, sVCAM-1, sE-selectin) on the surface of the endothelium and a decrease of sL-selectin, which are all involved in the rolling process of lymphocytes known to initiate the atherosclerotic process. Endothelial activation is also reflected by an alteration of the endothelially produced haemostatic factors (tPA-mass, PAI-1, VWF) towards a pro-coagulant state. This dysfunction of the endothelium can be identified first as a disturbed ED-FMD, followed by an increased IMT, the sub-clinical state of a future CVD, and finally as a CVD event such as an AMI or stroke. It appears that the inflammatory activity is added to traditional CVD risk factors in the progression of CVD in RA. Consistent relationships between traditional CVD risk factors, i.e., male sex, age, hypertension, hyperlipidaemia and smoking, and the extent of atherosclerosis in patients with RA were found, however the studies in this thesis were not designed to evaluate any additive effect of inflammation.

Future prospective studies on the progression of CVD in patients with RA are important to better understand the pathogenic mechanisms and to be able to reduce the contribution of CVD to the increased morbidity and mortality in this group of patients. An ongoing follow-up of the patients with RA and the controls in the studies on ultrasound assessed atherosclerosis five years after inclusion is in progress, and an additional follow-up ten years from inclusion is planned. The results from T18 can then be evaluated with greater confidence and the novel risk factors for CVD that have been revealed in the recent years can be included. From the studies presented in this thesis I can conclude that it is important to identify and prevent traditional CVD risk factors in patients with RA according to existing and future recommendations, and also to optimize the treatment and follow-up of CVD events in this group of patients. Indeed, the treatment of the inflammatory activity in patients with RA should be optimized to reduce the risk both for CVD and joint destruction.
Figure 10: A proposed explanation for the increased atherosclerosis in patients with RA emanating from the studies presented in this thesis.

An increase has been shown in the papers in this thesis
A relationship has been shown in the papers in this thesis
A decrease has been shown in the papers in this thesis
A relationship has been shown by others
CONCLUSIONS

In this thesis I aimed to discuss the epidemiology and pathogenesis of cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA) and I conclude the following:

- Patients with RA have an increased mortality in CVD due to a higher incidence of, as well as a higher case fatality after, an AMI or stroke compared with the general population.

- The homocysteine level in patients with stable RA is as easy to reduce as in the general population, and inflammatory activity has an influence on the homocysteine level in RA patients.

- No signs of increased atherosclerosis, as assessed by IMT and ED-FMD measurements, were found among patients with newly diagnosed RA compared with healthy controls. However, patients with early RA had a significant increase in atherosclerosis, measured by IMT, after only 18 months.

- Both IMT and ED-FMD were related to several biomarkers of endothelial activation, predominantly MCP-1 and sL-selectin, respectively, implicating an endothelial activation occurring very early in the course of RA despite low inflammatory activity in these patients.

- The results emphasize the necessity of optimising the preventive, diagnostic and caring strategies for CVD in patients with RA.
SAMMANFATTNING PÅ SVENSKA

Kronisk ledgångsreumatism (reumatoid artrit, RA) är en kronisk inflammatorisk, ledförstörande sjukdom som också kan vara förknippad med en förkortad livslängd. Hjärt- och kärlsjukdomar (kardiovaskulär sjuklighet, KVS) står bakom en stor del av denna ökade dödlighet. Publikationerna rörande KVS hos patienter med RA har ökat exponentiellt de senaste åren. Trots det är inte klart vilka mekanismer som ligger bakom detta samband mellan RA och KVS. Denna avhandling avsåg att försöka ytterligare klargöra dessa samband genom olika studier.

I delarbetena I och II visade jag att patienter med en mångårig RA insjuknade ca tre gånger så ofta i såväl hjärtinfarkt som stroke som normalbefolkningen. Dödligheten efter dessa kärlhändelser var också ökad jämfört med kontroller, då patienter med RA hade en 1.7 gånger ökad risk att dö jämfört med kontroller under de 10 första åren efter såväl en hjärtinfarkt som en stroke. Dessa studier gjordes på ett unikt material från MONICA projektet i norra Sverige där alla patienter som haft en misstänkt hjärtinfarkt eller stroke inkluderas i ett stort register. Patienterna med RA (640 st) bestod av en befolkningsbaserad kohort från 1979, och denna samkördes med MONICA registret, varför studierna kan sägas vara i stort sett befolkningsbaserade.

En ökad nivå av homocystein, ett ämne i blodet vars omsättning påverkas av bl. a. olika B-vitaminer, är förknippad med en ökad risk att insjukna i KVS i normalbefolkningen. Tidigare studier har visat att omsättningen av detta ämne är förändrad hos patienter med RA. I delarbete III behandlades 62 patienter med RA och lätt förhöjda nivåer av homocystein med en kombination av vitamin B6, B12 och folsyra eller placebo under 12 månader. Under uppföljningen sågs en betydande sänkning av nivåerna av homocystein hos gruppen behandlad med B-vitaminer redan efter 3 månader. Under uppföljningstiden var dock för kort för att se några effekter på KVS hos dessa patienter.


Det är sedan tidigare känt att traditionella riskfaktorer för KVS, som rökning, högt blodtryck och höga blodfetter, samverkar med inflammationen hos patienter med RA vid utvecklingen av KVS. Jag fann flera relationer med traditionella riskfaktorer för KVS och förekomsten av ateroskleros i mina studier. Det finns även ämnen i blodet som kan spegla en tidig aktivering av kärlväggens innersta lager, endotelet. Denna aktivering anses vara det första steget i aterosklerosprocessen. Vid första mättilfallet i ultraljudsstudien fann jag en ökad aktivering
av endotelet hos patienter med RA jämfört med kontroller. Dessa markörer för endotelaktivitet var också relaterade till förekomsten av ateroskleros i större utsträckning hos patienter med tidig RA än kontroller. Även en del markörer för inflammation hos patienter med RA var relaterade till dessa markörer för endotelaktivitet.

Sammanfattningsvis är det viktigt att identifiera och behandla traditionella riskfaktorer för KVS hos patienter med RA i enlighet med de riktlinjer som finns, samt att behandla och följa upp kärinhändelser inom denna patientgrupp på ett optimalt sätt. Viktigt är även att den inflammatoriska aktiviteten hos dessa patienter behandlas optimalt, för att minska risken både för KVS och för den leddestruktion som är förknippad med RA.
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