Monitoring Arthritis - Biochemical, Metodological and Clinical Aspects

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2009

Document Version:
Publisher's PDF, also known as Version of record

Från Instutionen för kliniska vetenskaper, Lund,
avdelningen för Reumatologi, Lunds Universitet, Lund

Monitoring Arthritis

– Biochemical, Methodological and Clinical Aspects

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Akademisk avhandling
Som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i Segerfalksalen, Wallenberg Neurocentrum, BMC, Sölvegatan 17, Lund, fredagen den 6 februari 2009, kl. 13.00

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Organization
LUND UNIVERSITY

Department of Clinical Sciences, Lund, section for Rheumatology, Lund University, Sweden

Document name
DOCTORAL DISSERTATION

Date of issue February 6, 2009

Sponsoring organization

Author(s)
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Title and subtitle

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Abstract

Arthritides are musculoskeletal diseases, which predominantly affect the joints e.g. osteoarthritis (OA) and rheumatoid arthritis (RA). Modern treatment strategies include early detection and careful monitoring of the disease course. Close monitoring is also the basis for individualized treatment. Monitoring should be done using methods reflecting different aspects of the disease to get a broad perspective of the disease course. It is of great importance that the methods used for monitoring arthritis are standardized.

In paper I, we studied the natural course of knee OA in a population based cohort of middle-aged individuals with chronic knee pain at inclusion, over a 12 year period with a structural method, radiography, and a clinical method. The main finding was that individuals with chronic knee pain were at high risk for developing radiographic knee OA. In paper II, we studied the presence of low-grade systemic inflammation in individuals with chronic knee pain with and without radiographically verified knee OA by measuring high-sensitivity (hs)CRP . We found that low-grade inflammation is a feature of knee OA especially in individuals developing OA in both tibiofemoral (TF) and patellofemoral (PF) compartments. In paper III the diurnal variation of serum COMP was studied in individuals with clinically verified OA and in patients with RA. We found that serum COMP has a half-life of about 7 hours and it has a significant diurnal variation but serum levels were stable during day-time. The objective of study IV was to monitor serum levels of COMP during a randomised controlled trial of physical exercise vs. standardised rest in individuals with symptomatic and radiographic knee OA. Serum COMP levels increased after one hour of physical exercise and decreased after one hour of rest. No increase was seen after a six-week exercise program. In a supplementary study in paper IV we monitored serum COMP with repeated measurements after the exercise session. Serum COMP levels increased immediately after exercise but 30 minutes after the exercise session the levels did not differ significantly from baseline levels. From these studies I conclude that knee pain is often the first sign of knee OA. Elevated levels of hsCRP in serum, particularly in individuals developing combined PF and TFOA, suggest that low-grade inflammation is a feature of early knee OA. COMP has a short half-life in serum and a significant diurnal variation. Serum COMP levels increase after physical exercise but normalize after about 30 minutes of rest. Samples for measuring serum COMP should be obtained at day-time after resting for at least 30 minutes in a sitting position.

Key words: Arthritis, Osteoarthritis, Rheumatoid Arthritis, Biomarkers, CRP, COMP, Monitoring, Clinical, Diurnal variation

Classification system and/or index termes (if any):

Supplementary bibliographical information:

Language
English

ISSN and key title:
1652-8220

ISBN
978-91-86059-98-9

Recipient's notes

Number of pages
131

Price

Security classification

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clinical sciences, Lund University, Sweden

Monitoring Arthritis

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Thesis 2009

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Cover by Annica Graffton
Layout by Media-Tryck

ISSN 1652-8220
ISBN 978-91-86059-98-9
Lund University, Faculty of Medicine Doctoral Dissertation Series 2009:11
Printed in Sweden
Media-Tryck
2008

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List of papers

I Thorstensson CA, Andersson M LE, Jönsson H, Saxne T, Petersson IF. *The natural course of knee osteoarthritis in middle-aged individuals with knee pain - A 12 year follow-up using clinical and radiographic criteria*

Ann Rheum Dis. 2008 Dec 3 [Epub ahead of print]

II Andersson M LE, Saxne T, Petersson IF. *C-reactive protein in individuals with developing knee joint osteoarthritis in a population-based cohort – a twelve year follow-up*

Manuscript submitted

III Andersson M LE, Petersson IF, Karlsson KE, Jonsson EN, Månsson B, Heinegård D, Saxne T. *Diurnal variation in serum levels of cartilage oligomeric matrix protein in patients with knee osteoarthritis or rheumatoid arthritis*. Ann Rheum Dis 2006;65;1490-1494

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IV Andersson M LE, Thorstensson CA, Roos EM, Petersson IF, Heinegård D and Saxne T. *Serum levels of cartilage oligomeric matrix protein (COMP) increase temporarily after physical exercise in patients with knee osteoarthritis*. BMC Musculoskeletal Disorders 2006, 7:98

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Some additional data, not previously presented have been included in the results and discussion section of this thesis

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Abbreviations

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
BMI	Body mass index
COMP	Cartilage oligomeric matrix protein
CRP	C-reactive protein
hsCRP	high sensitivity C-reactive Protein, a method of analysing small quantities of CRP in serum
IL-1	Interleukin 1
JSW	Joint space width
K&L	Kellgren and Lawrence radiographic grading system
MRI	Magnetic resonance imaging
OA	Osteoarthritis
PF	Patellofemoral
PFOA	Osteoarthritis in the patellofemoral compartment of the knee
TFPFOA	Osteoarthritis in both the tibiofemoral and patellofemoral compartments
TF	Tibiofemoral
TFOA	Osteoarthritis in the tibiofemoral compartment of the knee
TNF-α	Tumor necrosis factor α
RA	Rheumatoid arthritis

1. Introduction

1.1. Musculoskeletal diseases and arthritis

Musculoskeletal diseases are a major cause of disability and impaired health related quality of life worldwide, implying a huge impact on individuals, their families, societies and economies (Bone and Joint Decade 2008). Musculoskeletal diseases are sometimes associated with serious co-morbidities and also premature death. They account for about one third of the costs of health care and sick leave in Europe. The diseases predominantly affecting the joints include osteoarthritis (OA) and rheumatoid arthritis (RA). These arthritides affect individuals of all ages, but there is a higher prevalence among middle-aged and older individuals.

Modern strategies for early detection and treatment, as well as prevention, are employed in new paradigms of careful monitoring of the course of the disease and its consequences. Standardised monitoring using clinical, radiographical and biochemical procedures in clinical practice is advocated in international guidelines (ACR 2002; Zhang et al. 2008).

1.2. Osteoarthritis

OA is the most common musculoskeletal disease involving articular cartilage, the synovium, subchondral bone and ligaments, causing pain and disability. It is characterized by thinning and fibrillation of the cartilage, loss of joint space, osteophyte formation, subchondral bone sclerosis and cysts. Humans have suffered from OA throughout history, and it is not a new disease, although the joints affected have changed over the centuries and differ between ethnic groups (Rogers et al. 1981; Jurmain et al. 1995). OA was previously referred to as a degenerative joint disease. Recent studies have shown OA to be a disease with more of a systemic origin, in which low-grade inflammation could be one feature of the disease course (Spector et al. 1997; Pearle et al. 2007). One currently used definition was defined in 1994 “OA diseases are a result of both mechanical and biological events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes and extra cellular matrix, and subchondral bone”(Hart et al. 1995). In OA there is an imbalance between synthesis and degradation of cartilage components resulting in a breakdown of the cartilage. The reason for this imbalance is complex. Studies of mechanoreceptors in chondrocytes and the effect of adipokines on the articular tissue have demonstrated aspects of the complexity of the disease (Pottie et al. 2006). Certain factors act as triggers of the disease including joint injury or joint overload due to hard and intensive physical exercise or increased body weight (Cooper et al. 1994; Roos et al. 1995a; Sowers 2001; Biswal et al. 2002; Englund et al. 2003;

Lohmander et al. 2004). Some factors make the joints more sensitive to these triggers, thus increasing the risk of developing OA, such as age, genetic factors, gender, muscle weakness, inflammation, joint malalignment and obesity leading to degradation of the cartilage matrix by a complex interplay between factors regulating the balance between synthesis and degradation (Davis et al. 1988; Felson 1990; Slemenda et al. 1998; Baker et al. 2004; Thorstensson et al. 2004; D'Ambrosia 2005; Szoeke et al. 2006). A simplified picture of this scenario is shown in Figure 1.

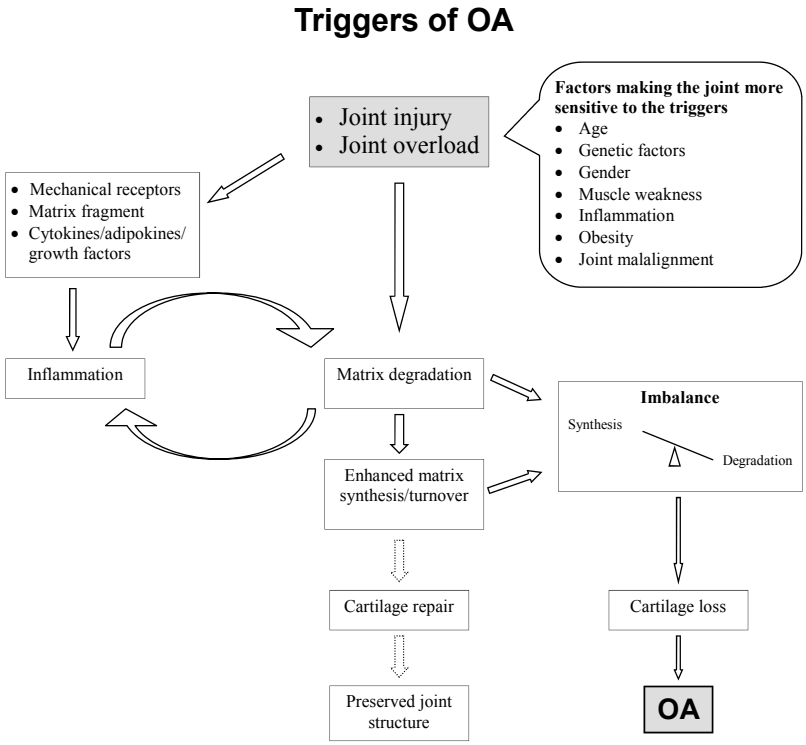

Figure 1

Schematic figure of triggers of the onset of OA, and of how the matrix turnover is disturbed leading to irriversible joint damage.

The surface of chondrocytes contains mechanoreceptors, sensitive to pressure. This could cause activation of signalling pathways within the cell, resulting in the release of cytokines, growth factors and metalloproteinaser (Guilak et al. 2004; Millward-Sadler et al. 2004). Experimental studies indicate that overload in certain situations might trigger inhibition of matrix synthesis and degradation of the cartilage (Wang et al. 1993).

OA is often characterized by signs of local inflammation, synovitis, and an increase in the amount of cytokines released into the synovial fluid where the most predominant cytokines are interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) (Goldring 2000). There are several possible explanations for this increase, one of which is activation of the mechanoreceptors. Various studies have shown that components derived from cartilage might trigger inflammation. One example is fibromodulin, which may activate the classical pathway of the complement system (Sjoberg et al. 2005). It is well established that collagen II can induce arthritis in animal models.
Obesity increases the risk of developing OA not only in weight-bearing joints such as the knee and hip, but also in joints of the hand (Felson et al. 1988; Cicuttini et al. 1996; Manninen et al. 1996; Felson et al. 1997). Adipose tissue is known to be a location of energy storage, and it is essential for normal glucose homeostasis. Recent studies have shown adipose tissue to be an endocrine and secretory organ, secreting adipokines, some of them with inflammatory properties e.g. IL-1, TNF-α, leptin and resistin (Trayhurn et al. 2001; Pottie et al. 2006; Ronti et al. 2006). Obesity has both systemic and mechanical effects on the joints. In this context OA is a complex musculo-skeletal disorder with systemic and mechanical involvement which results in a disequilibrium in the synthesis and degradation of cartilage components.

OA can be defined by radiographic or clinical criteria. The clinical criteria are usually defined by abnormalities consistent with OA found on physical examination (Altman et al. 1986)**.** Radiographic criteria have for a long time been the "golden standard" when classifying OA, and the Kellgren and Lawrence (K&L) grading scale has been used for more than four decades (Kellgren et al. 1957). New and more sensitive structural methods as magnetic resonance imaging (MRI) and biomarkers might change the criteria in the future.

The prevalence of OA differs depending on the method used to classify it (Duncan et al. 2006). When using the K&L grade 2 or more as a cut off for OA the prevalence of radiographic OA in individuals aged between 45 and 54 is about 14 %, in the knee (slightly lower in men and slightly higher in women), about 3 % in both men and women in the hip, and in the hand (DIP-joint) about 30 % in women and 19 % in men. The prevalence of OA as confirmed radiographically has been found to increase with age (van Saase et al. 1989; Petersson et al. 2002).

The knee joint is composed of two different compartments the tibiofemoral (TF) and the patellofemoral (PF). When using the classification according to K&L to classify knee OA the patellofemoral joint is not taken into account.

Using Joint Space Width (JSW) and a cut-off less then 3 mm, the prevalence of isolated radiographically assessed OA has been reported to be about 15 % in the PF compartment and about 35 % in both compartments in individuals older than 60 years (Davies et al. 2002).

The treatment strategies for patients with OA focus on information about the disease, weight-loss programmes and physical exercise. Pharmacological treatment of OA is symptom-based employing analgesics, non-steroidal anti-inflammatory drugs (NSAID) and/or intra-articular administration of steroids or hyaluronan (Guidelines 2000). Pharmacological treatment of OA can not yet be used in clinical practice to treat the actual disease or bring about structure modification. However, certain compounds (Brandt et al. 2005) as well as certain forms of exercise (Roos et al. 2005) have yielded interesting results regarding structure modification of the cartilage in knee OA. Treatment with autologous chondrocyte transplantations is under development for patients with cartilage defects but its role in the treatment of OA remains unclear (Brittberg 2008). For the patients with the most severe forms of knee OA joint replacement is the ultimate treatment.

1.3 Rheumatoid Arthritis

RA is the most common chronic inflammatory joint disease with a peak onset in middle age (55 years of age). RA is more common in women than in men and the prevalence increases with age. Between the ages of 45 and 64 the prevalence is 1.6 % in women and 0.6 % in men (Symmons 2002). Historically there are no valid descriptions of the disease before the nineteenth century. There may, therefore, be a relation between the urbanization/ industrialization and the RA occurance and RA can thus be classified as a rather "modern disease" (Rothschild 2001). RA is characterized by morning stiffness, symmetrical arthritis in the hands/fingers, elbows, hip joints, the knees and feet/toes, accompanied by radiographical changes, often positive rheumatoid factor (RF) (van Schaardenburg et al. 1993) and anti-CCP antibodies and occasionally rheumatoid nodules. A number of factors which increase the risk for developing RA have been identified, including genetic factors, environmental factors, age, gender (hormonal factors), infection/ immunization, lifestyle factors (e.g. smoking) (Saag et al. 1997)) and trauma (physical or psychological). Several genetic factors are associated with RA; the major histocompatibility complex (MCH) being one of the main contributors, often with involvement of the HLA-DRB1 locus (Stastny 1978; Gregersen et al. 2006). RA not only affects the joints but is also systemic with cardiovascular involvement, nodules or lung involvement (Zvaifler 2006). Modern treatment of RA comprises combinations of information and self care, physical therapy and exercise, pharmacological treatment and sometimes surgery

and assistive devices. For many years the pharmacological treatment focused on reducing pain and stiffness. Over the past 25 years there has been a shift towards modification of the disease process and its consequences, mainly the destruction of cartilage, bone and soft tissues. Targeted therapies, such as anti-cytokine drugs, offering a rapid, significant effect on almost all physical aspects of the disease, have been introduced during the past decade. It is thus necessary to be able to make timely decisions regarding changes in pharmacological therapy in order to optimize the positive effects and reduce side effects. This calls for new, more tailored methods of monitoring the disease (ACR 2002; Jordan et al. 2003b).

1.4 Cartilage

Cartilage is an avascular tissue found in several parts of the body. There are three types of cartilage: elastic, fibro- and hyaline cartilage with different morphologies and functions. Elastic cartilage is found in the external ear, larynx and epiglottis. Fibrocartilage is found in the meniscus, pubic symphysis and between the intevertebral discs. Hyaline cartilage is found in the larynx, trachea, in the ribs and on the articular surfaces of the bones in adults i.e. in all synovial joints. It is also found in the growth plates in children. The task of hyaline cartilage is to be resilient, to distribute loads and to lower the friction between the bones in joints. This distribution of load and increased friction protects the underlying bone and facilitates movement.

The hyaline cartilage consists of three layers with different morphology. Nearest to the joint cavity is the superficial layer, with chondrocytes and collagen fibrils running parallel to the surface. The next layer is the intermediate layer where the chondrocytes and the collagen fibres are more randomly distributed. In the deep layer next to the bone the chondrocytes and the collagen fibres are organized perpendicular to the joint surface (Franzen et al. 1981).

The macromolecules in hyaline cartilage give the tissue its specific characteristics. The major constituent is collagen fibres forming a network, which gives the cartilage its tensile strength. The collagen found in hyaline cartilage is mostly collagen II, but collagens XI, VI and IX are also present. Collagen IX is thought to have a regulating effect on the fibre thickness by preventing more collagen molecules from binding to the fibre.
The proteoglycan aggrecan is the most abundant non-collagenous molecule in cartilage. Aggrecan is a large negatively charged molecule with the ability to imbibe water, forming a gel-like substance with a good ability to distribute load and withstand compressive forces. Cartilage contains other non-collagenous, less frequent, molecules, all with different functions in maintaining the tissue properties. One group of non-collagenous proteins is the leucine-rich repeats

(LRR) –family, which includes decorin, biglycan, asporin, fibromodulin, lumican, PRELP (proline/arginine-rich end leucine-rich repeat protein) and chondroadherin. These proteins play a roll in stabilizing the collagen network and regulating the formation of the collagen fibrils and the assembly of the matrix (Heinegard et al. 2007). Another non-collagenous protein found in cartilage is cartilage oligomeric matrix protein (COMP) further described in a subsequent section. Figure 2 shows a schematic drawing of the cartilage matrix. The figure is produced by Dick Heinegård.

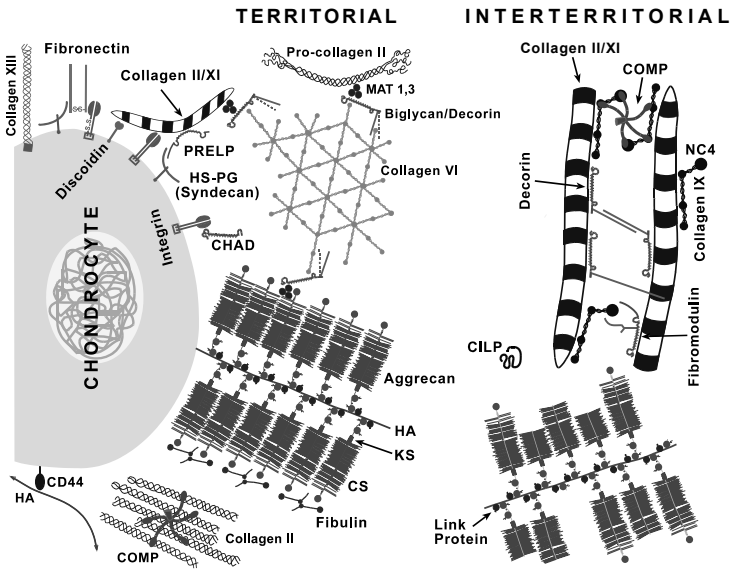

Figure 2

Schematic illustration of the chondrocyte and the matrix constituents in cartilage. Courtesy of Dick Heinegård/Pilar Lorenzo

1.5 Methods for monitoring arthritis

Close monitoring is important in both clinical practice and research concerning patients with chronic arthritis in order to be able to follow the course of the disease, and the effects of treatment. Reliable methods for early diagnosis are crucial. The methods used can be divided into three groups: 1. clinical methods, based on signs and symptoms, 2. structural methods such as radiography and magnetic resonance imaging (MRI) and 3. methods reflecting ongoing tissue processes such as biomarkers (Poole 2000). All methods used in clinical research

and by clinicians should be validated to allow comparisons between studies and patients.

1.5.1 Clinical methods

Clinical methods can be defined in this context as structured, validated examination methods based on clinical examination by a physician. The clinical methods described below are examples of methods used in clinical practice and research when evaluating patients with different forms of chronic arthritis.

The American College of Rheumatology (ACR) criteria for classification of OA in the knee were developed in 1986 and are based on clinical symptoms, where the main symptom is knee pain, followed by bony enlargement, morning stiffness < 30 minutes and age ≥ 38 years, figure 3 (Altman et al. 1986).

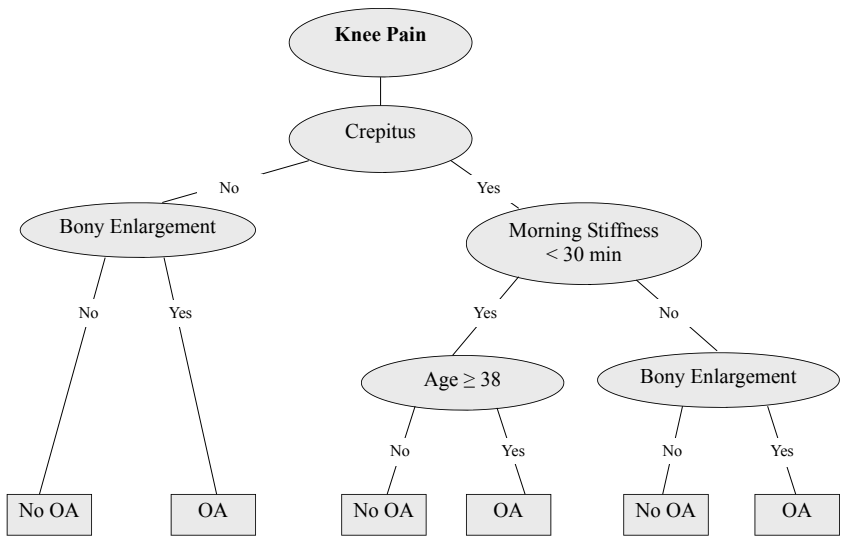

Figure 3

Classification tree of knee osteoarthritis according to Altman et al (1986) for clinical diagnosis of OA.

The ACR criteria for the classification of RA are based on the presence of the following symptoms: 1. morning stiffness in and around joints lasting at least 1 hour before maximal improvement; 2. soft tissue swelling (arthritis) of 3 or more joint areas observed by a physician; 3. swelling (arthritis) of the proximal interphalangeal, metacarpophalangeal, or wrist joints; 4. symmetric swelling (arthritis); 5. rheumatoid nodules; 6. the presence of rheumatoid factor; and

7. radiographic erosions and/or periarticular osteopenia in hand and/or wrist joints. Criteria 1 through 4 must have been present for at least 6 weeks. RA is defined by the presence of 4 or more criteria (Arnett et al. 1988).
The twenty-eight joint count disease activity score (DAS28) is a method of measuring disease activity in patients with RA. This method can discriminate between high and low disease activity. The scoring system includes the assessment of twenty-eight predetermined joints together with measurement of (Erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) to estimate the degree of inflammation, and a pain measurement using a visual analog scale (VAS) (Prevoo et al. 1995; Smolen et al. 1995).

The methods mentioned above are based on clinical examination by a physician. In addition patient reported outcomes measures as the knee injury and osteoarthritis outcome score (KOOS) (Roos et al. 1998), the Western Ontario and McMaster Universities (WOMAC) osteoarthritis index (Bellamy et al. 1988) and the rheumatoid and arthritis outcome score (RAOS) (Bremander et al. 2003) could be used in clinical practice and in research to assess changes induced by treatment or by the disease process for clinical evaluation of arthritis (Garratt et al. 2004).

1.5.2 Structural methods

Structural methods used to monitor arthritis are MRI and plain radiographs. MRI is a detailed but expensive imaging method providing detailed images with high contrast in any plane. It will not be described in detail, since it is not used in this thesis.

Radiography is probably the most commonly used structural method in clinical practice and research for diagnos and classification of chronic arthritis. There are mainly three classification scales used to grade radiographs in knee OA.

1. The Kellgren & Lawrence (K&L) grading scale is based on plain radiographs of the TF joint in supine position. This scale focuses on the appearance of osteophytes, joint space narrowing (JSN), sclerosis and cysts with a range from 1 to 4. 1 – doubtful, minute osteophyte doubtful significance, 2 – minimal, definite osteophyte, unimpaired joint space, 3 – moderate, moderate diminuition of joint space and 4 – severe, joint space greatly impaired with sclerosis of subchondral bone. This grading scale is weighted towards the presens of osteophytes (Kellgren et al. 1957).
2. The classification according to Ahlbäck is a five graded scale based on plain radiographs under weight-bearing conditions of the TF joint. The Ahlbäck grading scale reaches from I to V, I – joint space narrowing less than 3 mm,

II – joint space obliterated or almost obliterated, III – minor bone attrition (0-5 mm), IV – moderate bone atrittion (5-15 mm) and V – severe bone attrition (>5 mm) (Ahlback 1968).

3. Classification of knee OA according to joint space width (JSW) is based on plain radiographs of both TF and PF joint under weight-bearing conditions, with the knee in a semi-flexed position. The cut-off for OA in the TF joint is a JSW of 3 mm or less and in the PF joint 5 mm or less (Boegard et al. 1997; Boegard et al. 1998).

The correlations between these three structural methods have been described by Petersson and coworkers in 1997 (Petersson et al. 1997). Ahlbäck grade I is comparable with K&L grade 3 and JSW <3mm in the TF compartment, table 1.

Ahlbäck grade	Ahlbäck definition	Kellgren& Lawrence grade	Kellgren & Lawrence definition
		Grade 1 "doubtful"	Minute osteophyte, doubtful significance
		Grade 2 " minimal"	Definite osteophyte, unimpared joint space
Grade I	Joint space narrowing (joint space <3 mm)	Grade 3 "Moderate"	Moderate dimunition of joint space
Grade II	Joint space obliteration	Grade 4 "Severe"	Joint space greatly impared with sclerosis of subchondral bone
Grade III	Minor bone attrition (0-5 mm)	Grade 4 "Severe"	Joint space greatly impared with sclerosis of subchondral bone
Grade IV	Moderate bone attrition (5-10 mm)	Grade 4 "Severe"	Joint space greatly impared with sclerosis of subchondral bone
Grade V	Severe bone attrition (>10 mm)	Grade 4 "Severe"	Joint space greatly impared with sclerosis of subchondral bone

Table 1

Comparison between Ahlbäck and Kellgren & Lawrence grading systems for radiographic knee OA. From Petersson, I.F., Boegard, T., Saxne, T., Silman, A.J. and Svensson, B. (1997). "Radiographic osteoarthritis of the knee classified by the Ahlback and Kellgren & Lawrence systems for the tibiofemoral joint in people aged 35-54 years with chronic knee pain." Ann Rheum Dis 56(8): 493-6.

RA is usually classified by inspecting plain radiographs of the hands and feet. The classifications commonly used are the Larsen or Sharp score with modifications. In the Larsen method the joint destruction is evaluated on a score between 0 and 5 in relation to reference radiographs (Larsen 1973). In the Sharp method,

the erosion of each joint is evaluated on a score between 0 and 5 and a score for JSN between 0 and 4 is assigned to each joint (Sharp et al. 1985).

1.5.3 Biological markers

A biological marker (or biomarker) can be defined as, "a compound that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes or as a response to a therapeutic intervention" (Atkinson et al. 2001).

The human body consists of an almost infinitely large number of molecules, many of which could be potential biomarkers for different processes in the body (Saxne et al. 1995). Techniques employing biomarkers are widely used when diagnosing and monitoring different diseases, and provide useful tools as they are faster, less invasive, and less expensive than most other methods. In acute coronary disease, for example, the measurement of the biomarker troponin (Cummins et al. 1987) together with EKG is the most important tool for rapid diagnosis, which is crucial in ensuring rapid and appropriate treatment. Several kinds of markers have been studied with regard to processes affecting synovial joints: for inflammation, for processes in bone, for processes in cartilage, table 2 (Otterness et al. 2000; Poole 2000; Garnero et al. 2003; Kraus 2005; Wieland et al. 2005; Saxne et al. 2006; Maksymowych et al. 2007).
In this thesis two biomarkers have been more thoroughly studied: C-reactive protein, a non-specific marker of systemic inflammation and cartilage oligomeric matrix protein, a marker reflecting turnover processes in the cartilage.

Biomarkers for processes affecting synovial joints – key examples

Biomarker	Tissue process	Source
Collagen type II c-propeptide	Cartilage synthesis	Serum, synovial fluid
Collagen type II cross links (CTX-II)	Cartilage degradation	Urine
Collagen II collagenase generated cleavage products (C2C, C1, 2C)	Cartilage degradation	Urine, serum, synovial fluid
Aggrecan core protein epitope	Cartilage turnover	Synovial fluid
Aggrecan, keratan sulphate (KS) epitopes	Cartilage turnover	Synovial fluid
Aggrecan, chondroitin sulphate (CS) epitopes, 846 and 3B3	Cartilage turnover/synthesis	Serum, synovial fluid
Cartilage Oligomeric Matrix Protein (COMP)	Cartilage turnover	Serum, synovial fluid
Cartilage Intermediate Layer protein (CILP)	Cartilage turnover/synthesis	Serum, synovial fluid
Collagen type I N-and C- (carboxy) propeptides	Bone turnover	Serum
Collagen type I c-telopeptide fragments (CTX-I)	Bone remodelling	Urine
Osteocalcin	Bone assembly/turnover	Serum
Bone Sialoprotein (BSP)	Bone assembly/turnover	Serum
C-Reactive Protein	Systemic inflammation	Serum

Table 2

Biomarkers for processes affecting synovial joints – key examples adapted from Saxne, T., Månsson, B., Heinegård, D. (2006). Molecular markers for assessment of cartilage damage in rheumatoid arthritis. Rheumatoid Arthritist. Firestein, G.S., Panayi, G.S., Wollheim,F.A. New York, Oxford University Press: 301-316.

1.5.3.1 C-Reactive Protein

C - reactive protein (CRP) is an evolutionary ancient protein conserved through the evolution of vertebrates. CRP was first described by Tillett and Francis in 1930 and was named for its ability to bind to C-polysaccharide on the cell wall of pneumococci (Tillett et al. 1930). CRP is a protein within the pentraxin family composed of five identical non-glycosylated subunits arranged in a pentameric, disc like structure, and has a molecular weight of 105 500 Da. CRP is synthesized in the hepatocytes in response to IL-1 and Interleukin-6 (IL-6). CRP takes part in the innate immune response and can bind to a wide range of substances from damaged autologous cells and microorganisms. CRP is able to activate the classical pathway of the complement system by activating C1q, causing lysis of the cell. CRP also opsonizes cells through binding to Fcγ-receptors, facilitating phagocytosis. This interaction leads to production of cytokines and mobilizes the adaptive immune response (Pepys 1981; Du Clos 2000; Mortensen 2001; Du Clos et al. 2004).

CRP is an acute-phase protein with elevated levels in diseases characterized by cell disintegration, in bacterial infections and in chronic inflammations. C-reactive protein is a non-specific marker of systemic inflammation, with a rather quick clearance from the system ($T_{½}$ 19 h). It is widely used in clinical practice in screening for diseases and for monitoring disease activity in infectious, inflammatory and immunological disorders (Pepys 1981). Recent studies have suggested low-grade inflammation to be a feature of other diseases such as cardiovascular diseases and OA, based on measurements of high sensitivity C-reactive protein (hsCRP), and it has also been suggested that increased hsCRP in patients with OA is predictive of a progressive disease (Spector et al. 1997; Sharif et al. 2000; Sowers et al. 2002; Saxne et al. 2003; Tousoulis et al. 2008)

1.5.3.2 Cartilage Oligomeric Matrix Protein

As the name implies, cartilage oligomeric matrix protein (COMP) is a protein primarily detected in cartilage but is also present in low amounts in the synovium and tendons (Hedbom et al. 1992). COMP is a 435 kDa non-collagenous protein within the thrombospondin family, also called thrombospondin-5, synthesized by chondrocytes. COMP is composed of five identical subunits with a globular domain at the C-terminal part of the molecule, joined together with a coil-coil structure, stabilized with disulphide bridges, forming a molecule shaped like a bouquet of tulips, figure 4. COMP interacts with collagen and it has been suggested that it plays a role in the regulation of fibril assembly as well as a structural role in the maintenance of the mature collagen network. (Rosenberg et al. 1998; Rosenberg 2001; Halasz et al. 2007). The COMP gene is located on chromosome 19, region 13.1. Mutations of the COMP gene are seen in human

diseases such as pseudochondroplasia (PSACH) and multiple dysplasia (MED) and result in disturbed skeletal growth, leading to short limb dwarfism in humans (Newton et al. 1994; Briggs et al. 1998). However, in COMP deficient mice there are not any anatomical, histological or ultrastructural abnormalities and no clinical signs of PSACH or MED (Svensson et al. 2002).

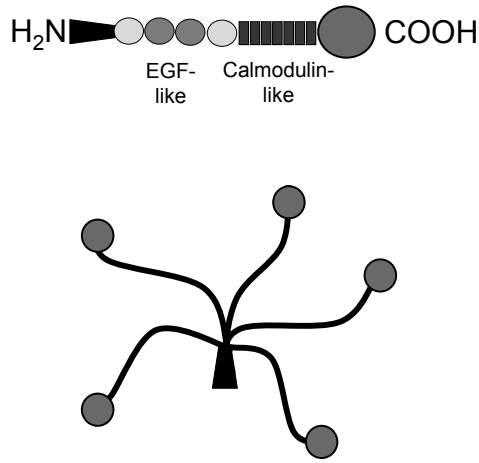

Figure 4

Schematic picture of the domain structure of COMP and how the five monomers are joined together at the N-terminal. EGF= epidermal growth factor. Courtesy of Dick Heinegård/Pilar Lorenzo

COMP is a biomarker reflecting processes in cartilage and is one of the most thoroughly studied cartilage biomarkers (Williams et al. 2008) and has in studies been shown to reflect cartilage turnover related to structural damage in both RA and OA (Mansson et al. 1995; Sharif et al. 1995a; Larsson et al. 1997; Mansson et al. 1997; Conrozier et al. 1998; Petersson et al. 1998a; Petersson et al. 1998b; Clark et al. 1999; Marti et al. 1999; Larsson et al. 2002; Sharif et al. 2004; Sharif et al. 2006; Hunter et al. 2007). Some studies have shown raised serum levels of COMP in patients with OA compared to individuals without OA (Neidhart et al. 1997; Clark et al. 1999). It has also been shown that middle-aged individuals with chronic knee pain at baseline, who had developed radiographic OA at the follow-up after 3 years, had higher serum COMP levels at baseline than the individuals without any radiographic signs of OA (Petersson et al. 1998b). Patients with knee OA whose disease was seen to progress radiographically over five years showed a higher increase in serum COMP than non-progressors (Sharif et al. 1995b; Sharif et al. 2004). Increased serum COMP levels at the onset of RA is

a predictor of a more severe disease course (Mansson et al. 1995; Lindqvist et al. 2005). Treating RA patients with TNF-γblockade normalizes serum COMP levels (Crnkic et al. 2003). Corticosteroids have been shown to reduce the cartilage destruction and decrease the serum COMP levels both in experimental arthritis and in patients with RA (Larsson et al. 2004; Skoumal et al. 2006).

1.5.3.3 Factors influencing the results when measuring biomarkers

1.5.3.3.1. Pre-analytical variations

Pre-analytical factors are phenomena occuring before or during the blood sampling occasion which could have an effect on the result of the analysis. Examples of preanalytical factors are the time point for the sampling, food intake, smoking habits, physical exercise, the body position e.g. sitting or lying down and the sampling procedure e.g. venous or capillary, with or without tourniquet. Fore example prolonged tourniquet could increase the concentrations of protein or protein-bound constituents by 15 %. The handling and the storage of the samples could also influence the result. It is important to be aware of these factors and control for them if possible by using standardised procedures. (Statland et al. 1974; Young et al. 2006a; Young et al. 2006b).

1.5.3.3.2. Analytical variation

The analytical variation is the generic term for methodological bias and random errors. Methodological bias results from systematic faults such as problems associated with the analytical procedure e.g. calibration problems or problem with the analytical equipment, which can be identified and remedied. Generally the analytical variation should be less than half of the within-subject variation. Random errors can not be corrected for, their effects can be reduced by performing several measurements and reporting the mean value (Linnet et al. 2006). Participation in standardization programs as EQUALIS (External Quality Assurance in Laboratory Medicine In Sweden) and similar international programs (CLSI 2008) improves the quality of the analyzes and increase the patient safety by comparing methodologies and measurement results from different laboratories and detecting and elucidating sources of error and methodology problems (EQUALIS 2008).

1.5.3.3.3. Biological variation

Biological variations represent the preanalytical factors including within-subject variation and between-subject variation. The within-subject variations refer to a setting point, specific for each individual and it could be cyclic or random. Examples of cyclic variations are diurnal, between meals, monthly or seasonal variations. Random variations can be seen when analysing substances with intermittently internal secretion and a short half-life (Statland et al. 1973a; Bokelund et

al. 1974; Statland et al. 1974; Winkel et al. 1974; Young et al. 2006a). For some substances external circumstances e.g. physical exercise could have major effects on the day-to-day variation. In a study by Statland and co-workers 30 minutes exercise gave an increase in serum concentrations of alanine aminotransferase (ALAT) by 40 % and in aspartate aminotransferase (ASAT) by 30 %, at the time point 15 minutes after stopping the exercise (Statland et al. 1973b).

The between-subject variation could be influenced by endogenous factors such as gender, age, obesity, body position (e.g. standing, sitting or lying down) or ethnic origin. For example, a change from the supine to the upright position has been reported to result in a 10 % increase in the plasma protein concentration, which is normalized after 30 minutes (Felding et al. 1980; Hyltoft Petersen et al. 1980). Another biological factor is the clearance time from the system, which depends for example, on the condition of the liver and kidneys (Young et al. 2006a).

1.5.3.3.4. Validation of biomarkers in arthritis

All methods of analysing markers for clinical use or in research should be standardised to minimize the bias and also to make it possible to compare different studies or samples taken on different occasions (Fraser et al. 1989). Criteria for validation of biomarkers to be considered as biomarkers reflecting structural damage in OA and RA have been developed. Validation of a biomarker should include studies demonstrating reliability, discrimination and feasibility, table 3. There should be a biological rationale for using a particular biomarker. Furthermore, knowledge about the function of the marker in the joint tissue and its release during tissue turnover must be obtained and the characteristics of the marker in relation to established biomarkers must be determined. The assay used for measurements should provide a sufficiently high level of discrimination. This includes the reproducibility of the assay, effects of pre-analytical factors of the measurement and descriptions of biological variations.
The feasibility of the assay should also be high i.e. it must be simple to perform (methodologically), internationally standardized, and stability of the specimen both at room temperature and frozen, should be known (Poole 2000; Maksymowych et al. 2007).

A. Truth

1. Evidence that the biomarker reflects tissue remodelling in animal models of disease.
2. The biomarker has been immunohistochemically localized to joint tissues.
3. The biomarker demonstrates sensitivity and specificity for target of joint tissue origin.
4. Relation of biomarker to synthesis, degradation, turnover of joint tissue components has been characterized.
5. Levels of the biomarker correlate with scores for other biomarkers that have been established as possesing predictive validity for structural damage.

B. Discrimination

6. The assay for measurement of the biomarker is reproducable (coefficient of variation: assay < 10%, interassay < 15%).
7. The effects of the following sources of variability on levels of the biomarker in normal individuals are known: age, sex, menopause, circadian rhythms, body mass index, physical activity, NSAID, renal and hepatic disease, contribution of different affected joints.
8. The metabolism, clearence, and half-life of the biomarkerhave been characterized in normal individuals and in patients with arthritis.
9. The biomarker demonstrates high sensitivity and specificity in comparisions of the disease population with age and sex matched healthy controls.
10. The biomarker demonstrates independent association with the structural damage end point (van der heijde modification of Sharp score for RA, mSASSS for AS, joint space narrowing for OA) at the level of both absolute and relative change in (A) a clinically well defined prospectivecohort, (B) a randomized controlled trial, (C) a clinically well defined prospective cohort of patients with preradiographic disease of adequate sample size and followed for a suffucient duration to detect change in radiographic damage score (3 criteria).

C. Feasibility

11. The assay for measurment of the biomarker has been well characterized, is internationally standardized (availability of reference standards), and is methodologically simple.
12. Stability of the biomarker at room temperature and in frozen specimen has been documented.

Table 3

Suggested validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage in RA/SpA generated prior to consensus voting by Delphi technique. From Maksymowych, W.P., Landewe, R., Boers, M., Garnero, P., Geusens, P., El-Gabalawy, H., Heinegard, D., Kraus, V.B., Lohmander, S., Matyas, J., Saxne, T. and van der Heijde, D. (2007). "Development of draft validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage endpoints in rheumatoid arthritis and spondyloarthritis clinical trials." J Rheumatol 34(3): 634-40.

Much of the validation process of biomarkers is a part of the research process. Certain initiatives for standardising this development and evaluation of biomarkers in arthritis have been initiated (Bauer et al. 2006).

2. Aims of the thesis

2.1. General

The overall purpose of this thesis was to study and evaluate methods for monitoring arthritis in research and clinical practice and to study the disease course of early symptomatic knee OA.

2.2. Specific

- To study the development of knee OA in a middle-aged population with chronic knee pain with the hypothesis that idiopathic chronic knee pain is an early sign of knee OA. *(The natural course of OA development over 12-year in patients with knee pain)*

- To study serum levels of high sensitivity C-reactive protein (hsCRP) over a 12-year period in a cohort of individuals with chronic knee pain with and without radiographic knee OA as an indicator of low-grade inflammation and possible marker of developing OA. *(Low-grade inflammation in OA development in patients with knee pain)*

- To monitor changes in serum concentrations of cartilage oligomeric matrix protein (COMP) during a 24-h period to determine any diurnal variation, and to estimate the half life of COMP in the circulation in patients with symptomatic knee OA and in those with RA. *(Diurnal variation of COMP in serum)*

- To monitor serum levels of COMP during a randomised controlled trial of physical exercise vs. standardised rest in individuals with symptomatic and radiographic knee OA. *(The influence of physical exercise on serum COMP levels)*

3. Ethical considerations

Ethical approval was obtained from the Ethics Committee, Lund University, (LU 312-90, LU 684-00, LU 421-02, LU 947-02). The studies followed the guidelines from the Helsinki Declaration. Written consent was obtained from the participants. The blood samples are saved in a biobank at Spenshult Hospital for Rheumatic Diseases, which is registered at the Swedish Board of Health and Wellfare (Biobank no. 85).

4. Methods and results

4.1. Definitions

In this thesis the following definitions have been used:

- RA, according to the ACR 1987 criteria (Arnett et al. 1988)
- Clinical OA, according to the Altman criteria, figure 3, (Altman et al. 1987)
- Radiographic knee OA
  a. TFOA
  - K&L grade 1 or worse (paper I)
  - K&L grade 2 or worse (paper III and IV) (Kellgren et al. 1957)
  - JSW in the tibiofemoral compartment < 3 mm (paper II) (Boegard et al. 1998)

  b. PFOA
  - JSW in the patellofemoral compartment < 5 mm (paper II) (Boegard 1998)

4.2. A knee pain cohort (the Spenshult cohort) for monitoring OA development (papers I and II)

The Spenshult cohort is a population-based cohort created in 1990 to study the features and early course of symptomatic knee OA in middle-aged individuals (35-54 years of age at inclusion) with chronic knee pain.

In 1990 a questionnaire was sent to 2000 people aged between 35 and 54 living in the district of Laholm, a rural district in the southwest part of Sweden**.** Over 92 % of the individuals (1853) responded to the questionnaire, and of them 279 had had knee pain for at least three months. Seventy-five of the individuals with

knee pain declined further participation, six had had major knee trauma and 14 had other rheumatic disorders, and one was misclassified, thus 183 individuals were available for inclusion in the study (Petersson 1997). Regular follow-ups involving the collection of clinical, biochemical and imaging data have been performed in this group over 12 years**,** figure 5.

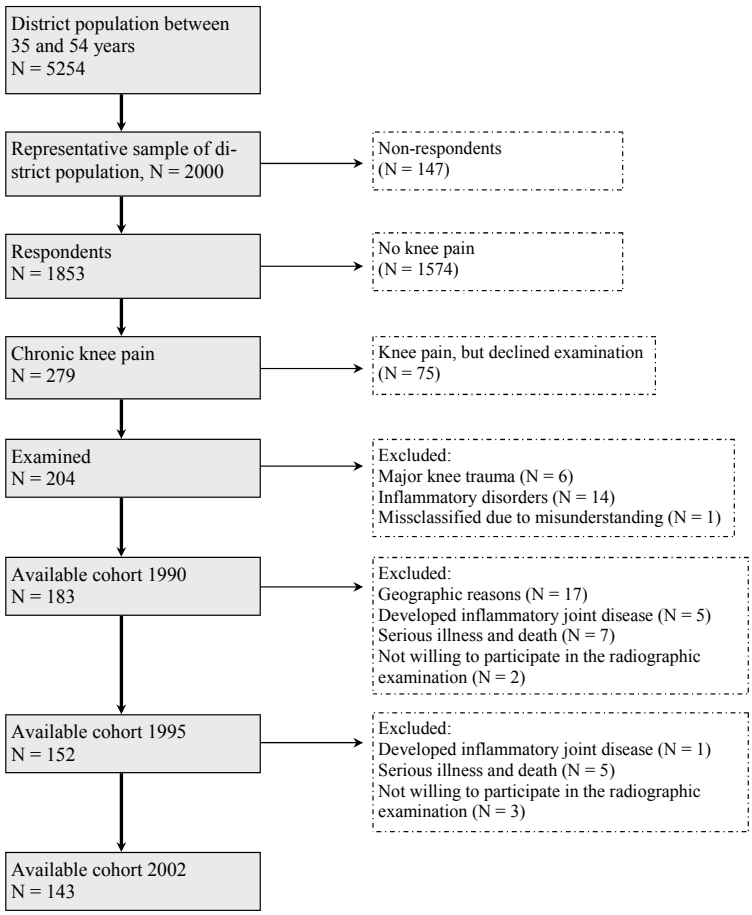

Figure 5

The Spenhult cohort a population-based cohort of middle-aged individuals with chronic knee pain recruited in 1990.

4.2.1. The natural course of OA development over 12 years in patients with knee pain (Paper I)

4.2.1.1. Method

The participants in the study described in paper I were the 143 individuals from the Spenshult cohort, 63 women and 80 men, who were included in 1990 and had participated in the 12-year follow-up in 2002. At the inclusion the participants underwent a clinical examination by a rheumatologist and posterior-anterior radiographs were obtained of both TF compartments, with straight legs in weight-bearing position and with the weight equally distributed on both legs. At the follow-ups after 5 and 12 years, posterior-anterior radiographs were taken of the TF compartments in both knees in a weight-bearing semi-flexed position and radiographs in a skyline view of the PF compartments were taken. For the present study, data on TF compartments from baseline and the 12 year follow up were used. Patellofemoral compartments were examined at five and twelve years follow up only. The radiographs of the TF compartments from the two occasions were classified according to Kellgren & Lawrence. Radiographically OA in the TF compartments was defined as K&L grade 1 or more in at least one knee. Incidence and progression were defined as a change in grade of one or more in at least one knee between the baseline examination and the 12-year follow-up. OA in the PF compartments was defined as JSW less than 5 mm in at least one knee. The clinical method used for defining clinical OA was the classification according to Altman The clinical criteria described by Altman are; knee pain; crepitus; morning stiffness < 30 minutes; bony enlargement; age > 38 (Altman et al. 1987), figure 6.

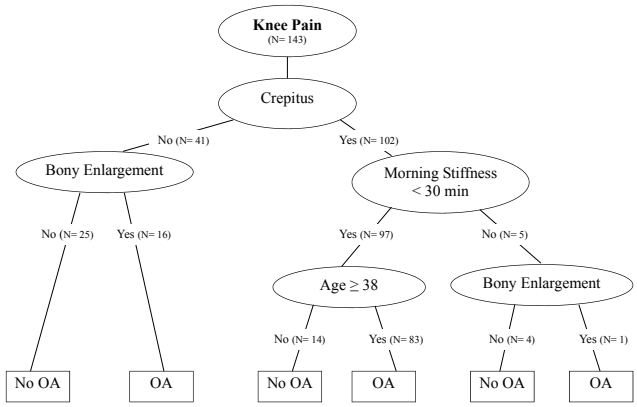

Figure 6

Classification scheme of clinical knee OA according to Altman et al (1986) as applied in the Spenshult cohort in paper I.

This study included four groups of individuals, those with no radiographically or clinically defined OA, those with clinically confirmed OA, those with radiographically confirmed OA and individuals with both clinically and radiographically confirmed OA, figure 7.

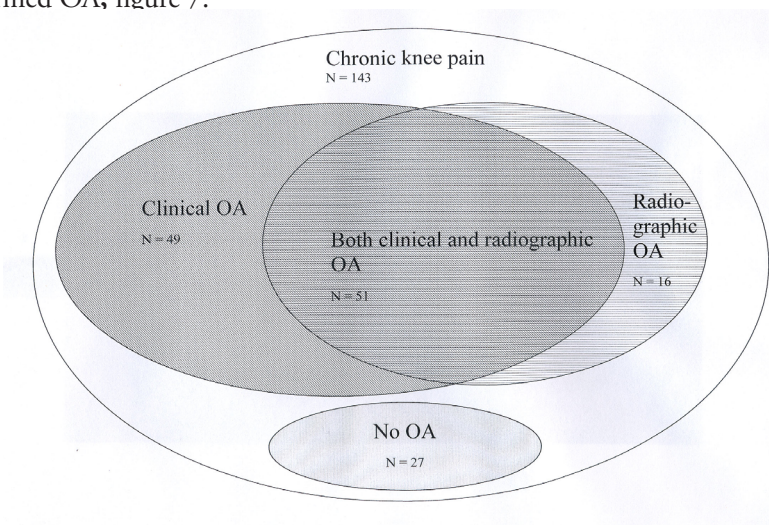

Figure 7

Four groups of individuals with and without clinically verified OA and/or with and without radiografically confirmed OA at inclusion in the Spenshult cohort in paper I.

4.2.1.2. Results

At the 12-year follow-up 4 % (6) of the patients had no signs of radiographic OA in either compartment of the knee.

In the group of 76 individuals with no radiographic changes at baseline, 65 individuals (87%) developed incident TFOA over the 12 years, table 4.

	Age (range)	**BMI*** (range)	**No progress** (no change in K&L†)	**Progress** (change K&L† ≥ 1)
No OA baseline (N=27, 13 men, 14 women)	40 (35-54)	25.0 (18.4-36.0)	6	21
Clinical OA baseline (N=49, 29 men, 20 women)	46 (35-54)	25.6 (18.3-37.0)	5	44
Radiographic OA baseline (KL ≥1) (N=16, 7 men, 9 women)	41 (35-54)	23.6 (20.3-32.3)	1	15
Both clinical and radiographic OA baseline (N=51, 31 men, 20 women)	47 (37-55)	25.9 (19.3-37.5)	1	50

* Body Mass Index

† Kellgren and Lawrence

Table 4

Development of radiographic tibiofemoral osteoarthritis (OA) over twelve years in individuals with or without radiographic OA and/or clinical OA at baseline from the cohort studied in Paper I (n=143)

At the inclusion there were 67 individuals, who had radiographic changes corresponding to K&L grade 1 or more. At the 12 year follow-up 65 (97 %) of them had progressed radiographically over 12 years, table 4 (data not included in manuscript).

Using the clinical criteria according to Altman, 49 of the 143 had clinical OA without radiographically verified knee OA at inclusion. Twenty-seven of the 143 individuals had neither clinically nor radiographically confirmed knee OA. At the follow-up 12 years later, 90 % (44) of the individuals with clinical OA had developed radiographic changes corresponding to K&L grade 1 or more and 78 % (21) of the individuals without knee OA had radiographically verified knee OA, figure 8.

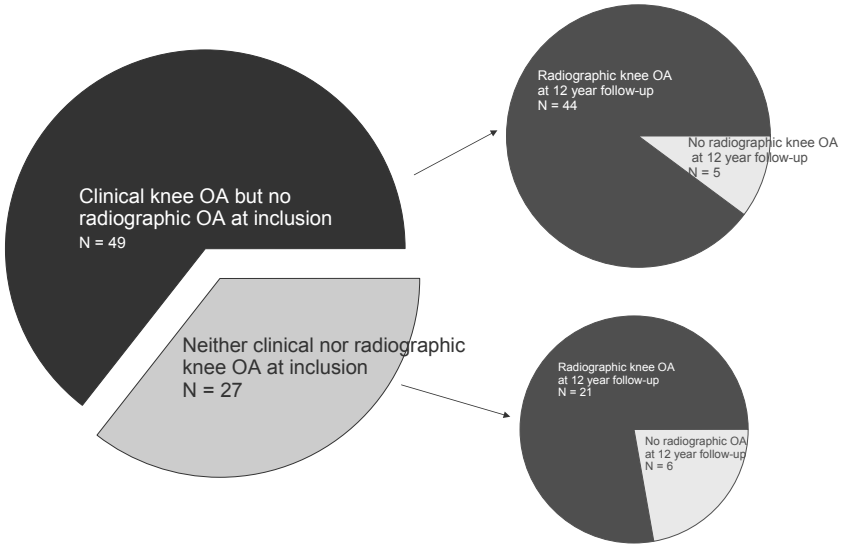

Figure 8

The incidence,at the 12-year follow-up, of radiographic knee OA in participants with and without clinically verified knee OA at baseline.

At the five-year follow-up 84 of the 125 examined individuals had no radiographically confirmed OA in the PF compartment, while seven years later 26 (31 %) had developed PF OA, table 5 (data not included in manuscript).

5-year follow-up	12-year follow-up	
	No PF OA	**PF OA**
No PF OA (N=84, 60 men, 24 women)	58 (48 men, 10 women)	26 (12 men, 14 women)
PF OA (N=41, 12 men, 29 women)	2 (2 women)	39 (12 men, 27 women)

Table 5

Development of radiographic patellofemoral (PF) osteoarthritis (OA), classified as joint space width (JSW) < 5 mm, from the 5-year to the 12-year follow-up of the Spenshult cohort (n=125)

4.2.2. Low-grade inflammation in OA development in patients with knee pain (Paper II)

4.2.2.1. Method

The participants in this study were 124 individuals from the Spenshult cohort included in 1990, who had participated in the follow-ups in both 1995 and 2002 and where both radiographs and blood samples were available. Posterior-anterior radiographs were obtained of the TF compartments in both knees in weight-bearing semi-flexed position and in a skyline view of the PF compartments. TFOA in the was defined as JSW less than 3 mm in at least one knee, and PFOA was defined as JSW less than 5 mm in at least one knee. Serum levels of CRP were analysed with high sensitivity rate turbidimetry. The detection limit was <0.2 mg/L and the coefficient of variation 6%.

4.2.2.2. Results

The patients were divided into four different groups according to their JSW at the 12-year follow-up, 1. No OA (NOA-12), 2. OA in the tibiofemoral compartment (TFOA-12), 3. OA in the patellofemoral compartment (PFOA-12) and 4. OA in both compartments (TFPFOA-12). There were some differences in the gender distribution between the groups. There were more men in group 1 and group 2 than in the other groups ($p \leq 0.003$) and the group with TFPFOA had higher body mass index (BMI) than the other groups ($p < 0.020$), table 6.

Radiographic classification at 12 year follow-up		Descriptives			hsCRP 1990	hsCRP 1995	hsCRP 2002
		BMI 1990	BMI 2002	Age 2002	(mg/L)	(mg/L)	(mg/L)
All	N=124 (70 men, 54 women)						
	Median	**25.31**	**27.4**	**56.2**	**0.90**	**1.25**	**1.40**
	Min	18.29	17.2	46.7	0.20	0.20	0.20
	Max	37.47	41.8	66.1	27.50	31.80	38.70
NOA-12	N = 42 (33 men, 9 women)						
	Median	**25.01**	**27.0**	**55.8**	**0.80**	**1.10**	**1.10**
	Min	20.22	21.9	46.7	0.20	0.20	0.20
	Max	33.95	41.8	65.3	6.40	12.30	9.10
TFOA-12	N = 17 (14 men, 3 women)						
	Median	**23.85**	**26.0**	**59.5**	**0.80**	**1.10**	**1.10**
	Min	20.85	17.2	49.6	0.20	0.20	0.20
	Max	32.31	36.3	64.9	4.80	3.50	5.30
PFOA-12	N =51 (21 men, 30 women)						
	Median	**25.64**	**27.5**	**54.3**	**1.00**	**1.25**	**1.70**
	Min	18.29	19.2	46.9	0.20	0.20	0.20
	Max	34.49	40.3	65.6	5.20	31.80	8.50
TFPFOA-12	N = 14 (2 men, 12 women)						
	Median	**26.27**	**29.9**	**61.5**	**2.40**	**2.55**	**2.10**
	Min	19.27	19.7	47.7	0.40	0.50	0.30
	Max	37.47	36.4	66.1	27.50	16.20	38.70

Table 6

The median values of serum hsCRP (mg/L) in the different groups from 1990 to 2002. The table also shows descriptive characteristics of the individuals in each group for age and BMI

The group with TFPFOA had a higher CRP level than the other groups although this was not statistically significant on all sampling occasions, figure 9. Significant changes within the groups were seen in the group with OA in the PF compartment where the serum levels increased significantly from the baseline in 1990 to the follow-up 12 years later in 2002 (p=0.005). Serum levels of hsCRP showed significant relations to BMI (p≤0.025) at baseline and at the 12-year follow-up both in the group with PFOA and in the group without radiographically confirmed OA.

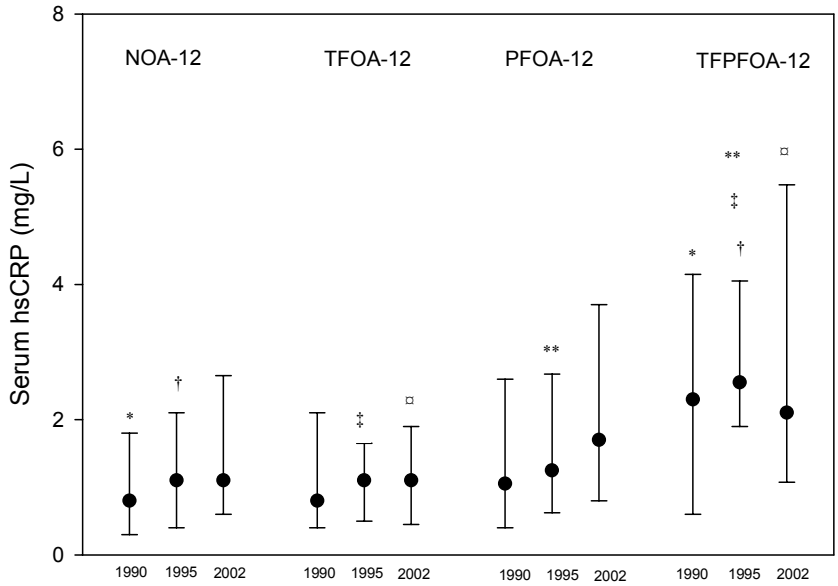

Figure 9

The median (25, 75 percentile) serum hsCRP values at baseline (1990) and at follow-up in 1995 and 2002. The signs *, †, ‡, **, ¤ denote the different comparisons between groups that showed significant differences for hsCRP levels (p<0.05).

4.3. Biological variations of serum COMP in arthritis patients (Papers III and IV)

4.3.1. Diurnal variation of COMP in serum (Paper III)

4.3.1.1. Participants

The participants in this study consisted of two groups, one with symptomatic OA and the other with RA. The OA group consisted of 10 individuals (5 men/5 women) recruited from the Spenshult cohort with symptomatic OA. Seven of them had also radiographically confirmed OA (K&L grade 2 or more). They all fulfilled the clinical criteria for OA (Altman et al. 1987). The median age was 62.4 years (range 60-64). The RA group consisted of 14 consecutively enrolled inpatients at Spenshult Hospital (2 men/12 women) with erosive RA, median age 66.0 years (range 45-82) and median disease duration of 20 years (range 1-30). All RA patients fulfilled the ACR 1987 criteria for RA (Arnett et al. 1988; Bremander et al. 2003). Eight patients were treated with disease modifying anti-rheumatic drugs (DMARD´s), two were given only low-dose prednisolone and four were treated with both DMARD´s and low-dose prednisolone.

4.3.1.2. Method

Venous blood samples were obtained on seven occasions during a 24-hour period, i.e. every 4 hours. Serum COMP was analysed with a sandwich ELISA (AnaMar Medical, Lund). Two different statistical analyses were performed. The first was based on Friedmann´s test and the Wilcoxon matched pair test, and the second was based on non-linear fixed effects modelling, as implemented in the computer program NONMEM with the first-order conditional estimation method with interaction. The purpose of the second analysis was to further explore the diurnal variation and to compute the fractional turnover rate of COMP.

4.3.1.3. Results

No significant changes were seen in serum COMP levels during day-time. The principal findings are shown in figure 10. At night during bed rest, a significant decrease was observed reaching the lowest level between 4 a.m. and 5 a.m. Neither was any significant difference seen in the diurnal variation between patients with and without radiographically confirmed knee OA nor between RA patients treated with low-dose prednisolone and those without such treatment. Patients with RA had significantly lower COMP levels than individuals with OA ($p<0.05$). The estimated half life of COMP was 7.4 hours (SD 0.1).

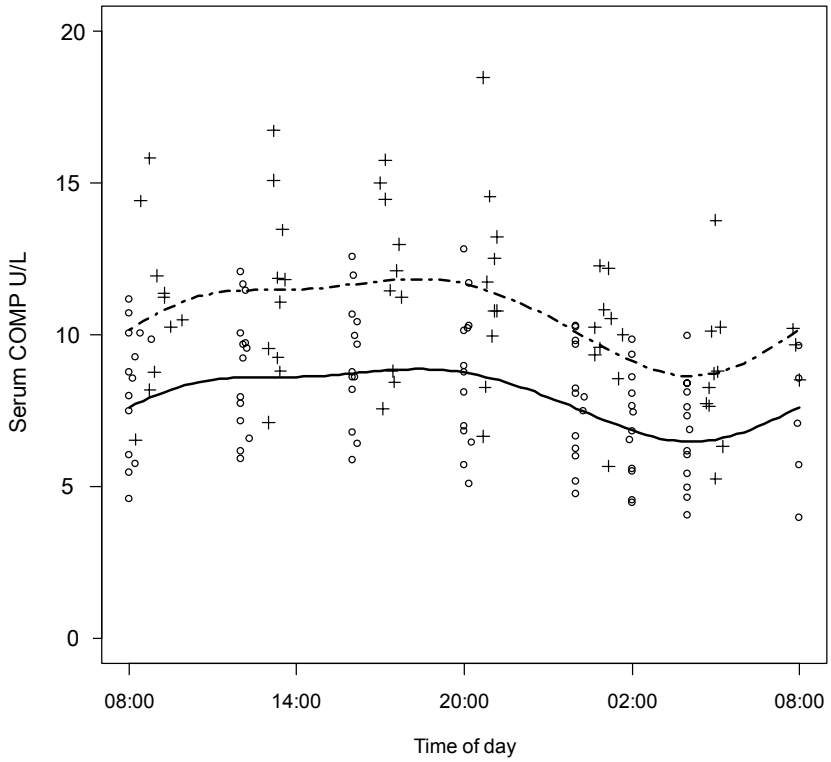

Figure 10

Calculated 24-h COMP level curves typical for patients with OA (dashed line) and RA (solid line). Also displayed are the observed COMP levels for patients with OA (crosses) and RA (open circles).

4.3.2. The influence of physical exercise on serum COMP levels (Paper IV)

4.3.2.1. Participant in study 1

The participants in this study were 58 individuals included in a randomized controlled trial on the effects of physical exercise in knee OA (Thorstensson et al. 2005). The patients were recruited from the Department of Radiology at the Halmstad County Hospital in Sweden and all had been referred for radiographic knee joint examination due to knee pain and all included had radiographic knee OA, K&L grade 2 or more.. The patients were randomised to an exercise group (n=29) or to a control group (n=29). There were no significant differences between the groups regarding age or BMI.

4.3.2.2. Participants in study 2

Seven patients from study 1 were recruited to study 2 by asking every fourth patient from the exercise-group if they were able to participate.

4.3.2.3. Method

In study 1, the patients in the exercise group followed a predefined exercise programme including supervised, high intensity, weight-bearing exercises aimed at increasing postural control, endurance and strength in the lower extremities. Blood samples were collected at predetermined occasions before intervention (-3 weeks), at the start of intervention (0 weeks), at the end of intervention (6 weeks) and after 24 weeks. On each occasion two blood samples were obtained, an hour apart. At weeks -3 and 24 both groups were resting between blood sampling, at weeks 0 and 6 the control group was resting and the exercise group was exercising for a one-hour, supervised high intensity session, figure 11.

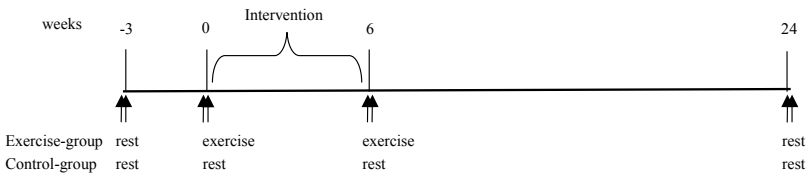

Figure 11

Blood-sampling procedures during the study from study baseline at -3 weeks to the end of the study at 24 weeks. Blood samples were obtained before and after 60 minutes exercise/rest at each occasion. The arrows indicates blood sampling occasions. Two blood samples at each occasion, with an hour apart.

In study 2, the patients exercised for a one-hour, supervised high intensity session, as performed in study 1. Blood samples were collected before, immediately after, 30 and 60 minutes after and then with 60 minutes interval for another five hours. Between the blood sampling procedures the patients were waiting in the hospital waiting room.

4.3.2.4. Results study 1

There were no significant differences between the serum levels of COMP in the two groups in study 1 three weeks before the start of the intervention at week 0. A slight decrease was seen in serum COMP levels after one hour of rest ($p<0.001$), figure 12. No significant differences were seen in the serum levels of

COMP between the groups before exercise/rest at week 0 and 6. After exercise the serum level of COMP increased (median increase 1.3 U/L) while the level decreased in the group resting (median decrease 0.6 U/L), figure 13**.** The serum levels of COMP decreased after rest in both groups at week 24 in a similar manner to those in week -3. There were no significant differences between the levels of serum COMP between the start and end of the study. Neither did the levels differ between the groups, figure 14.

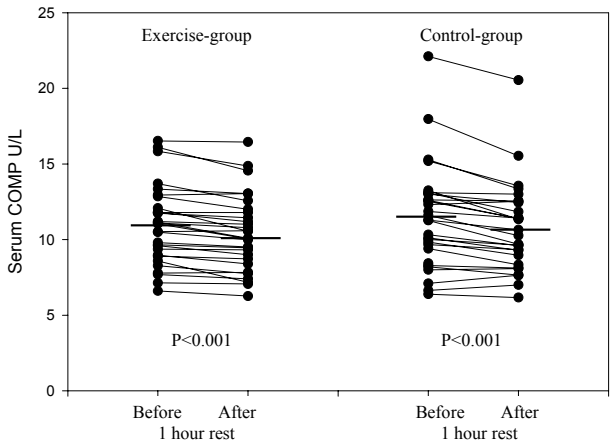

Figure 12

Serum COMP at week -3, before intervention, in study 1. Each line represents an individual patient. Horizontal bars show median values for each group.

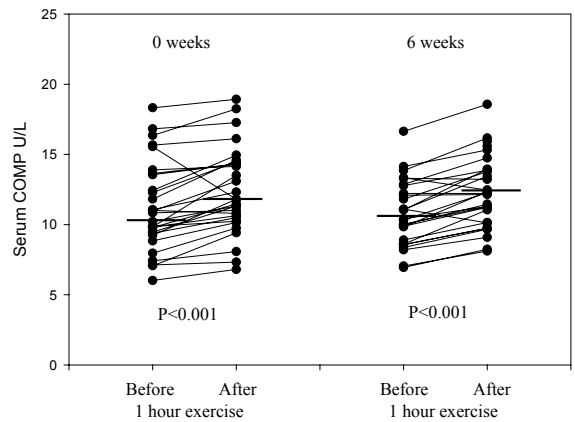

Figure 13

Serum COMP at weeks 0 and 6, before and afterintervention, in the exercise group in study 1. Each line represents an individual patient. Horizontal bars show median values for each sampling occasion.

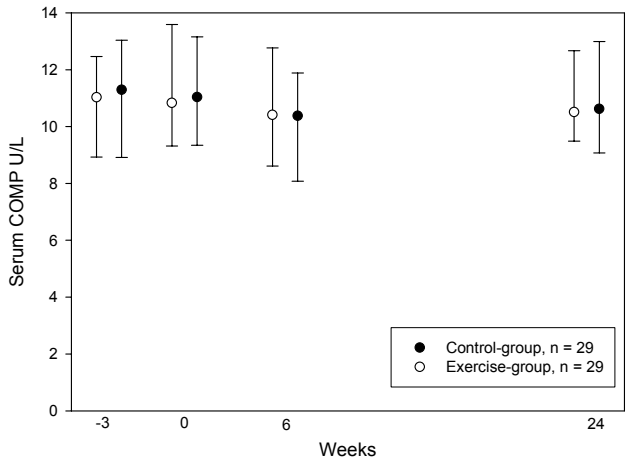

Figure 14

Serum COMP over 27 weeks in both groups (-3 to 24 weeks). There were no significant differences in serum COMP levels between the two groups before exercise or resting sessions at any occasion, including the 25 and 75 percentile.

4.3.2.5. Result study 2

In study 2, the serum COMP levels increased immediately after the exercise session and then decreased. After 30 minutes the levels did not differ significantly from baseline values, figure 15.

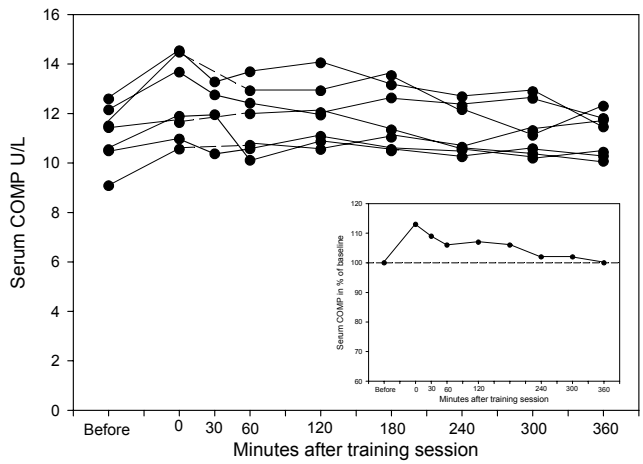

Figure 15

Serum COMP after standardised exercise in seven patients with knee OA, study 2. Each line represents an individual patient. The inserted figure shows median serum COMP concentration in percent of baseline after a one-hour supervised exercise session.

5. Discussion

5.1. Papers I and II

Monitoring diseases over time is time-consuming, expensive, but nevertheless very important. The information obtained from longitudinal studies such as those presented here, is often very useful both in research and to practitioners. Different types of longitudinal studies can be identified in clinical practice: studies of an established disease, often with the aim of studying different treatment strategies; or studies of the natural course of the disease i.e. the onset and development of the disease. When creating patient cohorts for longitudinal studies in established disease, for example in RA, validated criteria such as the ACR-criteria, are used to define the cohort. In studies of the natural course of diseases it is important to begin the study as early in the course of disease as possible. The most common method of classifying OA in cohort studies is radiography, often using different classification methods, which makes it difficult to compare the studies. However, using radiographic criteria for diagnosis of, for example OA, will not identify individuals at risk or in early stage of the disease. One solution is to use clinical criteria such as the Altman criteria (Altman et al. 1987) when establishing patient cohorts in early disease. This criteria has a high sensitivity but is confined by low specificity for radiographic OA, which can influence the interpretation in studies of this patient group.

The results obtained from the Spenshult cohort, presented in Paper I, showed that using clinical criteria identified 77 % of the individuals with OA in the TF compartment, (incidence or progression over 12 year), while radiographic criteria identified 52 % in spite of the fact that the cut-off for radiographic OA in that study was lowered to K&L grade 1. Radiographic imaging, may thus not be the best structural method when identifying OA, and other more sophisticated structural methods such as MRI, should perhaps be used for detecting structural changes in joint tissue. However, MRI is too expensive to use for screening patients in large cohort studies. Biomarkers are also conceivable, as they are inexpensive, non- invasive and easy to use. However, these methods have not yet been fully evaluated in screening procedures.

When monitoring OA over time it is important to include as many aspects of the disease as possible. This would be possible using clinical and structural methods together with biomarkers. The advantage of applying both structural methods and biomarkers is that they measure different aspects of the disease process. Biomarkers measure processes at the time point when the sample is obtained, while structural methods show the results of processes that have already taken place.

Different biomarkers provide a measure of different processes in the tissue, thus reflecting different stages of the disease process**.** The best solution would be to use a panel of biomarkers to reflect the progression of the disease (Saxne et al. 2006)**.** Measuring biomarkers over time in order to monitor the course of the disease is also a part of the validation process for new biomarkers. A common strategy when validating biomarkers is to compare the measured levels to different radiography classifications taken at the same occasion. It is important to be aware of the differences described above. Radiography is perhaps not the most sophisticated structural method available, but it has been used as a "golden standard" for many years and allows the results from "new" studies to be compared with studies performed in the past, but new structural methods should also be considered.

Not only methods used, but also the individuals participating in long-term follow-ups affect the result. A patient cohort is by definition a group of individuals from a given population experiencing an event in a particular time span. A well-defined patient cohort, which is followed over time, could be a useful tool when evaluating procedures for monitoring diseases, for studies regarding the disease course, and for the clinical evaluation of treatment. When performing this type of study, which often lasts several years, it is important to minimize the drop-out rate as much as possible. There is, of course, a natural drop-out in long-term studies, especially in cohorts of elderly people, but it is important to encourage the participants to come to the follow-up examination. Most patients agree to participate as long as the methods used are proven not to be harmful and not cause pain or discomfort. However, it is important to point out that continued participation benefits the whole patient-group and not only the individual. Regular information to the participants on the results of the study encourages participation. A low drop-out frequency strengthens the results in studies within the cohort.

The participation in the follow-ups of the Spenshult cohort has been good. 70 % of the individuals included in 1990 have been followed over 12 years as described in figure 5.

In papers I and II we have monitored middle-aged individuals with chronic knee pain during 12 years with different methods. These different methods mirror different aspects of the disease process.

We have chosen two different radiographic classifications in these two papers. In paper I we have used the K&L classification because in 1990 when the Spenshult cohort was initiated only radiographs of the TF compartment were taken, which

were the common way to diagnose radiographic knee OA at the time, not including the PF joint. At the follow-ups in 1995 and 2002 radiographs of both the PF compartment were included.

In paper II the OA classifications were done with the JSW classification because we were interested to see if there were differences in the grade of systemic inflammation between individuals developing OA in the different compartments of the knee and the Ahlbäck and K&L classifications are not described for radiographs of the PF compartment. Studies of other biomarkers had indicated differences in the biomarker profile between individuals with PFOA and individuals with TFOA (Kumm et al. 2006; Sharif et al. 2006).

In paper I we found a rather high incidence of radiographically verified knee OA in this group of individuals when using the K&L classification and a cut-off of 1 grade or more. Even if we used a lower cut-off than other studies of knee OA incidence, we also found that almost all (97 %) of the individuals who had radiographically verified OA at inclusion progressed one grade or more over 12 years. The crucial factor for individuals with chronic knee pain developing knee OA seems to be time. In this context individuals with knee pain should be considered as individuals developing knee OA and they should be treated according to the recommendations for knee OA with information, weight reduction and physical exercise (Zhang et al. 2008).

In paper II we have looked into another aspect of the disease course measuring the grade of systemic inflammation in individuals developing knee OA. In this study we found increased levels of CRP in serum in individuals developing combined knee OA and increasing levels in individuals developing PFOA which indicates increased systemic inflammatory activity in the disease progress in this individuals. The individuals with combined OA were slightly older and had a higher BMI than the individuals without OA at the twelve year follow-up ($p \leq 0.05$). Increased BMI has been shown to associate to knee OA in all compartments, but with a stronger association to PFOA and combined OA than to TFOA (McAlindon et al. 1996). A study by Rawson and coworkers showed an association between BMI and CRP with a significantly higher CRP in obese people (BMI > 25 kg/m^2). In our study the median BMI at the twelve year follow-up is more than 25 kg/m^2 in all groups. There was a correlation between BMI and the CRP level at baseline and at twelve year follow-up in the group without OA and in the group with OA in the patellofemoral compartment. There was no significant correlation between BMI and CRP in the group with combined OA or in the group with TFOA in this study, which might be explained by the small number of patients in this group.

There are other studies showing increased levels of CRP in patients developing knee OA (Spector et al. 1997; Sharif et al. 2000; Sturmer et al. 2004). A recent study by Engström and coworkers found no associations between the incidence of knee or hip OA and plasma CRP levels. In this study individuals with TFOA and PFOA or combined OA were not separated. This could perhaps explain why the results from this study differ from the results in our study (Engstrom et al. 2008).

5.2 Paper III and IV

Knowledge of factors that may influence the results when measuring biomarkers is necessary for correct interpretation of the results and for standardization of sampling procedures. Preanalytical factors such as age, gender and smoking habits need not be standardized in within-patient measurements. If these factors influence the results they must be taken into account in between-patients comparisons. Biological variations such as diurnal and seasonal variations, or variations due to physical exercise must be considered both in within-patient and between-patients measurements.

The preanalytical factors affecting serum COMP measurements are rather well documented. Age is one factor influencing the value of serum COMP, increasing in those aged over 65. It is also well known that women have lower serum COMP levels than men (Clark et al. 1999). Some studies have also demonstrated differences between ethnic groups, e.g. Jordan and coworkers (Jordan et al. 2003a). Results from 124 individuals in the Spenshult cohort also indicated differences in serum levels of COMP between smokers and non-smokers (unpublished results). The serum COMP was found to be lower in smoking individuals with symptomatic knee OA than in non-smoking individuals with symptomatic knee OA, figure 16. The reason why smoking habits influence the COMP levels in serum is not known.

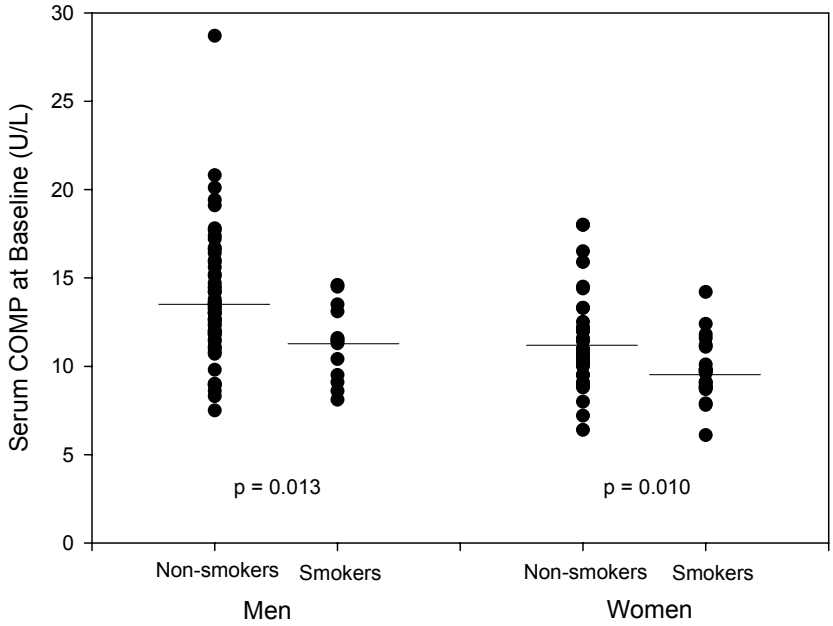

Figure 16

Serum COMP levels at baseline in smoking or non-smoking individuals with chronic knee pain in the Spenshult cohort.

Serum COMP has a rather short half-life of about 7 hours, and a diurnal variation with a significant decrease in early morning, but is stable during the day-time. Many compounds in body fluids vary throughout the day for example serum concentrations of ALAT and iron have been found to vary by 50 % between 8 a.m. and 2 p.m. Serum cortisol is another example of an analyte showing a diurnal variation, the highest concentration being seen between 6 a.m. and 8 a.m. Factors responsible for these variations could be body position, activity, food ingestion, stress, daylight/darkness and sleep/wakefulness.

Other compounds show a seasonal variation. Seasonal variation of body constituents is known to be small compared with those related to body position or improper use of the tourniquet when collecting samples. Serum concentrations of urea and urate are, for example, higher in summer than in winter (Letellier et al. 1982). We have in a study (unpublished results) of 38 participants (12 men, 17 women, median age 57.0 years range 38.6-67.3, median BMI 27.6, range 22.1-48.2, with radiographically confirmed OA (K/L grade 2 or more)) obtained blood samples each month for six months and then one year after inclusion. A

significant decrease was seen in serum COMP levels from November to March ($p<0.01$), suggesting a possible seasonal variation in serum COMP levels. However this remains to be verified in other studies.

The knowledge of the influencing factors for levels of other cartilage biomarkers is rather limited. In a recent study Kong and coworkers found a diurnal variation in cartilage marker such as COMP, keratan sulfate (KS-5D4), aggrecan neoepitope (CS846), collagen II collagenase cleavage products (C2C, C1, 2C) and type II procollagen carboxy-propeptide (CPII) in serum, as well as C-terminal telopeptides of CII (CTX-II) and C2C in urine, and they recommended samples to be obtained in late midday (Kong et al. 2006). The results from this study concerning COMP is in line with the results from our study in paper III

The effect of physical exercise on cartilage biomarkers such as aggrecan, hyaluronan and keratan sulfate was studied by Roos and co-workers, who found an increasing trend after exercise (Roos et al. 1995b). The effects of physical exercise on serum COMP have also been studied and levels found to increase after one hour of physical exercise, with a normalization time of 30 minutes, in paper IV. Mündermann and coworkers had shown that even moderate walking can significantly influence the serum COMP levels (Mundermann et al. 2005). Levels of other serum components e.g. ALAT, ASAT and creatinine, have also been found to increase after exercise (Young et al. 2006a).

Factors influencing the analytical procedures
Our data can be regarded as valid in these aspects as we tried to standardize and record all procedures: sampling standardization, technical handling, storage and appropriate and valid analytical procedures.

In paper III the participants were resting in sitting position for at least 15 minutes before the sampling and the samples were obtained with a minimum of tourniquet. The samples were allowed to clot for 60 minutes at room temperature and then centrifuged at 2000 g for 10 minutes at +4° C. The samples were stored at -80ºC until analyzis. The samples were thawed in room temperature and then mixed thoroughly before analyzed. The ELISA method used is a sandwich-ELISA by AnaMar Medical. This method is based on monoclonal antibodies and the detection limit is 0.1 U/l, and the intra-assay and inter-assay coefficient of variation is 5%. The samples were analysed in duplicate and the mean value were used in the calculations. All analyses were carried out without the knowledge of patient characteristics and all samples from each patient were tested on the same plate. Standard curves and two controls were included in each plate.

In paper IV the participants were exercising or resting before sampling. The samples obtained after exercise were taken as soon as possible after the exercise session in the exercise room in sitting position with a minimum of tourniquet. The samples obtained after rest were taken in sitting position with a minimum of tourniquet. The analyzis were done with the same method as in paper III.

Factors influencing the interpretation
In paper III we discuss that the most reasonable interpretation of these data are that we can use them for calculating both the half-life turnover and the diurnal variation in two patient groups with chronic arthritis and that the levels of COMP is stable during day time.

In paper IV we found data suggesting that serum COMP levels increase after 1 hour of physical exercise with normalization about 30 minutes after the session and decrease after 1 hour rest. Taken into account possible other factors we decide to regard the data as reflecting the normal turnover changes in rest and exercise.

6. General discussion and conclusions

The purpose of this thesis was to evaluate methods for monitoring arthritis in research and clinical practice. Monitoring arthritis is a crucial part of clinical care of patients with musculoskeletal disorders in relation to prognosis, choice of therapy strategies and outcome evaluation. The choice of methods when monitoring arthritis is important and to be aware of the influencing factors of each method is crucial to be able to identify significant changes in test results in patients. The methods used should be standardised and evaluated. The methods chosen for monitoring arthritis should be able to mirror different aspects of the disease over time and also detect effects due to treatment strategies. It is important to be aware of the differences when comparing methods from the different groups mentioned in this thesis i.e. structural, clinical and biomarkers. Pain often appears in early stage of the disease whereas, structural methods reflect the result of the disease process and biomarkers measures the disease processes at the time when the sample is obtained. Figure 17 is a schematic illustration of when in the disease process the methods could be used to detect symptoms/changes in the joints due to the disease.

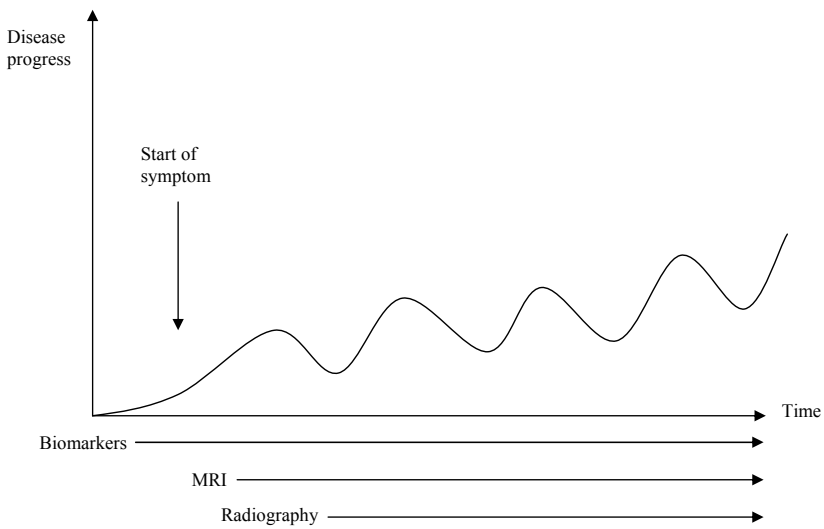

Figure 17

Schematic illustration of when in the disease processes the methods could be used to detect symptoms/changes in the joints due to the disease.

The conlusions in this thesis were:

- A majority of the individuals with chronic knee pain developed knee OA over 12 years. We conclude that knee pain is often the first sign of knee OA

- Elevated levels of hsCRP in serum, particularly in individuals developing combined PF and TFOA, suggest that low-grade inflammation is a feature of early knee OA.

- During normal daytime activities, serum COMP levels are constant. The decrease during the night indicates a rapid elimination of COMP once it has reached the circulation. The stable COMP levels during the day suggest that it is not necessary to further standardise the time of serum sampling in clinical practice.

- Serum COMP has a short half-life of about 7 hours.

- Serum COMP levels increased during exercise in individuals with knee OA, whereas levels decreased during rest. The increased serum COMP levels were normalized 30 minutes after an exercise session. No increase was seen after a six-week exercise program indicating that any effect of individualized supervised exercise on cartilage turnover is transient

- Samples for measuring serum COMP should be obtained at day-time after resting at least 30 minutes in a sitting position.

Summary in English

Arthritides are musculoskeletal diseases, which predominantly affect the joints e.g. osteoarthritis (OA) and rheumatoid arthritis (RA). Modern treatment strategies include early detection and careful monitoring of the disease course. Close monitoring is also the basis for individualized treatment. Monitoring should be done using methods reflecting different aspects of the disease to get a broad perspective of the disease course. It is of great importance that the methods used for monitoring arthritis are standardized.

In paper I, we studied the natural course of knee OA in a population based cohort of middle-aged individuals with chronic knee pain at inclusion, over a 12 year period with a structural method, radiography, and a clinical method. The main finding was that individuals with chronic knee pain were at high risk for developing radiographic knee OA. In paper II, we studied the presence of low-grade systemic inflammation in individuals with chronic knee pain with and without radiographically verified knee OA by measuring high-sensitivity (hs) CRP . We found that low-grade inflammation is a feature of knee OA especially in individuals developing OA in both tibiofemoral (TF) and patellofemoral (PF) compartments. In paper III the diurnal variation of serum COMP was studied in individuals with clinically verified OA and in patients with RA. We found that serum COMP has a half-life of about 7 hours and it has a significant diurnal variation but serum levels were stable during day-time. The objective of study IV was to monitor serum levels of COMP during a randomised controlled trial of physical exercise vs. standardised rest in individuals with symptomatic and radiographic knee OA. Serum COMP levels increased after one hour of physical exercise and decreased after one hour of rest. No increase was seen after a six-week exercise program. In a supplementary study in paper IV we monitored serum COMP with repeated measurements after the exercise session. Serum COMP levels increased immediately after exercise but 30 minutes after the exercise session the levels did not differ significantly from baseline levels. From these studies I conclude that knee pain is often the first sign of knee OA. Elevated levels of hsCRP in serum, particularly in individuals developing combined PF and TFOA, suggest that low-grade inflammation is a feature of early knee OA. COMP has a short half-life in serum and a significant diurnal variation. Serum COMP levels increase after physical exercise but normalize after about 30 minutes of rest. Samples for measuring serum COMP should be obtained at day-time after resting for at least 30 minutes in a sitting position.

Summary in Swedish – Populärvetenskaplig sammanfattning på svenska

Sjukdomar i leder och muskler är mycket vanliga och orsakar invaliditet och försämrad hälsorelaterad livskvalité för personer i hela världen. Artros och reumatoid artrit (ledgångsreumatism) är två av de vanligaste ledsjukdomarna. Den moderna behandlingen och omhändertagandet av patienter med dessa sjukdomar är beroende av att sjukdomen upptäcks och diagnostiseras i ett tidigt skede och att man kan följa sjukdomsförloppet över tid. För att få ett brett perspektiv på sjukdomsförloppet bör man använda sig av olika metoder som speglar olika aspekter i olika skeden av sjukdomen. Det är viktigt att de metoder som används är standardiserade och att man känner till faktorer som påverkar mätningen för att kunna värdera resultatet och kunna identifiera relevanta förändringar.

Genom att följa grupper av patienter kan man få värdefull information och kunskap om till exempel sjukdomars naturalförlopp samt hur olika behandlingsstrategier kan påverka sjukdomsförloppet.

I den första studien följde vi 143 medelålders individer med kronisk knäsmärta under tolv år för att se om de utvecklade artros mätt dels med kliniska metoder, dels med röntgen. Vår hypotes var att kronisk knäsmärta är ett första tecken på artros. Deltagarna röntgades vid tre tillfällen under dessa tolv år, vid start av sudien samt efter fem och tolv år. Vid studiestarten gjordes också klinisk undersökning av läkare och sjukgymnast. Femtio-tre procent (76/143) av deltagarna i studien hade inte artros enligt röntgen vid start, 49 av dessa hade dock artros vid den kliniska undersökningen.
Vid tolvårsuppföljningen hade 65 av de 76 (86 %) utvecklat artros enligt röntgen. Av de 67 som redan vid starten hade artros enligt röntgen hade 97 % försämrats på röntgenbilderna vid tolvårsuppföljningen. Vår slutsats i denna studie är att knäsmärta ofta är första tecknet på knäartros

I studie II undersökte vi om låggradig inflammation kan vara ett inslag i sjukdomsbilden vid utveckling av knäartros. Vi följde 124 individer med kronisk knäsmärta med och utan röntgenverifierad artros under en tolvårsperiod. Blodprov och röntgen togs vid tre tillfällen, vid studiestart samt efter fem och tolv år. En inflammationmarkör, C-reaktivt protein (CRP), analyserades med en speciellt känslig metod (hsCRP) och individerna grupperades enligt röntgen vid tolvårsuppföljningen. Studien visade att personer med artros i både tibiofemoralleden (lårbensleden) och patellofemoralleden (knäskålsleden) har en högre CRP koncentration i blodet jämfört med dem som inte har röntgenverifierad artros

samt jämfört med dem som har artros enbart i tibiofemoral- eller patellofemoralleden. Vi konkluderar således att det finns ett inslag av låggradig inflammation vid utveckling av artros hos vissa personer med kronisk knäsmärta.

Syftet med studie III var att följa nivåerna av serum COMP, en broskmarkör, under 24 timmar för att fastställa dygnsvariationen samt beräkna halveringstiden av COMP i blod hos patienter med artros eller reumatoid artrit. Blodprover togs var 4:e timme under ett dygn på 10 personer med klinisk knäartros samt på 14 personer med reumatoid artrit. I denna studie fann vi att COMP är stabilt dagtid med en lägsta nivå under de tidiga morgontimmarna. Halveringstiden för COMP beräknades till ungefär 7 timmar.

I studie IV studerade vi effekten av fysisk träning på serum COMP-nivåerna i en randomiserad, kontrollerad studie av 58 personer med kliniska och röntgenmässiga tecken till artros. Patienterna lottades till en kontrollgrupp eller till en träningsgrupp. Träningsgruppen tränade intensivt under 6 veckor medan kontrollgruppen levde som vanligt. Nivåerna av serum COMP mättes före och efter träningsperioden samt 24 veckor efter studiens start. Vid varje tillfälle togs två blodprover med en timmes mellanrum. Under timmen mellan provtagningarna tränade träningsgruppen medan kontrollgruppen vilade. Vi såg att COMP sjönk under vila och steg vid träning. COMP förändrades dock inte långsiktigt dvs från studiens start till uppföljningen efter 24 veckor.
Vi gjorde även en kompletterande studie i vilken ingick sju personer där vi studerade serum COMP före och efter en timmes träning och sedan följde vi COMP-nivåerna under fem timmar. Vi fann då att COMP steg efter en timmes träning, men var tillbaka på ursprungsnivån efter 30 minuter.

Baserat på resultaten från studierna i denna avhandling drar jag slutsatserna att:

- Långvarig knäsmärta är ofta första tecknet på knäartros
- Låggradig inflammation kan vara en del av sjukdomsbilden hos patienter med knäartros, speciellt hos personer som utvecklar artros både i ”knäskålsleden” och ”lårbensleden”.
- Serum COMP nivån är stabil under dagtid hos personer med kliniskt verifierad artros samt hos personer med reumatoid artrit..
- Serum COMP har en kort halveringstid på ungefär 7 timmar.
- Serum COMP koncentrationen sjunker efter en timmes vila och stiger efter en timmes fysisk träning, men normaliseras efter 30 minuter.
- Blodprov för mätning av serum COMP bör tas dagtid efter att patienten vilat, sittande under 30 minuter.

Acknowledgements

Many people have contributed to the work with this thesis and I would like to express my sincere gratitude and special thanks to:

All patients participating in these studies and enduring all sampling procedures and staff concerned at Spenshult Hospital for help with the bloodsampling.

Ingemar Petersson my supervisor, for introducing me to the "world of science" with great knowledge and visions, always with a humorous glint in your eye. Eventhough the road to the goal sometimes has been winding.

Tore Saxne my co-advisor, for sharing your great knowledge in the field of cartilage biomarkers and for being supportive and patient guiding me through the difficulties and giving me the possibility to work with research within this field.

Dick Heinegård, co-author, for support and guidance especially in the beginning of this journey.

Carina Thorstenson, co-author, colleague and friend for support, good ideas, help, talks and laughs.

Mette Lindell, for skilful help at the laboratory and with the analyses.

Niclas Jonsson and *Kristin Karlsson*, co-authors, for skilful help with the calculations of the diurnal variation.

Bengt Månsson, co-author, for support and help at the laboratory

Torsten Boegård, for skilful reading of all the radiographs

Jan-Åke Nilsson, for helping me understand the "world of statistics".

Ewa Roos, co-author for good collaboration and for sharing your knowledge and for interesting discussions in the Ph.D. student network

Hanna Jönsson, co-author for good collaboration

Pia Andersson, colleague and friend for all help and support during these years and interesting talks and discussions

Stefan Bergman, head of the research and development centre at Spenshult Hospital for rheumatic diseases, for your support and good advice.

Ann Bremander, for wise words and help in big and small.

Siv Norén, colleague, roommate and friend, for help with the blood sampling and for support and nice small talk.

Ulrika, Ingrid, Susann, Emma, Maria S, Annika and Anna, for support, talks and laughter during breaks and lunches.

To my lovely family, my parents Sven-Erik and Britta and my sisters and brothers-in-laws *Kattis, Henke, Lisa, Patrik* and *Maggan* for always being there with

a helping hand when ever I needed and for support in prosperity and adversity. *Håkan* for love, friendship, support and good advice and sometimes telling me to think twice before acting (I need that). *Alexander* my son, who has put up with an absent mother for many years. I could not have done this work without you all.

These studies were supported by grants from King Gustaf V 80-year Found, The Swedish
Rheumatism Association, Spenshult's Research Foundations, Golje and Lundström foundation, Capio research foundation, The Swedish Medical Research Council, NIAMS, National Institute of Health Grant U01-AR050926 and the Faculty of Medicine, Lund University.

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