Clinical study on osteoporosis in ankylosing spondylitis

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ABSTRACT

Ankylosing spondylitis (AS) is a disease characterized by chronic inflammation and osteoproliferation in the spine, leading to bony fusion (ankylosis) of the sacroiliacal joints, the growth of bony spurs (syndesmophytes) between the vertebras and impairment of back-mobility. Paradoxically AS patients also have an increased risk of osteoporosis and vertebral fractures.

In this cross-sectional study on 210 included AS patients (New York criteria) from West Sweden we found that osteoporosis and vertebral fractures were common but often not diagnosed or treated. Osteoporosis (WHO definition) was found in 21 % and osteopenia in 44 % of patients 50 years or older and bone mineral density (BMD) below expected range for age was found in 5% of patients younger than 50 years. Totally 42 vertebral fractures were diagnosed in 24 patients (12%). Osteoporosis was associated with old age, long disease duration, advanced chronic AS related changes in the spine, impairment of back-mobility, history of coxitis, glucocorticoid use, elevated inflammatory parameters, low BMI and menopause. Vertebral fractures were associated with old age, long disease duration, advanced chronic AS related changes in the spine, impairment of back-mobility, poor self-estimated general health, smoking, menopause and low BMD.

The osteoproliferation in AS can cause artifactual increase of lumbar BMD when measured in anteroposterior (AP) projection with dual-energy x-ray absorptiometry (DXA). Lumbar BMD can also be measured in the vertebral bodies using lateral projection. Comparing lateral with AP DXA we found that lateral lumbar DXA was more sensitive in detecting low BMD, less affected by the osteoproliferation in AS and more closely associated with vertebral

fractures. Combining AP and lateral lumbar DXA also allows for the estimation of volumetric BMD (vBMD).

There is a lack of biomarkers for osteoproliferation and osteoporosis in AS. We analysed serum levels of the following biomarkers for bone metabolism in relation to disease activity, back mobility, osteoproliferation and BMD: Wingless proteins (Wnt-3a, Wnt-5a), Dickkopf-1 (Dkk-1), sclerostin, soluble receptor activator for nuclear factor-κB ligand (sRANKL) and osteoprotegerin (OPG). We found that the AS patients in comparison with healthy controls had significantly higher serum levels of Wnt-3a, but lower serum levels of sclerostin and sRANKL. Elevated serum levels of Wnt-3a were associated with osteoproliferation and impairment of back-mobility, independent of age, suggesting that Wnt-3a could be a marker for the osteoproliferative process. High CRP was associated with lower levels of the Wnt inhibitors Dkk-1 and sclerostin. BMD of femoral neck was negatively correlated with Wnt3a and OPG and positively correlated with sRANKL in the univariate analyses, but positively associated with sclerostin after adjusting for age in multiple regression. Osteoproliferation and impairment of back mobility and function were in addition associated with smoking.

To study peripheral bone microarchitecture in relation to osteoproliferation, fractures and vBMD of the spine 69 male AS patients were randomized to undergo assessment with High Resolution peripheral Quantitative Computed Tomography (HRpQCT) of the ultra-distal radius and tibia and QCT of the lumbar spine. We found strong correlations between trabecular vBMD in lumbar spine and radius and tibia, indicating coupling of trabecular bone loss in axial and peripheral skeleton. Low lumbar vBMD, vertebral fractures and osteoproliferation were in addition associated with deterioration of the bone microarchitecture of the peripheral skeleton. In lumbar spine decreasing trabecular vBMD was associated with increasing cortical vBMD, suggesting that cortical bone is appositioned as part of the osteoproliferative process meanwhile trabecular bone is lost in the vertebral bodies.

AS is closely related to inflammatory bowel disease (IBD) and subclinical intestinal inflammation has been detected in many AS patients. We measured fecal calprotectin, a marker for neutrophil inflammation, to indirectly study the prevalence of gut inflammation in AS. We found elevated levels of fecal calprotectin in 68% of the AS patients, without association with gastrointestinal symptoms. Fecal calprotectin was higher in users of non-steroidal anti-inflammatory drugs (NSAIDs) in a dose dependent manner, but lower in patients treated with methotrexate or TNFα-blockers. No association was found between fecal calprotectin and BMD.

List of publications

I.   Klingberg E, Lorentzon M, Mellström D, Geijer M, Göthlin J, Hilme
      E, Hedberg M, Carlsten H, Forsblad-d'Elia H: **Osteoporosis in
      ankylosing spondylitis – prevalence, risk factors and methods of
      assessment.** Arthritis Research & Therapy 2012, 14(3):R108

II.   Klingberg E, Geijer M, Göthlin J, Mellström D, Lorentzon M, Hilme
      E, Hedberg M, Carlsten H, Forsblad-d'Elia H: **Vertebral fractures in
      ankylosing spondylitis are associated with lower bone mineral
      density in both central and peripheral skeleton.** The Journal of
      Rheumatology 2012, **39**(10):1987-1995.

III. Klingberg E, Nurkkala M, Carlsten H, Forsblad-d'Elia H: **Biomarkers
      of bone metabolism in ankylosing spondylitis in relation to
      osteoproliferation and osteoporosis.** Submitted.

IV.   Klingberg E, Lorentzon M, Göthlin J, Mellström D, Geijer M, Ohlsson
      C, Atkinson EJ, Khosla S, Carlsten H, Forsblad-d'Elia H: **Bone
      microarchitecture in ankylosing spondylitis and the association
      with bone mineral density, fractures and syndesmophyte
      formation.** In manuscript.

V.   Klingberg E, Carlsten H, Hilme E, Hedberg M, Forsblad-d'Elia H:
      **Calprotectin in ankylosing spondylitis – frequently elevated in
      feces, but normal in serum.** Scandinavian Journal of
      Gastroenterology 2012, Apr 47(4): 435-44

CONTENTS

ABBREVIATIONS

aBMD	areal bone mineral density
ALT	alanine aminotransferase
AP	anteroposterior
AR	androgen receptor
AS	ankylosing spondylitis
ASAS	The Assessment of SpondyloArthritis international Society
ASDAS	Ankylosing Spondylitis Disease Activity Score
AV	atrio-ventricular
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BASFI	Bath Ankylosing Spondylitis Functional Index
BAS-G1	Bath Ankylosing Spondylitis patient Global score (last week)
BAS-G2	Bath Ankylosing Spondylitis patient Global score (last 6 months)
BMC	bone mineral content
BMD	bone mineral density
BMP	bone morphogenic protein
BMU	basic multicellular units
BV/TV	trabecular bone volume fraction
CRP	c-reactive protein
Cort	cortical
CTX	cross-linked c-telopeptides
Dkk-1	Dickkopf-1
DXA	dual energy x-ray absorptiometry
DCort	volumetric BMD of cortical bone
DMARD	disease modifying anti-rheumatic drug
DTrab	volumetric BMD of trabecular bone
CortCSA	cortical cross-sectional area
CortPm	cortical periosteal circumference
CortTh	cortical thickness
CtPo	cortical porosity
CtPoDiam	mean pore diameter
ELISA	enzyme-linked immunosorbent assay
ER	estrogen receptor
ER	endoplasmic reticulum
ESSG	European Spondylarthropathy Study Group
ESR	erythrocyte sedimentation rate
FRAX	fracture risk assessment
Hb	haemoglobin
Hh	hedgehog
HLA-B27	human leukocyte antigen-B27
HRpQCT	high-resolution peripheral quantitative computed tomography
IBD	inflammatory bowel disease

IBP	inflammatory back pain
IL	interleukin
ISCD	International Society for Clinical Densitometry
LLD	lower limit of detection
LTPAI	Leisure Time Physical Activity Instrument
LRP	low-density lipoprotein receptor-related protein
M-CSF	macrophage colony stimulating factor
MFI-20	The 20-item Multiple Fatigue Inventory
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
mSASSS	modified Stoke Ankylosing Spondylitis Spine Score
NSAID	non-steroidal anti-inflammatory drug
OMERACT	Outcome Measures in Rheumatology
OPG	osteoprotegerin
PAHWI	Physical Activity at Home and Work Instrument
PG	prostaglandin
PPI	proton pump inhibitor
QCT	quantitative computed tomography
RA	rheumatoid arthritis
ROI	region of interest
SF-36	The 36-item Short Form Health Survey
SHBG	sex hormone binding globulin
SEC	synovio-entheseal complex
SpA	spondarthritis
SOST	sclerosteosis
Sfrp	secreted frizzled related protein
sRANKL	soluble receptor activator of nuclear factor-κB ligand
TbN	trabecular number
TbSp	trabecular separation
TbTh	trabecular thickness
TGF-β	transforming growth factor β
TNF	tumour necrosis factor
Trab	trabecular
UPR	unfolding protein response
VAS	visual analogue scale
vBMD	volumetric bone mineral density
Wnt	wingless protein

INTRODUCTION

Ankylosing spondylitis

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease which predominantly affects the spinal column. The name derives from the Greek words anchylosis meaning "bent" or "crooked" and spondyl meaning "vertebra". The term ankylosis is used for bony fusions.

The earliest known humans with AS were found in the ancient Egypt. Radiographic examinations of the royal Egyptian mummies have revealed chronic AS related changes in at least three of the pharaohs, including Ramses II (1314-1224 B.C.), who is considered contemporary with Moses and the creator of many great temples in for example Luxor, Karnak and Abu Simbel.[1]
The disease was first described in 1693 by Bernard Connor (1666-1698), an Irish physician who wrote a report on a skeleton, probably found in a church grave yard, with a completely ankylosed spine and the iliac bones fused to the sacrum. [2] Vladimir Bechterew (1857-1927), was a Russian psychiatrist and neurologist, who in 1892 presented five cases with spinal stiffness and sensory and motoric neurologic deficiencies. Although the described symptoms were not typical for AS, the disease is often called Bechterew's disease. In 1927 Bechterew suddenly died, short after having diagnosed Josef Stalin as paranoiac. Allegedly Bechterew was murdered by poisoning. [3]

AS belongs to a group of inter-related diseases named the spondyloarhtritides. Other diseases belonging to the same entity are psoriatic arthritis, reactive arthritis, arthritis associated with inflammatory bowel disease (IBD), juvenil spondylarthritis (SpA) and undifferentiated SpA.[4]

Epidemiology

The prevalence of AS in different regions correlates with the presence of HLA-B27 positivity in the local population, with a peak prevalence of 6% among male individuals of the Canadian Haida Indians, where HLA-B27 positivity is found in about 50%.[5] In the north of Norway where HLA-B27 positivity is frequent (14-16%) two studies from 1985 and 2005 reported the AS prevalence to be 1.1-1.4% and 0.4% respectively.[6, 7] In mid-Europe a prevalence in the range of 0.3 to 0.5% has been found.[8] The disease is uncommon in African sub-Saharan populations.[9] A south Swedish study from 2011 based on the health care register found the prevalence of Spa and AS to be 0.45% and 0.14% respectively.[10] The diverging results from different studies regarding epidemiology can, besides presence of HLA-B27, be explained by differences in target populations, recruitment methods and diagnosis criteria.

AS is more common in males than in females with a ratio of about 2-3:1. Onset of disease occurs before the age of 45 in 95%, with mean age at onset of 26 years.[11]

Clinical presentation

Spine

The onset of disease is classically insidious and chronic back pain (lasting more than three months) is the first symptom in most of the cases. The pain typically comes at night, is worsen by rest, relieved by exercise and is accompanied by morning stiffness. This pattern of back pain is named inflammatory back pain (IBP), a concept defined in a subsequent chapter (4.4.1.). The pain is caused by inflammation, which often starts at the sacroiliacal joints and may translocate cranially to other parts of the spine during the course of the disease. The disease process eventually leads to pathologic new bone formation with obliteration of the sacroiliacal joints (ankylosis) and growth of bone spurs (syndesmophytes) between the vertebras, resulting in impairment of back-mobility and an often bent forward habitus. Spinal stiffness and successive impairment of function is caused both by osteoproliferation, inflammation and alterations in muscular tonicity. [12]

Peripheral joints

The peripheral arthritis in AS is typically a mono-arthritis or asymmetrical oligo-arthritis and is predominantly found in the lower limbs.[13] Arthritis affects approximately 25% of the AS population. Involvement of the hip joints occurs in 10%, can lead to joint destruction, need for prosthetic surgery and is a negative prognostic factor.[14]
Inflammations of entheses are also typical features of the disease including Achilles tendinitis, plantar fasciitis, heel pain, epicondylitis, trochanteritis and shoulder tendinitis.
The peripheral joint inflammation in AS is accompanied by an anabolic bone response (subchondral sclerosis) and the growth of bone spurs in joints (osteophytes) and in tendon insertions (enthesophytes).[15]

Gut

A close relationship exists between AS and intestinal inflammation. In IBD, sacroiliitis is seen in 10–20% of the patients and peripheral joint disease in 17–20%. Of patients with SpA, 6% eventually develop IBD.[16, 17]

Ileo-colonoscopic studies have demonstrated that 40-60% of AS patients have microscopic or macroscopic signs of inflammation in the gut.[18-21] The inflammation is often localized to the terminal ileum or colon and is in most cases asymptomatic. Histopathologically the inflammation can resemble Crohn's disease, with microgranulomas, giant cells and aphthoid ulceration, but the inflammation does not lead to strictures and has not been proven to exacerbate due to treatment with non-steroidal anti-inflammatory drugs (NSAIDs). Patients with subclinical gut inflammation are at increased risk of developing overt Crohn's disease.[22, 23] An increased prevalence of intestinal inflammation has also been found in first-degree relatives to patients with AS.[24]

Calprotectin belongs to the family of calcium binding calgranulins (or S-100 proteins) and consists of heterodimers of the two proteins S100A8 and S100A9. Calgranulins have anti-microbial and both pro-inflammatory and regulatory properties. Calprotectin is an abundant protein in the neutrophils constituting up to 40–60% of the cytosolic protein content. It is also found in gut epithelial cells, monocytes and macrophages.[25, 26]

The level of calprotectin in feces is proportional to the level of neutrophil inflammation in the gut. Fecal calprotectin is elevated in IBD, gut adenocarcinomas and in NSAID-users. Fecal calprotectin is clinically used to discriminate IBD from irritable bowel syndrome and correlates well with clinical, endoscopic and histologic measures of disease activity in IBD.[27, 28] Fecal calprotectin is resistant to bacterial degradation, homogenously distributed and stable in stool for up to one week in room temperature.[29]

We found elevation of fecal calprotectin in 68% of the AS patients. Elevated levels of fecal calprotectin were found in both users and non-users of NSAIDs, but were significantly higher in the NSAID-users. The levels of serum calprotectin were normal in most AS patients. (Paper V)[30]

Eye

Patients with AS have a 20–30% risk of developing an uveitis during the course of their disease.[31] Anterior uveitis is an inflammation that involves the iris or ciliary body and accounts for 90% of the cases of uveitis in AS. Typical symptoms of an anterior uveitis are recurrent unilateral eye-pain, redness and photophobia. If inadequately treated, it can result in synechia, hypopyon, cataract, glaucoma and reduction of sight. Posterior uveitis, which engages the choroidea and the retina, occurs in AS, but is unusual. Uveitis is highly associated with HLA-B27. Recurrent episodes occur more frequently in the male patients.[32]

Heart

Cardiac involvement, typically consisting of conduction abnormalities and/or lone aortic valve insufficiency (without stenosis) has been reported in 10-30 % of AS patients. Typical conduction abnormalities in AS are bundle-branch blocks, intra-ventricular blocks and atrio-ventricular (AV) blocks, which can require pacemaker treatment.[33] The aortic valve insufficiency can be caused either by aortic dilatation, fibrotic thickening and retraction of the cusps or inward rolling of the edges of the cusps.[34] Fibrosis and an obliterative (occlusive) endarteritis of small vessels have been found in the aortic root and the AV-node histologically.[35, 36]

In comparison with the general population patients with AS have an increased mortality, which predominantly is caused by cardiovascular comorbidity.[37-39] The prevalence of myocardial infarctions is increased about 2-3 fold compared with the general population.[40]

Diagnosis

The diagnosis of AS is in many cases delayed. The time span between onset of symptoms and time of diagnosis is in average 7-10 years.[6] In the current study the diagnosis of AS was in average delayed 9.5 years. One reason is that it takes several years until structural changes appear on radiographs. The magnetic resonance imaging (MRI) technique however allows the detection of inflammation in the sacroiliac joints earlier in the disease course, when no structural changes are visible on conventional radiographs.

Inflammatory back pain

The first definition of IBP was made by Calin et al in 1977.[41] The criteria were revised by the Assessment of SpondyloArthritis international Society (ASAS) in 2009.[42] The current ASAS Inflammatory Back Pain Criteria have a sensitivity of 80% and a specificity of 72%. Table 1.

Table 1: The ASAS Inflammatory Back Pain Criteria (2009)
   IBP is present if at least 4 of 5 criteria are fulfilled.

- Age at onset < 40 years
- Insidious onset
- Improvement by exercise
- No improvement with rest
- Pain at night (with improvement upon getting up)

AS

Diagnostic criteria for AS were specified at the Rome conference in 1963.[43] The criteria were later revised and the New York Clinical Criteria for Ankylosing Spondylitis were formulated in 1966. In 1984 another revision was made and the current Modified New York criteria for Ankylosing Spondylitis, which are used in the present study, were defined.[44] (Table 2)

Table 2:
The Modified New York Criteria for Ankylosing Spondylitis (1984)

1. Clinical criteria:
a. Low back pain and stiffness for more than 3 months which improves with exercise, but is not relieved by rest
b. Limitation of motion of the lumbar spine in both the sagittal and frontal planes
c. Limitation of chest expansion relative to normal values correlated for age and sex

2. Radiological criterion:
Sacroileitis grade ≥ 2 bilaterally or grade 3-4 unilaterally

Definite ankylosing spondylitis if the radiological criterion is associated with at least one clinical criterion.

Grading of Radiographic Sacroileitis (1966)

- Grade 0: normal
- Grade 1: suspicious changes
- Grade 2: minimal abnormality – small localized areas with erosion or sclerosis, without alteration in the joint width
- Grade 3: unequivocal abnormality – moderate or advanced sacroileitis with one or more of: erosions, evidence of sclerosis, widening, narrowing, or partial ankylosis
- Grade 4: severe abnormality- total ankylosis

Classification Criteria for Spondyloarthritis (Spa)

The first diagnostic criteria for SpA were elaborated in 1991 by the European Spondylarthropathy Study Group (ESSG).[13] Diagnostic criteria already existed for most disorders belonging to the SpA group, but the new SpA criteria were constructed in order to also encompass patients with undifferentiated SpA. The current ASAS Classification Criteria for Axial Spondyloarthritis were developed in 2009 in order to include patients with both radiographic and non-radiographic SpA and to enable diagnosing earlier in the course of the disease.[45, 46] The new criteria were shown to have a sensitivity of 83% and specificity of 84%. New items introduced were MRI, CRP-levels and HLA-B27.

Table 3:
The ASAS Classification Criteria for Axial Spondyloarthritis (SpA) (2009)

In patients with ≥3 months back pain and age at onset <45 years

> ➢ Sacroiliitis on imaging plus ≥1 Spa feature
>
> or
>
> ➢ HLA-B27 plus ≥2 other Spa features

SpA features
- inflammatory back pain
- arthritis
- enthesitis (heel)
- uveitis
- dactylitis
- psoriasis
- Crohn's/colitis
- good response to NSAIDs
- family history for SpA
- HLA-B27
- elevated CRP

Sacroiliitis on imaging
- active (acute) inflammation on MRI highly suggestive of sacroiliitis associated with SpA
- definite radiographic sacroiliitis according to modified New York criteria

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Table 4:
The ASAS Classification Criteria for Peripheral Spondyloarthritis (2011)

Arthritis or enthesitis or dactylitis

plus

≥ 1 SpA feature

- uveitis
- psoriasis
- Crohn's/colitis
- preceding infection
- HLA-B27
- sacroiliitis on imaging

or

≥ 2 other SpA features

- arthritis
- enthesitis
- dactylitis
- inflammatory back pain
- family history of SpA

Pathogenesis

HLA-B27

Approximately 95 to 98% of AS patients display human leukocyte antigen-B27 (HLA-B27), a striking association first reported in 1973.[47, 48] HLA-B27 belongs to the major histocompatibility complex (MHC) class I receptors, present on all nucleated cells and responsible for antigen presentation for CD8+ cytotoxic T-cells and NK-cells. There are at present 100 known subtypes of HLA-B27, of which several subtypes including B2702, B2704 and B2705 are strongly associated with AS. [49] HLA-B2705 is considered to be the original form of the molecule and is the most common type, present in 90% of HLA-B27 positive individuals in Northern Europe.[50]

HLA-B27 is the strongest known genetic factor for AS, contributing to 20-40% of the genetic susceptibility of the disease. HLA-B27 positive individuals have about 5% risk of developing AS, whereas HLA-B27 positive first degree relatives of AS patients have approximately 20-40% risk of developing the disease.[51] The concordance for AS in monozygotic twins is about 63% and 23% in dizygotic twins.

Figure 1: The HLA-B27 molecule
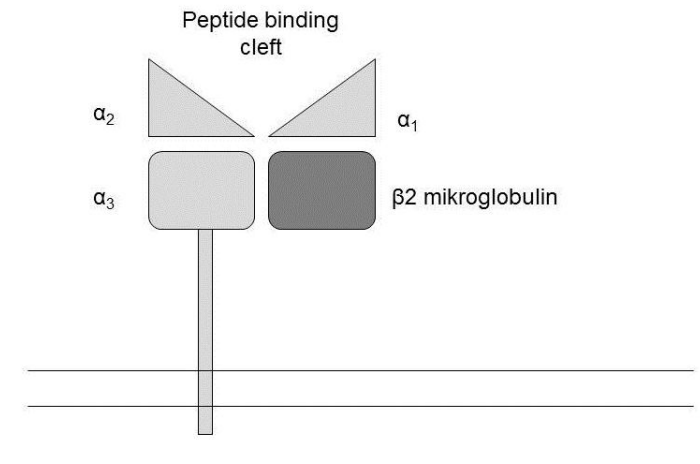
The HLA-B27 molecule consisting of three α-helixes and β2-microglobuline

Various theories for the pathogenic role of HLA-B27 have been presented. Molecular mimicry between foreign and self-peptide can cause a cross-reaction where cytotoxic T-cells activated by an "arthritogenic peptide" start an autoimmune destruction of self-tissue. A number of viral and bacterial species have been proven to evoke such a response, including Chlamydia trachomatis, Campylobacter, Yersinia, Shigella and Epstein-Barr virus. The concept of a cross-reaction between infectious antigens and self-peptides is supported by various observations: Disease causing HLA-B27-subtypes differ from non-disease associated B27-subtypes only by a few residues in the protein-binding groove of the molecule. The difference at these residuals alters the antigen presentation properties of the receptor and also affect T cell recognition.[50] HLA-B27 transgenic rats raised in a germfree environment do not develop inflammatory intestinal or peripheral joint disease, but their skin and nail disease is unaffected. [52, 53] Grouped caging of male DBA-1 and HLA-B27 transgenic mice (mouse models for SpA) increases their liability to develop inflammatory joint disease. [54] About 10% of HLA-B27 positive patients with reactive arthritis develop AS. The finding that disease can develop in HLA-B27 transgenic rats without functioning CD8+ T-cells has however cast doubt on the molecular mimicry hypothesis.[55]

Intestinal inflammation leads to increased permeability and leakage from the gut mucosa, which amplifies the interaction between the immune system and gut bacteria. HLA-B27 positive patients with Crohn's disease have about 50% risk of developing AS, whereas only 3% of HLA-B27 negative Crohn's patients develop the disease.[56]
Increased incidence of Klebsiella pneumonia has been found in the intestinal flora of AS patients with active disease.[57] Monoclonal antibodies against HLA-B27 have also been found to cross-react with antigens from the Klebsiella bacteria.[58]

After reaching the cell surface HLA-B27 molecules can also form heavy chain disulphide-linked dimers which can be recognized by cell pattern recognition receptors and trigger an autoinflammatory response.

HLA-B27 has a tendency to be processed more slowly than other class 1 MHC molecules in the endoplasmic reticulum (ER), which may cause misfolding of the molecule.[59] This can result in a reaction called the "unfolding protein response" (UPR), a control mechanism of the ER which reduces protein synthesis and upregulates enzymes and structural components in the ER, as observed in HLAB27 transgenic rats.[52] Chronic UPR activation leads to a pro-inflammatory status and increased expression of IL-23.

Cytokines

Elevated serum levels of TNF-α, interleukine (IL) -6 and IL-1 have been demonstrated in AS. TNF-inhibitors are well-established as treatment for AS with good effect on back pain, arthritis, back mobility, inflammatory parameters, quality of life, working ability and inflammation visible on MRI.[60, 61] Trials on blockers of IL-6 (tocilizumab) and IL-1 (anakinra) have however been disappointing.[62]

Elevated serum levels of IL-23 and IL-17 have also been demonstrated in AS and in addition polymorphisms in the IL-23 receptor.[63, 64] T-cells responsive to IL-23 have recently been found in the entheses and the aortic root in a mouse model for SpA. Overexpression of IL-23 resulted in enthesitis in these mice.[65] IL-23 is produced by the gut and up-regulation of IL-23 transcription has been found in gut biopsies from AS and Crohn's patients.[66] Trials on inhibition of IL-17 (secukinumab) and IL-23/IL-12 (ustekinumab) in AS are on-going. Preliminary reports on the effect of secukinumab have been positive.[62]

The enthesis

The inflammation in AS is often located to entheses, which are the attachments of tendons, ligaments or joint capsules to bone or cartilage. The enthesis provides anchorage for the tendon by spicules extending like the roots of a tree into the trabecular bone, where it also comes in contact with the bone marrow and the immune system. The terminal end of a tendon contains fibrocartilage, which serves as an absorber of stress in the junction between hard and soft tissue.[67] The entheses are regions of "wear and tear" where stress concentrates and micro damage frequently occurs. A synovio-entheseal complex (SEC) is a concept of entheseal and synovial tissue in close vicinity affecting one another. [68] In this concept the pathogenesis of SpA is biomechanical and autoinflammatory rather than autoimmune. The damage and subsequent repair response in the enthesis are thought to induce inflammation in the nearby synovium. MRI studies have shown that enthesitis precedes synovitis especially in the knee joint in SpA.[69] Mice models of SpA have also shown evidence of enthesitis in combination with synovitis. Unloading of the hind limbs by tail suspension in TNFΔARE mice, a mouse model which develops colitis, spondylitis, peripheral arthritis and sacroileitis, resulted in abolishment of new bone formation and arthritis.

Both endochondral, membranous and chondroidal ossification in tendons are normal age-related changes as evidenced by cadaver studies of entheseal spurs.[70, 71] (Different mechanism for bone formation is described in chapter. 4.6.4.)[62]

Inflammation

The inflammation in AS is typically accompanied by a local bone marrow oedema which is detectable on MRI. Immunohistological studies have shown oedema and invasion of inflammatory infiltrates in the bone marrow component of the enthesis of SpA patients, with CD8+ and CD3+ T-cells being predominant.[72] Biopsy studies of femoral heads, intervertebral discs, sacroiliacal joints and the manubriosternal junction have shown inflammation with macrophages, osteoclasts and CD4+, CD8+ and CD3+ T-cells located in the interface between bone and cartilage, which led to the hypothesis that the disease is caused by an autoimmune reaction to cartilage, especially fibrocartilage.[73-76]

Osteoproliferation

Osteoproliferation is the formation of new bone outside the borders of the normal shape. In AS new bone is built at the outer surface of the cortical bone in the spine, meanwhile trabecular bone is degraded in the central parts of the vertebral bodies. Both endochondral and membranous bone formation contribute to the ankylosis in AS.[77]
The arthritis in AS is different from the one of rheumatoid arthritis (RA). Whereas the synovitis in RA leads to erosions and periarticular bone loss, the arthritis in AS gives rise to an anabolic response and osteoproliferation. Mechanical forces are considered as important triggers for the osteoproliferation.

Inflammation and new bone formation are at least partially uncoupled events in AS, as osteoproliferation can occur even if the inflammatory activity is low. New syndesmophytes sometimes evolve from vertebral corners where inflammation has been visible on MRI, but do frequently develop from vertebral corners without inflammation too.[78] TNF-inhibition has not been proven to prevent radiographic progression in AS, although it's an effective treatment for inflammation.[79, 80] TNF-inhibition does not affect the severity of joint ankylosis in DBA-1 mice either.[81] In fact syndesmophytes have been shown to develop more frequently where inflammation has resolved after TNF-inhibition.[78, 82, 83] It has been hypothesised that this could be caused by increased Wingless protein (Wnt) signalling and that TNF acts as a brake on bone repair and remodelling by stimulating the expression of the Wnt antagonist Dickkopf-1 (Dkk-1). TNF-inhibition loosens this brake by lowering the levels of

Dkk-1. [84, 85] Blocking of Dkk-1 in TNF transgenic mice resulted in protection from inflammatory bone loss, increased expression of β-cathenin and osteoprotegerin and lowered expression of sclerostin. [86]

There has however not been any evidence of an accelerated osteoproliferation in patients treated with TNF-inhibition. It is speculated that early and sustained treatment with TNF-blockers could prevent the initial inflammatory lesions and thereby hamper osteoproliferation and studies with TNF-inhibition in early axial SpA are on-going.[87]

Until now, daily or high use of NSAIDs is the only treatment which has been associated with retardation of syndesmophyte growth.[88-90] Prostaglandin E_2 (PGE_2) is produced by osteocytes in response to mechanical load. PGE_2 promotes osteocyte viability and stimulates osteoblast maturation by activating the Wnt/β-catenin pathway (described in chapter 4.7.3.).[91, 92] In orthopaedics NSAIDs are known to retard early bone formation following fractures, delay fracture healing and increase the risk of endoprostethic loosening. Heterotopic ossification is ectopic bone formation in soft tissue which can develop after trauma. NSAIDs have been shown to impede heterotopic ossification after prosthetic hip surgery.[93]

Bone formation is governed by several protein mediators which affect gene transcription via intracellular signalling pathways, including the Wnts (described in chapter 4.7.3.), the bone morphogenic proteins (BMPs) and the Hedgehogs (Hh).

BMPs are members of the transforming growth factor β (TGF-β) superfamily. BMPs are important regulators of cell proliferation, differentiation and death.[94] BMPs can induce ectopic endochondral bone formation. BMP signalling is enhanced by cytokines such as TNFα an IL-1 and inhibited by extracellular antagonists, including noggin.[95] Increased expression of BMPs has been found in biopsies of Achilles tendons from SpA patients and in ankylosing enthesitis in DBA-1 mice. Overexpression of noggin reduced the development of bone formation and joint ankylosis and severity of arthritis in DBA-1 mice.[96] Pro-inflammatory cytokines such as TNF-α and IL-1β enhance the expression of BMPs in arthritic synovia.[97]

Hhs are a protein family consisting of Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh). Hhs govern embryonic skeletal development and growth via the promotion of endochondral ossification. Hhs could be interesting targets for therapies against osteoproliferation, since they influence endochondral ossification, which only occurs as pathologic new bone formation in adult life, but have no effect on physiologic bone remodelling. Blocking of Hh signalling in C57/BL6 mice, another mouse model for SpA, resulted in inhibition of osteophyte formation without affecting severity of arthritis or bone density.[98]

Bone

Bone physiology

The skeleton has many important functions including providing mechanical support to soft tissues and serving as levers for muscle action, enabling longitudinal growth and maintaining the calcium homeostasis, housing of the bone marrow and providing protection for organs such as the brain, spinal cord, heart and lungs.

Bones consist of trabecular and cortical bone tissue. The trabecular bone (also called spongious or cancellous), is located in the inner zones of the bones, comprises a lattice of small criss-crossing laths (trabeculae) and harbours the bone marrow. Due to its great total surface area the trabecular bone is very metabolically active and the first site of bone loss. The cortical bone (also called compact bone) is located in the outer zoon (cortex) and consists of dense bone tissue. The cortical bone is lined by two thin layers of fibrous tissue containing osteoblast precursors; the periosteum on the outer surface and the endosteum on the inner surface.

Mechanical strength of the skeleton

The mechanical strength of the skeleton depends on the bone microarchitecture, bone geometry and bone mineral content (BMC). Important microarchitectural features are the number, thickness and connectivity of the trabeculae and the thickness and porosity of the cortex.[99] Geometrical factors of importance for biomechanical strength in the spine are the cross-sectional area of the vertebral bodies and the length of the processus spinosus lever. The cross-sectional area, neck-shaft angle and the length of the femoral neck are of importance for femoral neck fractures, whereas the outer diameter of the long bones is an important factor for bending strength and long-bone fractures.[100]

The bone cells

The bone cells come from two mayor cell lines; the osteoblast and the osteoclast lineage. The osteoblast lineage has mesenchymal origin and comprises the preosteoblasts, osteoblasts, bone-lining cells and osteocytes.[101]

The osteoblasts, which are located at the bone surfaces, synthesize osteoid, the organic part of the bone matrix, consisting largely of type 1 collagen and chondroitin sulphate, but also containing alkaline phosphatase and osteocalcin. As the osteoid becomes mineralized by deposition of hydroxyapatite (calcium and phosphate ions) the osteoblasts are embedded in bone matrix and some develop into osteocytes which lie in small chambers (lacunae) within in the bone. Others turn into resting bone-lining cells.

The osteocytes have long dendritic processes which stretch out through small canals (canaliculi) out on the bone surface and in to the bone marrow.[91] Osteocytes are mechanosensors which react upon mechanic strain or fluid flow within the canaliculi; additionally they are reactive to inflammatory mediators. They translate mechanical loading into biochemical signals that regulate bone remodelling.[91]

The osteoclasts have bone marrow origin and derive from the monocyte lineage. The osteoclasts are multinucleated cells located on the bone surface in small cavities and are responsible for bone resorption.[102] The cells pump hydrogen ions into the sealed cavity through a specialized folded cell membrane, called the ruffled border, and hereby dissolve the bone minerals. Degrading enzymes, such as cathepsin K and matrix metalloproteinases, are also released via lysosomes to catabolise the organic components of the matrix.

Bone modeling

Bone modeling is the formation of bones during development and skeletal growth. Bones form via two different mechanisms; endochondral and intramembranous ossification. During endochondral bone formation, mesenchymal progenitor cells condense and differentiate into chondroblasts and chondrocytes forming a cartilage template. As the chondrocytes later undergo hypertrophy, the template is invaded by vessels and the cartilage is successively degraded by osteoclast-like cells (chondroclasts) and replaced by osteoid forming osteoblasts.[101] In intramembranous bone formation, which occurs only in certain locations like the skull and clavicles, condensed mesenchymal progenitor cells differentiate directly into osteoblasts.

Bone remodeling

Bone remodeling is the continuous renewal of bone by which 10% of the skeleton is replaced by new bone yearly. A bone remodelling cycle starts with bone resorption followed by bone deposition, which are locally coupled events in so called basic multicellular units (BMU). When resorption increases, deposition usually follows, but the resorption is much faster than the deposition. It takes about 4-6 months to rebuild bone resorbed in 4-6 weeks, which explains why increased remodelling rate often results in bone loss. The balance between osteoclastic bone resorption and osteoblastic bone formation is tightly regulated by only partly known paracrine, endocrine, neurologic and mechanic feedback mechanisms.

Sexual differences during growth and ageing

The greater strength in bones in men compared with women depends on bone size and geometry rather than bone density.[100] During childhood and adolescence boys grow larger long bones and vertebras than girls, but volumetric bone mineral density (vBMD) is almost constant and equal between sexes.(vBMD is defined in chapter 4.9.1.) [103] Androgens, growth hormone and insulin-like growth factor-1 stimulate periosteal apposition in males, whereas estrogens inhibit periosteal bone apposition resulting in narrower bones in females. At puberty vertebral trabecular vBMD increases due to increased trabecular width, but not number. The bone balance in the BMU is positive during growth and until the third decade, when the peak bone mass is reached. Bone loss begins in young adulthood but the loss is slow in the beginning, because the remodelling rate is low. During ageing the bone turnover rate increases. In men bone loss due to trabecular thinning dominates, whereas loss of trabecular number and connectivity dominates in women.[104] As the trabeculae disappear, more bone is lost from the cortical compartment resulting in increased cortical porosity. Periosteal bone apposition continues however to be greater in men than in women during ageing.

Biomarkers of bone metabolism (Fig 2)

Receptor activator of nuclear factor-κB and its ligand

The maturation of osteoclasts requires macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL), which is expressed both as a secreted protein, soluble RANKL (s-RANKL) and a membrane-bound protein by various cell types including osteocytes, osteoblasts, synovial cells, T- and B cells.[102, 105] RANKL binds to RANK on osteoclast precursors, inducing their transformation into mature osteoclasts and has thus a resorptive effect on bone. The RANKL expression is enhanced by pro-inflammatory cytokines, parathyroid hormone, 1,25-hydroxy vitamin-D and dexamethasone. Denosumab, a human monoclonal antibody to RANKL, has emerged as a new treatment option for osteoporosis.[106] RANKL knockout mouse develop osteopetrosis, a condition characterized by very high bone mass and dense skeleton. [101]

Osteoprotegerin

The RANK-RANKL interaction is inhibited by osteoprotegerin (OPG), a decoy receptor for RANKL, synthesized by many cell types including osteoblasts. OPG has an anti-resorptive effect on bone. The expression of OPG in

osteoblasts is enhanced by TGFβ, oestrogens, calcitonin, BMPs and Wnt-signalling.[102]

Wingless proteins

Wingless proteins (Wnts) are a family of 19 known secreted palmitoylated glycoproteins, which play important roles in embryogenesis, cell differentiation, apoptosis, gene expression and cancer development. The name "wingless" comes from a phenotype of Drosophila with no wings which had mutations in a gene, subsequently called wingless.[107] Mutations in Wnt genes in mouse cause serious malformations.[108] Wnts signal through at least four different pathways, of which the Wnt/β-catenin pathway, also known as the "canonical pathway", is best described. In the canonical pathway Wnts bind to cell surface receptors named Frizzled, low-density lipoprotein receptor-related protein 5/6 (LRP 5/6) and Kremen. The binding of Wnt to its' receptors stabilises β-catenin via inhibition of the kinase GSK-3β which otherwise would phosphorylate β-catenin and direct it to degradation. β-catenin accumulates in the cytoplasm and translocates to the cell nucleus, where it affects gene transcription.[109]

The canonical Wnt pathway is important for osteoblast differentiation, proliferation and survival. Gain or loss-of-function mutations in the LRP 5 receptor gene in mouse models have been shown to lead to high bone mass or osteoporosis respectively. [110] Wnt activation also blocks osteoclast maturation by increasing the synthesis of OPG in the osteoblasts. [111]

In this thesis the serum levels of two types of Wnts were measured; Wnt3a and Wnt5a.

Wnt3a was the first of the Wnts which could be isolated from cell cultures in its active form and characterised.[112] Wnt3a has been shown to activate both canonical and non-canonical Wnt pathways in mesenchymal stem cells and promote osteoblast differentiation, proliferation and survival.[113-115] Total knock-out (KO) of Wnt3a causes embryonic lethality in mice, but heterozygote KO mice display bone loss and low BMD and trabecular number.[116] Mutations in the Wnt3a gene in humans is known to cause Tetra-Amelia, a syndrome characterized by the complete absence of all four limbs and serious anomalies involving the cranium and inner organs.[117] Wnt3a has also been shown to inhibit chondrogenesis and to induce matrix loss and increased expression of catabolic proteases in cultured articular chondrocytes.[118] The effect of Wnt3a on chondrocytes has been showed to be enhanced by mechanical load.[119] Wnt3a activity is however reduced in osteoarthritis.[118, 120]

Wnt5a signals via non-canonical pathways.[121] Heterozygote Wnt5a KO mice also display bone loss.[116] Wnt5a has been shown to have inhibitory effects on chondrocytes.[118] Wnt5a was recently also shown to enhance RANK

expression on osteoclast precursors and promote osteoclastogenesis.[122] In rheumatoid arthritis (RA) an increased expression of Wnt-5a has been observed in synovial tissue, where it also has been shown to activate the pannus formation and increase the expression of inflammatory cytokines.[123, 124]

Wnt signalling is regulated by several secreted receptor antagonists including secreted frizzled related proteins (Sfrps), the Dickkopfs and sclerostin.

Dickkopf-1

Dickkopf-1 (Dkk-1) binds to the LRP 5 and Kremen receptors with high affinity and promotes the internalization of the complex, thus making the receptor unavailable for Wnt signalling. Dkk-1 is expressed in osteoblasts and osteocytes.[125] Dkk-1 expression is stimulated by TNF-α. In RA high levels of RANKL (activating osteoclasts), together with high levels of Dkk-1 (inhibiting osteoblast proliferation) switches the balance towards bone resorption, with articular erosions and periarticular osteoporosis as a result. Blocking of Dkk-1 by antibodies in a mouse arthritis models resulted in abolishment of erosions, despite persistent synovial inflammation. In one mouse model the Dkk-1 blocking reversed the RA-like bone-destructive pattern to a more osteoarthritis like bone-forming pattern with growth of osteophytes in the inflamed joints.[85] Blockage of Dkk-1 by antibodies has been shown to induce fusion of sacroiliacal joints in mice transgenic for TNF.[126]

Sclerostin

Sclerostin is another receptor inhibitor expressed exclusively in osteocytes. Mutation of the SOST gene, which encodes for sclerostin, causes sclerosteosis in humans, a rare condition characterized by endosteal hyperostosis of the mandible and skull in the absence of sclerostin secretion.[127] Mechanical loading on osteocytes reduces their expression of sclerostin and Dkk-1 thus promoting bone proliferation. [91, 128, 129]

Figure 2: Biomarkers for bone metabolism
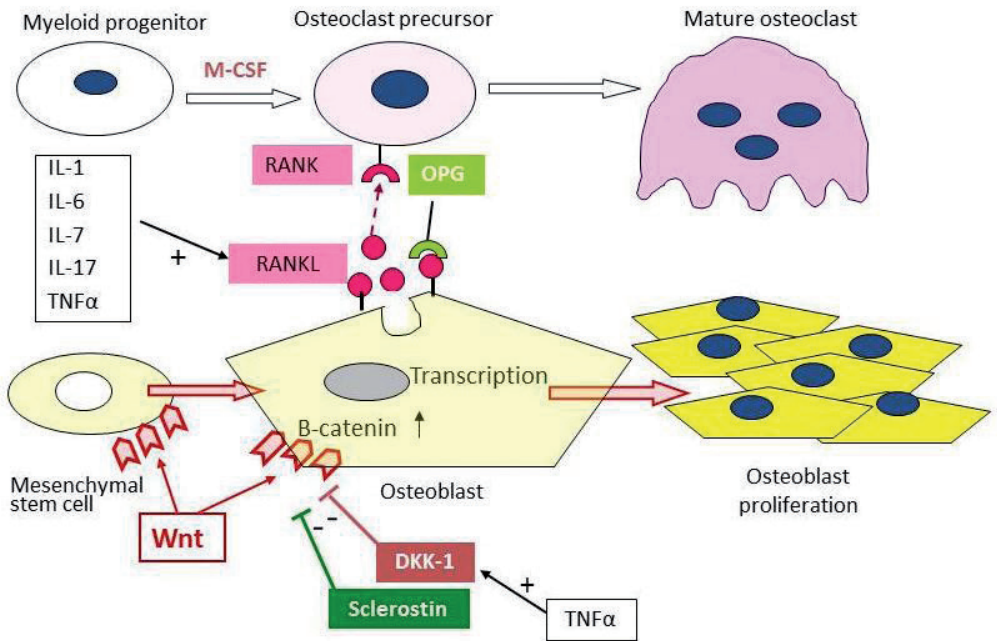
M-CSF and interaction between RANK and RANKL are required to induce expression of genes essential for osteoclastogenesis. OPG can bind and neutralize RANKL. The expression of RANKL is enhanced by cytokines, including IL-1,-6,-7,-17 and TNFα.

Wnt proteins bind to the frizzled/LRP receptor complex. As a result the degrading of β-cathenin is inhibited and the protein accumulates in the cytoplasm and nucleus, where it affects gene transcription. Wnt/β-cathenin signalling stimulates the differentiation and proliferation of osteoblasts. Wnt signalling is inhibited by receptor-antagonists Dkk-1 and sclerostin. Dkk-1 expression is enhanced by TNFα.

Osteoporosis

Definition

An internationally agreed description of osteoporosis is: "a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture".[130] The condition is asymptomatic until the occurrence of a fracture. Primary osteoporosis is caused by natural ageing, menopause or life style factors. Secondary osteoporosis is caused by drug treatment, such as glucocorticoids, or certain diseases, including inflammatory rheumatic diseases, IBD, malabsorption or thyroid illness.

The WHO definition of osteoporosis formulated in 1994 first only regarded postmenopausal women, but was later extended to also comprise men over the age of 50.[131, 132] (Table 5) The definition uses bone mineral density (BMD) measured with dual energy x-ray absorptiometry (DXA). The patients' BMD-value is compared with a standard score made from measurements of healthy young adults of the same sex, where zero represents the mean BMD-value of the reference population. The patients' BMD is converted into a T-score, which is the deviation from the mean of the standard score expressed in standard deviations (SD). The lowest T-score in lumbar spine, total hip or femoral neck is used to diagnose osteoporosis. Measurements of the radius should only be used in certain circumstances. The cut-off value of a T-score of 2.5 SD below the average BMD of healthy young adults identifies approximately 30% of postmenopausal women as having osteoporosis, which is equivalent to the lifetime risk of fractures at the hip, spine or forearm.[132] The age adjusted fracture risk increases 1.5- to 3-fold for each SD decrease in BMD in both sexes.

Table 5: The WHO-definition of osteoporosis for postmenopausal women and men older than 50 years

Normal BMD: T-score > -1.0 SD

Osteopenia: T-score ≤ - 1.0 to > - 2.5 SD

Osteoporosis: T-score ≤ - 2.5 SD

Severe/established osteoporosis: T-score ≤ - 2.5 SD in the presence of one or more fragility fractures.

The WHO definition only applies to patients older than 50 years. For individuals below the age of 50 the International Society for Clinical Densitometry (ISCD) recommends the following definition of low BMD based on Z-scores (the patients BMD compared with the average BMD of healthy age matched controls of the same sex): Table 6 [133]

Table 6:

BMD below expected range for age in patients below the age of 50

BMD within the expected range for age: Z-score above -2.0 SD

BMD below the expected range for age: Z-score of -2.0 SD or lower

---

Epidemiology

Osteoporosis and fragility fractures are major health problems in the western world, causing considerable suffering, disability and mortality and in addition high costs for the health care systems. One third of all Swedish women aged 70-79 are estimated to have osteoporosis when assessed in the hip. A total of 70 000 osteoporosis related fractures, whereof 18 000 hip fractures, occur yearly in Sweden.[134]

The incidence of hip fractures is sometimes used as an indicator of the prevalence of osteoporosis in a country. The hip fracture incidence has increased during the last 50 years, partly due to increased age expectancy, but varies about 10-fold between countries.[135] Sweden and the Scandinavian countries top the ranking list of 10-year probabilities for hip fracture.[136] At the age of 50 the lifetime risk for a hip fracture in Sweden has been estimated to be 28.5% for women and 13.1% for men.

Only about one third of all vertebral fractures come to clinical attention. The rates of vertebral fractures in Sweden have also been shown to be higher than in the rest of Europe.[137] The age-standardized yearly incidence of morphometric vertebral fractures in individuals older than 50 has been shown to be 10.7/1000 in women and 5.7/1000 in men in Europe.

Pathogenesis of osteoporosis

The bone mass of an individual in later life is the result of the peak bone mass accrued during childhood and adolescence as well as the subsequent rate of bone loss. The peak bone mass depends both on genetic factors and environmental conditions, such as nutritional status, physical activity and general health during childhood. Loss of trabecular bone starts already in the third decade, whereas cortical bone is lost from the fifth decade.[138]

In women the bone loss is accelerated after menopause in the estrogen deficient state. Estrogens and androgens are important for bone mass since they slow the rate of bone remodelling and prevent bone loss. The sex-hormones have a pro-apoptotic effect on osteoclasts and an anti-apoptotic effect on osteoblasts and osteocytes. Estrogens inhibit osteoclast maturation by stimulating the expression of OPG in osteoblasts and reducing the levels of inflammatory cytokines, such as IL-1, IL-6 and TNF, with a following decrease in RANK-RANKL interaction.[139] Estrogen loss leads to both increased osteblastogenesis and osteclastogenesis and rice in the rate of initiation of new bone remodelling cycles, which tilts the balance towards increased bone resorption. In addition the working life of the bone-resorbing osteoclasts is prolonged, whereas the working life of the bone-forming osteoblasts is shortened.

Estrogen deficiency plays an important role for bone loss also in men.[140] Estrogen receptors (ERα and β) and androgen receptors (AR) are expressed in chondrocytes, osteoclasts, osteoblasts and osteocytes in similar levels in women and men.[139] Sex hormone binding globulin (SHBG) increases with age in men and bioavailable (non-SHBG bound) levels of estradiol and testosterone decline.[141] Low serum estradiol is a better predictor for BMD and fracture risk in men than testosterone.[142, 143] Testosterone is however also important for bone formation and provides the necessary substrate for aromatization to estradiol in the testes and peripheral tissues in men. Testosterone is also essential for muscle strength and balance which impact the risk of falls.[144]

Risk factors for osteoporosis and fractures

Age is a strong risk factor for both osteoporosis and fragility fractures. The age-related increase in fracture risk cannot only be explained by the decline in bone density, but is also due to lower muscle strength, comorbidities and poorer balance leading to greater falling hazard. Other constitutional risk factors are previous fractures, heredity to fracture, female sex, early menopause, ethnicity and high body length.

Life style related risk factors for osteoporosis and fractures include smoking, physical inactivity, alcohol abuse, low calcium intake, underweight, low sun exposure and propensity to fall.[134]

Osteoporosis in ankylosing spondylitis

Patients with AS have an increased risk of developing osteoporosis compared with the general population.[145, 146] The bone loss can evolve already in early disease and affect young individuals.[147] A prevalence of osteoporosis from 19 to 62 % has been reported in earlier studies. [145, 148-151]. The diverging results between the studies can be explained by differences in the study cohorts regarding factors such as age, disease duration and severity of the rheumatic disease. We found a prevalence of osteoporosis of 20.8% and of osteopenia of 43.6% among AS patients of 50 years or older recruited from West Swedish rheumatology clinics. The prevalence of BMD below expected range for age was 5% in patients younger than 50. (Paper I)[152].

AS-specific risk factors for osteoporosis shown in earlier studies have been long disease duration, advanced syndesmophyte formation or ankylosis of the spine, poor back mobility, high disease activity and elevated serum markers of inflammation.[153-157] Low physical activity has not been proved to be a specific risk factor, as physical activity is not exceptionally low in AS in general.

The cause of osteoporosis in AS is multifactorial. Like in other inflammatory diseases increased levels of pro-inflammatory cytokines (IL-1, IL-6, IL-17, TNF-α), act on osteoblasts to promote RANKL expression, with subsequent osteoclast activation.[158] TNFα also inhibits proliferation and differentiation and increases apoptosis of osteoblasts.[159] Therapeutic blocking of TNF has been shown to improve BMD and to lower the serum levels of cross-linked c-telopeptides (CTX), a biomarker for bone resorption in Spa.[160-163] We found that TNF-inhibition was associated with increased serum levels of Wnt3a. (Paper III) [164] Spinal immobility and alterations of the biomechanical conditions of the spine could also contribute to the local osteoporosis in the vertebras. Subclinical gut inflammation leading to calcium and vitamin D malabsorption has been pointed out as another contributing factor. Additionally a polymorphisms of the vitamin D receptor gene has been shown to have association with lumbar BMD in AS.[165] Serum levels of dehydroepiandrosterone sulfate (DHEAS) are lower in osteoporotic males with AS, but conflicting results have been reported regarding the levels of testosterone.[166-169] Glucocorticoids are known to cause osteoporosis in AS and other diseases, but steroid use is however limited in AS.

Prior studies have reported that the osteoporosis in AS is primarily confined to the central skeleton.[155, 170, 171] In contrast we found almost as many patients with osteoporosis or BMD below the expected range for age at the radius (n = 17, 8%) as in the lumbar spine (n = 24, 12%) and also strong correlations between vBMD in lumbar spine and ultra-distal radius (r_S=0.762; p<0.001) and tibia (r_S=0.712; p<0.001) (Paper I and IV).[152, 172] Earlier

studies have shown increased incidence of vertebral, but not non-vertebral fractures in AS. We found 24 patients with vertebral fractures but only one patient with a hip fracture in our study. (Paper II)[173]

The bone disease in AS is paradoxical; pathologic new bone formation and enhanced bone loss are taking place in close vicinity. This creates diagnostic problems, since the syndesmophytes cause artifactual increase of lumbar bone mineral density (BMD) especially when measured in anteroposterior projection (AP). Using lumbar DXA in lateral projection or measuring vBMD with quantitative computed tomography (QCT) could be ways to diminish this problem. (These methods are described in chapter 4.9.1-2)

Vertebral fractures in ankylosing spondylitis

AS patients have an increased risk of vertebral fractures, with a reported prevalence from 9 to 42 % in studies on different AS cohorts. (Table 7) We identified a total of 42 vertebral fractures (Th4-L4) in 24 patients (11.8%) by radiography (Paper II).[173] The vertebral fractures often go undiagnosed since the symptoms may be disregarded as disease flares. The fractures can also be difficult to visualise in radiographs, due to unusual localisations or disturbing AS related changes.[149]

Unlike the stable compression fractures seen in age-related osteoporosis, the vertebral fractures in AS can be unstable and complicated with neurologic damage. Osteoporosis in combination with bridging syndesmophytes and ankylosis of the zygapophyseal joints leads to a rigid but brittle spine, with a propensity to fracture even after minor trauma. The fractures can run thru the intervertebral spaces, the dorsal arch or predicles resulting in injuries to the spinal cord or nerve roots and give rise to intra-spinal hematomas.[174-176]

Table 7. The prevalence of vertebral fractures found in different studies

Authors et al	Publ year	Land	Patients N	Men %	Diagnosis	Age years	Disease duration years	Prevalence of vertebral fractures %	Comment
Ralston	1990	UK	111	88	Spa	median 41	median 17	21	Morphometric fractures
Cooper	1994	USA	158	77	AS*	mean 34	no data	11	Clinical fractures
Donelly	1994	UK	87	71	Spa	mean 44	mean 16	9	Morphometric fractures
Sivri	1996	Turkey	22	91	AS*	mean 37	mean 10	41	Morphometric fractures
Mitra	2000	UK	66	100	AS*	median 38	median 10	17	Morphometric fractures
Lange	2005	Germany	84	63	AS*	mean 32-56 4 groups	mean 9-32 4 groups	11	Morphometric fractures
Jun	2006	South Korea	68	100	AS*	mean 31	mean 7	16	Morphometric fractures
Vosse	2006	Netherlands	135	67	AS*	mean 50	mean 9	31	Morphometric fractures
Ghozlani	2009	Morocco	80	84	AS*	mean 39	mean 11	42	Morphometric fractures VFA
Mermerci Baskan	2010	Turkey	100	75	AS*	mean 40	mean 11	19	Morphometric fractures
Klingberg	2012	Sweden	204	57	AS*	mean 50	mean 15	12	Morphometric fractures

*AS fulfilling the Modified New York criteria, VFA = vertebral fracture assessment using DXA. Morphometric fractures are vertebral fractures found on radiography when examining all study patients. Clinical fractures include only fractures which have come to clinical attention and are found in medical registers.

Assessment methods for BMD and bone morphology

BMD and bone morphology can be studied using different methods. Herein follows a description of the methods used in this thesis.

Dual energy X-ray Absorptiometry (DXA)

DXA is the most commonly used method to measure BMD and the basis for the WHO definition of osteoporosis. It measures areal BMD (aBMD) using two x-ray beams with different energy levels. The low energy beam is absorbed in the soft tissue, whereas the high energy beam gets absorbed by both soft tissue and the skeleton. The soft tissue absorption is then subtracted out from the high energy beam to calculate the amount of x-ray which is absorbed by bone alone, which is subsequently converted into the BMC by the software of the DXA-apparatus. The radiation dose for measurements in the hip and lumbar spine is approximately 0.01 mSv.

aBMD is the BMC divided per area of bone (mg/cm^2). It is measured in 2D without taking the depth of bone in the third dimension in account and depends both of bone density, mineralisation and bone size. Men have bigger bones than women and consequently higher aBMD.[177]

Volumetric BMD (vBMD) (expressed in mg/cm^3) is the BMC divided by the measured bone volume (cm^3). vBMD, a 3D mode to measure bone density, can be assessed by computed tomography or estimated using DXA in two perpendicular projections. vBMD is typically equal between the sexes.

Lumbar DXA is generally measured in AP or posterior-anterior projection. In AS the new bone formation particularly takes place on the anterior parts of the vertebral body and in the zygapophyseal joints, areas which are included in the scanning region when using AP DXA. This makes AP lumbar DXA unreliable, especially in patients with advanced ankylosis. The lateral sides of the vertebra bodies are however less affected by osteoproliferative changes. Lumbar DXA with lateral scanning measures BMD only in the vertebral body from a side view and the posterior vertebral parts are excluded, hence less AS specific osteoproliferative changes are included in this measurement. (Figure 3) Aortic calcifications and osteoarthritic changes in degenerative spinal disease are also excluded. Lateral lumbar DXA primarily assesses trabecular bone, since the vertebral body mainly consists of trabecular bone, whereas the posterior parts are rich in cortical bone. Consequently lateral lumbar BMD is lower than AP lumbar BMD. In addition, combining the two perpendicular areas from AP and lateral DXA creates a volume of interest in the vertebral body, in which vBMD can be estimated. The mayor disadvantage of lateral lumbar DXA is that it should not be used for diagnostic purposes, since the WHO definition of osteoporosis is based on posterior-anterior or AP scanning. Other drawbacks are availability problems and the current lack of a male reference data base.

Figure 3: Lumbar DXA in anteroposterior (AP) and lateral projections

Lumbar DXA in anteroposterior (AP) projection

Osteoproliferation

Lumbar DXA in lateral projection
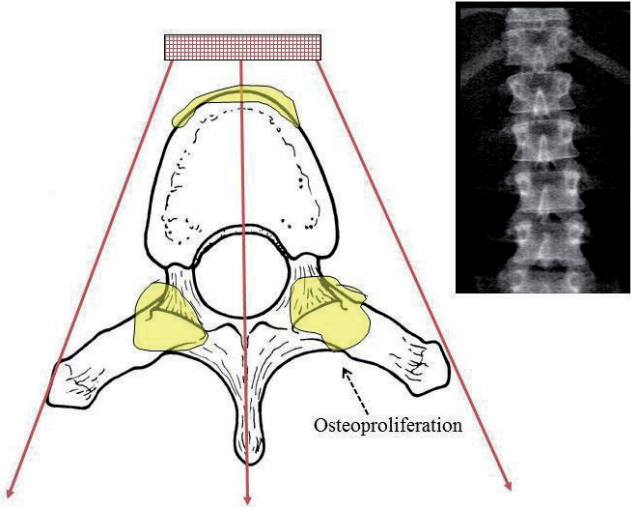
Osteoproliferation
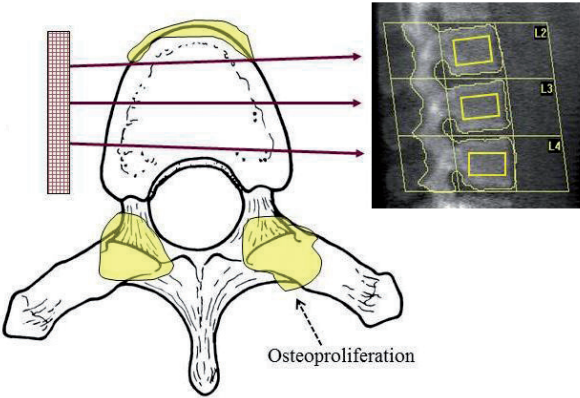
It has been recommended to use BMD measured in the hip region (femoral neck or total hip) in AS patients with extensive syndesmophyte formation.[149, 154] Hip BMD continues to descend during the development of osteoporosis, whereas AP lumbar BMD sometimes inaccurately increases due to osteoproliferation.

Quantitative Computed Tomography (QCT)

Lumbar QCT measures vBMD (expressed in mg/ cm^3 of calciumhydroxy-apatite) in 10 mm thick slices of each vertebra. The scanning is performed with a water- and bone-equivalent calibration phantom placed below the patient. The boundaries of the trabecular and cortical bone in each vertebra is outlined and separated in different regions of interest (ROIs). The radiation dose is 0.51 mSv for men and 0.81 mSv for women. (Figure 4)

Earlier studies have shown that vBMD measured with QCT is less affected by the osteoproliferative changes in AS than AP lumbar DXA and more sensitive in detecting osteoporosis.[150, 167, 178, 179]
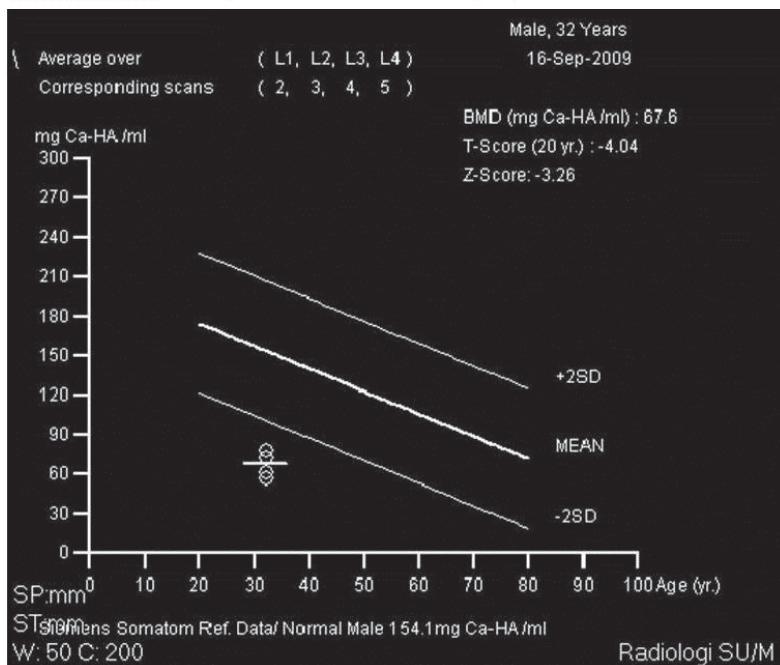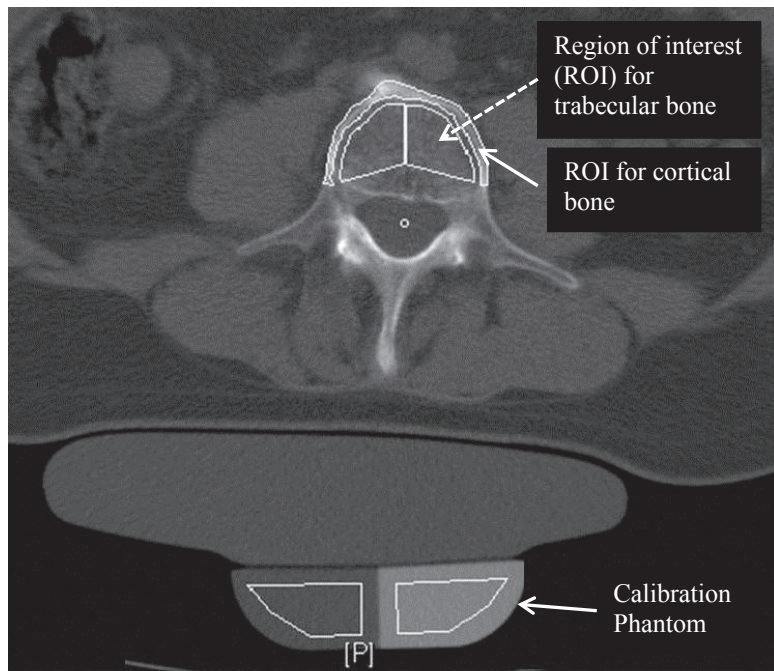
High Resolution peripheral Quantitative Computed Tomography (HRpQCT)

HRpQCT is a new method to study bone morphology. It gives a highly detailed 3D representation of bone and enables the study of bone microarchitecture in vivo without biopsy. (Figure 5) During the procedure the patient's forearm and leg are immobilized in especially designed carbon fibre shells. The radiation dose is approximately 0.01 mSv.

The volumes of interest, 9 mm sections of radius and tibia, are examined in 110 parallel slices (voxel size 82μm) and automatically separated into a trabecular and a cortical region. Some of the parameters are measured directly during the procedure and others are derived. The trabecular vBMD (DTrab; mg/cm^3) is measured directly and the trabecular bone volume/total volume (BV/TV; %) is derived assuming a mineral density of fully mineralized bone of 1.2 g hydroxyapatite/ cm^3. The trabecular number (TbN; 1/mm) is detected in the images using advanced data processing methods. The trabecular thickness (TbTh; μm) is calculated using the formula BV/TV $/$ TbN and the trabecular separation or spacing (TbSp; μm) using the formula (1-BV/TV) /TbN. The cortical vBMD (DCort; mg/cm^3), cortical bone cross-sectional area (CortCSA; mm^2) and the cortical periosteal circumference (CortPm; mm) are measured. The cortical thickness (CortTh; mm) is derived using the formula CortCSA /CortPm.

Figure 4: Quantitative computed tomography (QCT) of lumbar spine
Published with permission from the patient.

Lumbar vBMD of the patient in comparison with a standard score.

Figure 5: High Resolution peripheral Quantitative Computed Tomography (HRpQCT)
Published with permission from the patients.
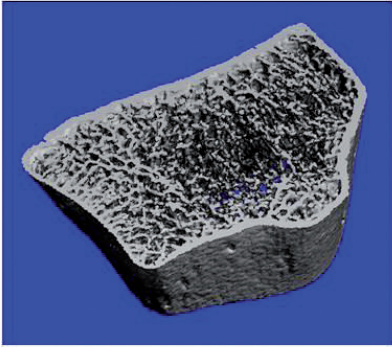
3D representation of ultra-distal radius. AS patient with osteoporosis.
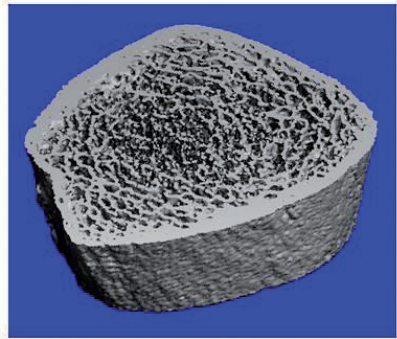
3D representation of ultra-distal tibia. AS patient. Normal BMD.
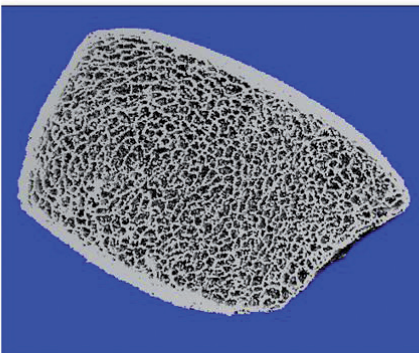
2D representation of ultra-distal tibia. AS patient with normal BMD.
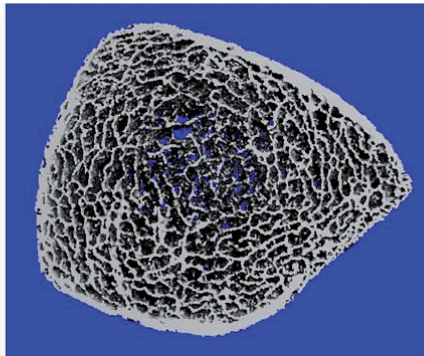
2D representation of ultra-distal tibia. AS patients with osteoporosis. Note the low trabecular number and thickness, high trabecular separation and low cortical thickness

AIMS

The main objective of this thesis were to study the prevalence of bone disease – both osteoporosis and osteoproliferation - in patients with ankylosing spondylitis and the association with disease activity, disease manifestations and risk factors using both conventional and new imaging techniques and biomarkers.

The specific aims were to study:

A. the prevalence of osteoporosis and vertebral fractures in a cohort of AS patients recruited from rheumatology clinics in West Sweden

B. demographic and disease related factors of importance for osteoporosis, osteoproliferation and vertebral fractures in AS

C. lumbar BMD measured with different techniques – QCT and DXA in anteroposterior and lateral projection- and to compare the different methods

D. serum levels of biomarkers reflecting bone metabolism in AS compared with the levels in healthy controls and investigate the relationship between these biomarkers and disease activity, back-mobility, chronic AS related changes in the spine and BMD

E. peripheral volumetric BMD in AS patients in comparison with healthy controls and explore the associations between bone microarchitecture assessed with HRpQCT and presence of vertebral fractures, syndesmophytes and lumbar BMD measures with DXA and QCT

F. the prevalence of intestinal inflammation in AS indirectly by measuring fecal calprotectin and to correlate the concentrations of calprotectin in feces and serum with reported gastrointestinal symptoms, medication, and measures of disease activity

METHODS

This thesis is based on a cross-sectional observation study.

Patients

Paper I-V. The patients were recruited from three participating centres in West Sweden; Sahlgrenska University Hospital and the Rheumatology Clinics in Borås and Alingsås. The patients were retrieved from the hospitals' databases. The medical records of all patients diagnosed with ankylosing spondylitis were checked and all patients fulfilling the modified New York criteria for ankylosing spondylitis were eligible for inclusion. Exclusion criteria were psoriasis, IBD, dementia, pregnancy and having another rheumatologic diagnosis. Due to the extensive questionnaires included in the study, patients with difficulties in understanding Swedish, needing an interpreter, were also excluded. The procedure of the trial, number of excluded patients, drop-outs and included patients are shown in figure 6. In short: 204 AS patients were included in the studies on BMD, vertebral fractures and biomarkers of bone metabolism, 205 patients in the calprotectin study and 69 male patients were randomised in an age-adjusted way to also undergo measurement of lumbar vBMD with QCT and assessment of peripheral vBMD and bone microarchitecture with HRpQCT.
The patients were all enrolled between February to April 2009. They underwent physical examination by the same physician (E.K.), including back mobility tests and 68/66 joints count for number of tender and swollen joints. The Bath Ankylosing Spondylitis Metrology Index (BASMI) was calculated using five clinical measurements that reflect axial mobility as described in the ASAS handbook: the cervical rotation, tragus to wall distance, intermalleolar distance, lateral lumbar flexion and lumbar flexion (modified Schobers test).[4, 180]
Blood samples were drawn.
Demographics and disease related parameters of the study population are demonstrated in table 8.

Controls
Paper III and V. Blood samples from 80 healthy blood donors were collected to use as controls to the AS patients. The blood donors were recruited when giving blood at Sahlgrenska University Hospital and answered questionnaires stating that they were in full health and not on medication.
Paper IV. The results of healthy male controls that had undergone measurement of peripheral vBMD with HRpQCT were provided by the Mayo Clinic, Rochester, Minnesota, USA in scientific collaboration. The controls were matched for age, length, weight BMI and race. The HRpQCT devise at the two centers, which are similar models from the same manufacturer, were calibrated with a phantom for vBMD measurements sent from Gothenburg to Rochester.

Figure 6: Procedure of the trial, drop-outs and patients who were excluded

538 AS patients registered at the clinics

177 patients did not meet with study criteria

361 patients invited to participate

72 patients declined participation

60 patients did not respond to invitation

19 patients showed exclusion criteria

210 patients included
phys.ex., questionnaires, blood samples

6 patients did not come to radiography or DXA

5 patients did not send in stool sample

DXA, spinal radiography

204 and 205 patients completed the BMD and calprotectin studies respectively
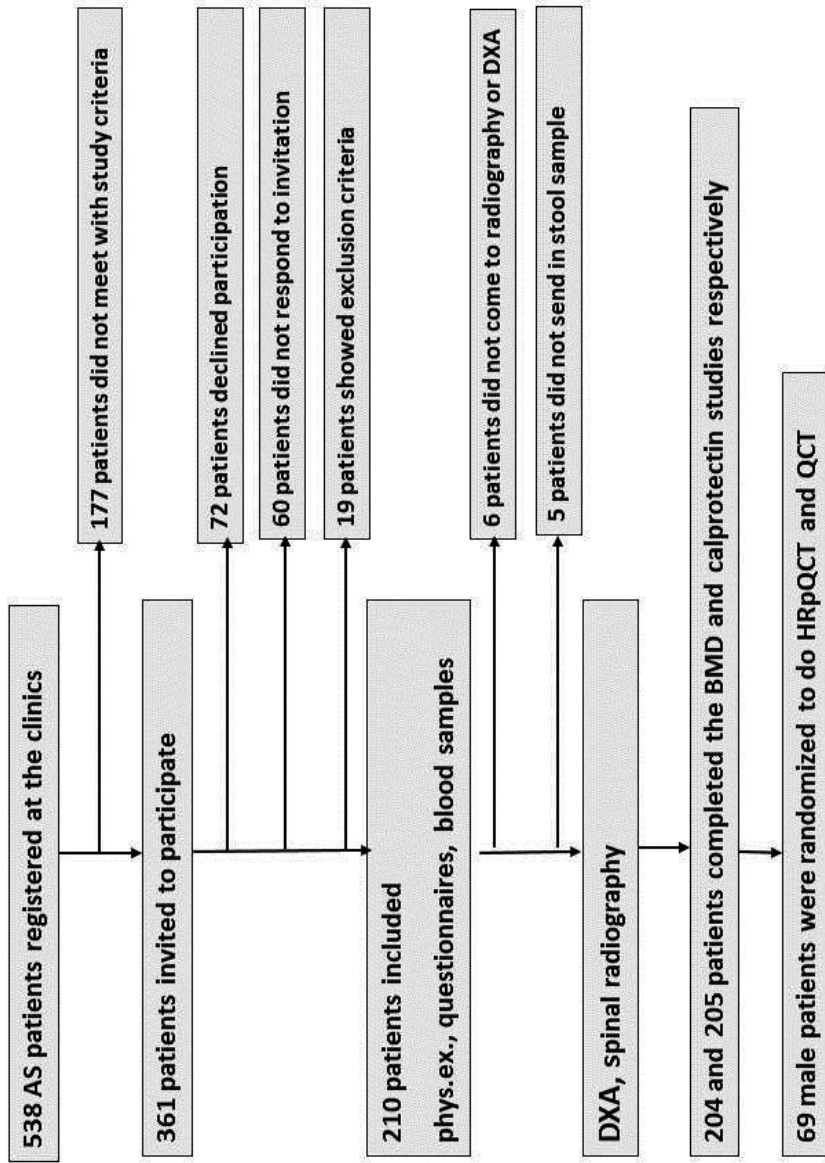
69 male patients were randomized to do HRpQCT and QCT

Table 8: Characteristics of 204 patients with ankylosing spondylitis in western Sweden

		Number of patients (%)	Median(range)	Mean±SD
Demographic variables				
Sex	Women	87 (43)		
	Men	117 (57)		
Age, years			49 (17, 78)	50±13
Postmenopausal women		45/87 (52)		
Heredity for fractures		57 (28)		
Current smokers		24 (12)		
Ever smokers (> 6 months)		101 (49.5)		
Daily calcium intake from dairy products			600 (0, 2640)	668±397
BMI			25 (19, 46)	26±4
LTPAI total, hours			6 (0, 42)	7.5±6
PAHWI total, hours			45 (0, 160)	40±21
Disease related variables				
Years since symptom onset			24 (2, 55)	24±13
Years since diagnosis			12 (1, 47)	15 ±11
Present or past anterior uveitis		102 (50)		
Present or past peripheral arthritis		120 (59)		
Present or past coxitis		17 (8)		
BASMI, score			3.0 (0.6, 7.4)	3.1±1.6
BASDAI, score			3.5 (0, 9.6)	3.6±2.1
BASFI, score			2.3 (0, 8.7)	2.7±2.1
BAS-G1, score			2.9 (0, 10)	3.4±2.6
BAS-G2, score			3.4 (0, 9.7)	3.8±2.6
ASDAS, score			2.3 (0.8, 5.9)	2.4±0.9
mSASSS, score			5.5 (0, 72)	14.2±19.2
Mean ESR 2004-2008, mm/h			16 (2, 102)	19±15
ESR, mm/h			11 (2, 105)	15±14
CRP, mg/L			5 (3, 80)	9±10
Hemoglobin, g/L			139 (105, 166)	139±13
WBC, x10^9 /L			6.7 (2.7, 18.1)	7.0±2.1
PLT, x10^9 /L			287 (133, 506)	299±75
Creatinine, μmol/L			70 (43, 148)	71±15
HLA-B27 positive		178 (87)		
Patients on NSAID		158 (77)		
Patients on DMARD		62 (30)		
Patients on TNF inhibitor		42 (21)		
Patients on glucocorticoids		7 (3)		
Prednisolone lifetime use, mg			100 (0, 56390)	1397±5775
Patients on bisphosphonates before study		8 (4)		
Patients on HRT		5 (2)		
Patients on calcium and vitamin D before study		24 (12)		

Ethics

Paper I-V. The study was approved by the regional ethics committee in Gothenburg and carried out in accordance with the principles of the Declaration of Helsinki. All the patients and blood donor controls gave their informed written consent.

Questionnaires

Paper I-V. The patients were asked to fill in an extensive questionnaire with queries concerning medical history, AS manifestations, current and previous medication, gastrointestinal symptoms and risk factors for osteoporosis including tobacco and alcohol use, daily intake of dairy products, hormonal treatments and menopausal status. The questionnaire also contained questions about educational, occupational and socioeconomic status.

Physical activity was divided into three levels of intensity (light, moderate and heavy) and reported in hours per week for leisure time, at home and at work, using two validated questionnaires; the Leisure Time Physical Activity Instrument (LTPAI) and Physical Activity at Home and Work Instrument (PAHWI).[181]

Questionnaires concerning disease activity and function

Paper I-V. The study protocol also contained the following questionnaires, in which the patients graded their symptoms from 0-100 using visual analogue scales (VAS):

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), with questions about pain in the back and peripheral joints, tenderness, fatigue and morning stiffness during the last week.[182] (Table 9)

The Bath Ankylosing Spondylitis patient Global score (BAS-G) where the patients grade their general health during the last week (BAS-G1) and last six months (BAS-G2).[183] (Table 10)

The Ankylosing Spondylitis Disease Activity Score (ASDAS) calculated using BASDAI questions 2, 3, 6, BAS-G and CRP. [184, 185] (Table 11)

The Bath Ankylosing Spondylitis Functional Index (BASFI), with questions concerning mobility and ability to perform tasks in daily life during the last week.[186] (Table 12)

Table 9. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

1. How would you describe the overall level of fatigue/tiredness you have experienced?

| None | 100 mm | Very severe |
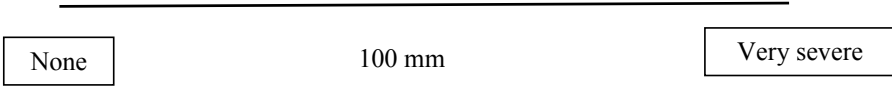
2. How would you describe the overall level of AS neck, back or hip pain you have had?

3. How would you describe the overall level of pain/swelling in joints other than neck, back or hip you have had?

4. How would you describe the level of discomfort you have had from an area tender to touch or pressure?

5. How would you describe the level of morning stiffness you have had from the time you wake up?

6. How long does the morning stiffness last from the time you wake up?

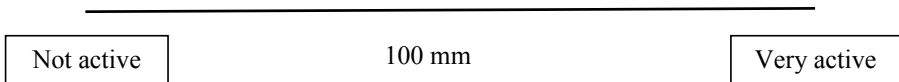$$BASDAI = \frac{Q1 + Q2 + Q3 + Q4 + \left(\frac{Q5 + Q6}{2}\right)}{5}$$

Table 10. Bath Ankylosing Spondylitis patient Global score (BAS-G)

BAS-G1

1. How active was your spondylitis on average during the last week?

| Not active | 100 mm | Very active |

BASG-2

2. How active was your spondylitis on average during the last 6 months?

Table 11. The Ankylosing Spondylitis Disease Activity Score (ASDAS)

The calculation of $ASDAS_{CRP}$:

(0.12 x BASDAI Q2) + (0.07 x BASDAI Q3) + (0.06 x BASDAI Q6) + (0.11 x BAS-G1) +
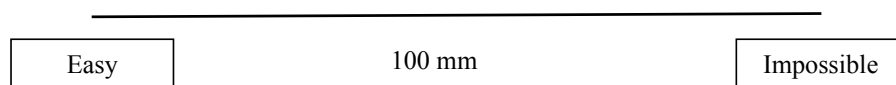
0.58 x Ln(CRP+1)

BASDAI Q2 = question 2 from BASDAI (see table 9)

Table 12: The Bath Ankylosing Spondylitis Functional Index (BASFI)

The questions regard the situation during last week.

Items to be scored by the patient:

1. Putting on your socks or tights without help or aids (eg, sock aid).

| Easy | 100 mm | Impossible |

2. Bending forward from the waist to pick up a pen from the floor without an aid.
3. Reaching up to a high shelf without help or aids (eg, helping hand).
4. Getting up out of an armless dining room chair without using your hands or any other help.
5. Getting up off the floor without help from lying on your back.
6. Standing unsupported for 10 min without discomfort.
7. Climbing 12 to 15 steps without using a handrail or walking aid. One foot at each step.
8. Looking over your shoulder without turning your body.
9. Doing physically demanding activities (eg, physiotherapy, exercises, gardening or sports).
10. Doing a full day's activities, whether it be at home or at work.

The BASFI is the mean of the 10 item scores.

Review of medical records

Paper I-V. The medical records of the patients were checked to verify that the inclusion criteria were fulfilled.

The lifetime use of glucocorticoids, converted into milligrams of prednisolone, was estimated both by reviewing patients' medical records and by asking the patients about previous glucocorticoid injections and oral prednisolone use.

The mean erythrocyte sedimentation rate (ESR) during the previous 5 years (2004-2008) was calculated using the first-noted ESR each year. An ESR test was excluded and the next consecutive ESR test was used if the medical records showed that the patient had had an infection at the time of the ESR test.

Laboratory analyses

Standard analyses
Paper I, II, III and V. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), creatinine, and alanine aminotransferase (ALT) were analyzed consecutively by standard laboratory techniques in the hospitals. Serum and plasma samples were obtained and stored at -20°C and -80°C until needed for analyses.

Biomarkers of bone metabolism
Paper III. Serum samples were assayed for biomarkers of bone metabolism using specific enzyme-linked immunosorbent assay (ELISA) kits according to manufacturers' instructions. The biomarkers measured were Wnt-3a and Wnt-5a (Uscn, Houston, Texas, US), Dkk-1 (R&D systems, Minneapolis, Minnesota, US), sclerostin (Biomedica gruppe, Vienna, Austria), sRANKL (Biovendor, Brno, Czech Republic) and OPG (Immunodiagnostic systems, Boldon, UK). The following values of sensitivity were reported by the manufacturers: Wnt3a lower limits of detection (LLD) =0,055 ng/mL; Wnt5a LLD=0,053 ng/mL; Dkk-1 LLD=4.2 pg/mL; sclerostin LLD=2.6 pmol/l; sRANKL LLD=0.4 pmol/L; OPG LLD=14 pmol/L.

Absorbance was read at 450 nm in SpectraMax 340PC[384] spectrophotometer (Molecular Devices). The software SoftMax Pro 5.2 was used to calculate the biomarker concentrations.

Calprotectin in feces and serum
Paper V. Stool samples were obtained for analysis of fecal calprotectin, using the standard laboratory technique of the hospital, a monoclonal antibody against calprotectin in an ELISA kit (Bühlmann Laboratories AG, Schönenbuch Basel, Switzerland). Normal range for fecal calprotectin is <50 mg/kg according to the manufacturer of the kit.

The patients who had a fecal calprotectin level above 500 mg/kg were asked to deliver another stool sample. If the level of fecal calprotectin remained over 500 mg/kg the patient was referred to colonoscopy.

Serum calprotectin was analyzed by ELISA (PhiCal, Immundiagnostic AG, Bensheim, Germany). Normal range for serum calprotectin using this kit is 500–3000 ng/mL.

Radiography
Paper I-V. All the patients underwent radiography of cervical-, thoracic- and lumbar spine.

Chronic AS related changes
Chronic AS related changes of the spine were estimated using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS), which assesses the anterior corners of vertebra C2 to T1 and T12 to S1. The corners are graded with 0-3 points each (0= normal, 1= erosion, sclerosis or squaring, 2= syndesmophyte, 3= bridging syndesmophyte) and the scale ranges from 0 to 72. The thoracic spine is excluded.[187] (Figure 7)

Vertebral fractures Genant score
Vertebral fractures were evaluated using the Genant score, which scores the vertebras Th4 to L4.[188] The vertebras are scored on visual inspection as normal (grade 0), mildly deformed (grade 1, 20%−25% reduction of the anterior, middle, or posterior height), moderately deformed (grade 2, 25%−40% reduction in any height), or severely deformed (grade 3, over 40% reduction in any height). The cervical spine excluded in this analysis. (Figure 8)

Figure 7: The mSASSS- score
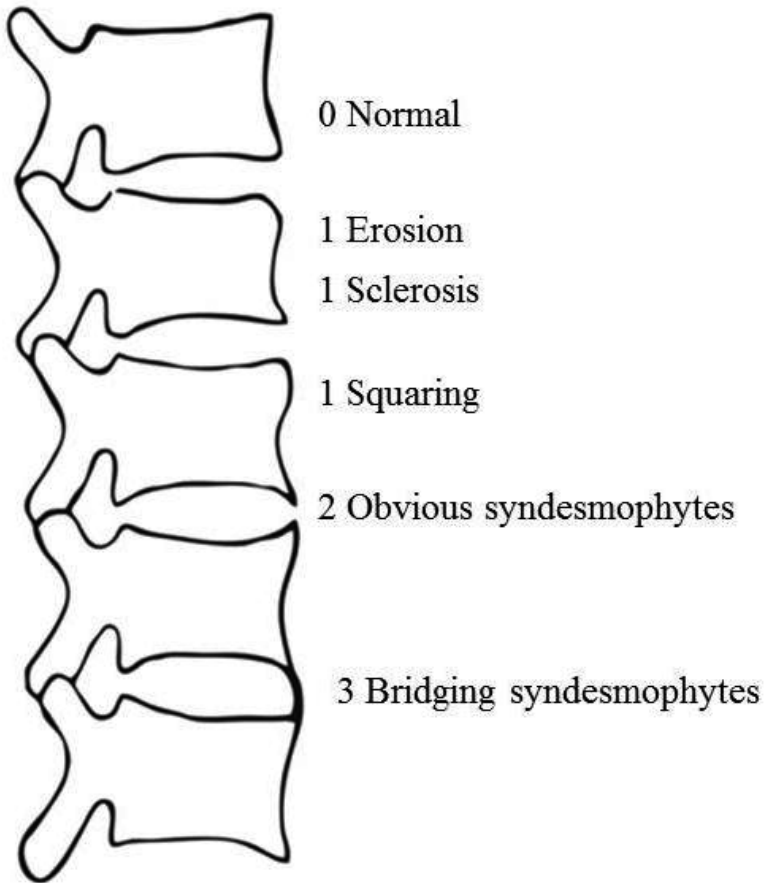
The score includes the anterior corners of vertebrae C2 to T1 and T12 to S1, which are graded with 0 to 3 points each (0 = normal, 1 = erosion, sclerosis or squaring, 2 = obvious syndesmophyte, 3 = bridging syndesmophyte). The remaining thoracic spine is not included in the score. The scoring scale ranges from 0 to 72.

Figure 8: The Genant score

Normal Grade 0

Wedge fracture Biconcave fracture Crush fracture

Mild fracture
Grade 1; 20-25%

Moderate fracture
Grade 2; 26-40%

Severe fracture
Grade 3; > 41%
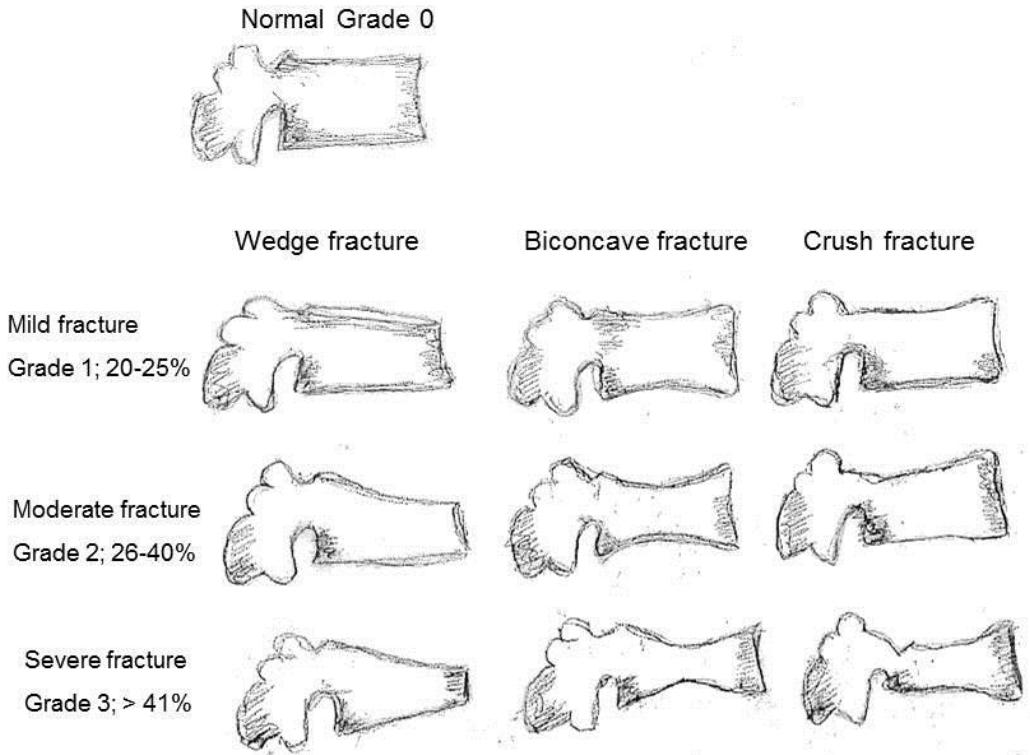
Bone mineral density

Dual energy X-ray Absorptiometry (DXA)
Paper I-V. BMD was measured with DXA (Hologic Discovery A, Hologic Inc, Bedford, MA, USA) in the non-dominant forearm, left hip (femoral neck and total hip regions) and in lumbar spine in anteroposterior (AP) (vertebras L1-L4) and lateral (L2-L4) projections with estimation of volumetric BMD.

Quantitative Computed Tomography (QCT)
Paper IV. A total of 69 male patients were randomised in an age-adjusted way to also measure lumbar vBMD in the vertebras L1-L4 with QCT (Siemens Somatom Sensation 16 with application Syngo Osteo CT, Siemens AG, Munich, Germany).

High Resolution peripheral Quantitative Computed Tomography (HRpQCT)
Paper IV. The 69 randomised male patients also underwent assessment of peripheral vBMD and bone microarchitecture with HRpQCT in the non-dominant ultra-distal radius and tibia (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland).

Statistical analysis

Paper I-V. Statistical analyses were performed using PASW Statistics 18.0 (SPSS Inc., IBM, Chicago USA). Descriptive statistics are presented as median and range and/or mean and standard deviation (SD). The t-test was used for comparison of normally distributed demographic and disease-related variables and the Mann-Whitney U-test was used to analyse variables that were not normally distributed. The chi-square test was used to compare categorical variables. Correlations were calculated using Spearman's correlation (r_s). For dichotomous variables, yes was coded 1 and no was coded 0. All tests were two-tailed and $p < 0.05$ was considered statistically significant.
Multiple linear regressions were run with continuous variables as outcome and logistic regression with categorical variables as outcome.
Paper I: Linear regression was run with BMD at different measurement sites as the outcome and logistic regression was run with the categorical variable low BMD T-score < -1.0 (yes/no) as the outcome. Covariates in both calculations were the variables that were significantly correlated with BMD in the first analyses.
Paper II: Logistic regression with a forward stepwise conditional method was run with the presence of a vertebral fracture as binary outcome. BMD and the demographic and disease-related variables significantly correlated with vertebral

fractures were entered as covariates. To elucidate the effect of vertebral fractures and chronic AS related changes of the spine on back-mobility multiple linear regression was run with BASMI as dependent variable and age, Genant score and mSASSS as independent covariates.

Paper III: Multiple linear regressions were run using a stepwise method with BASMI, mSASSS and BMD at different measuring sites as outcome. In the model where mSASSS was the outcome, mSASSS was normalised using a log-transformation and patients with only sacroileitis (mSASSS=0) were excluded, leaving data from 148 patients ready to analyse. Covariates in all multiple linear regressions were age, sex, pack-years of cigarettes, CRP and the analysed biomarkers.

Paper IV: Logistic regressions with a forward conditional method were run with presence of a syndesmophyte (yes/no) as the binary outcome.
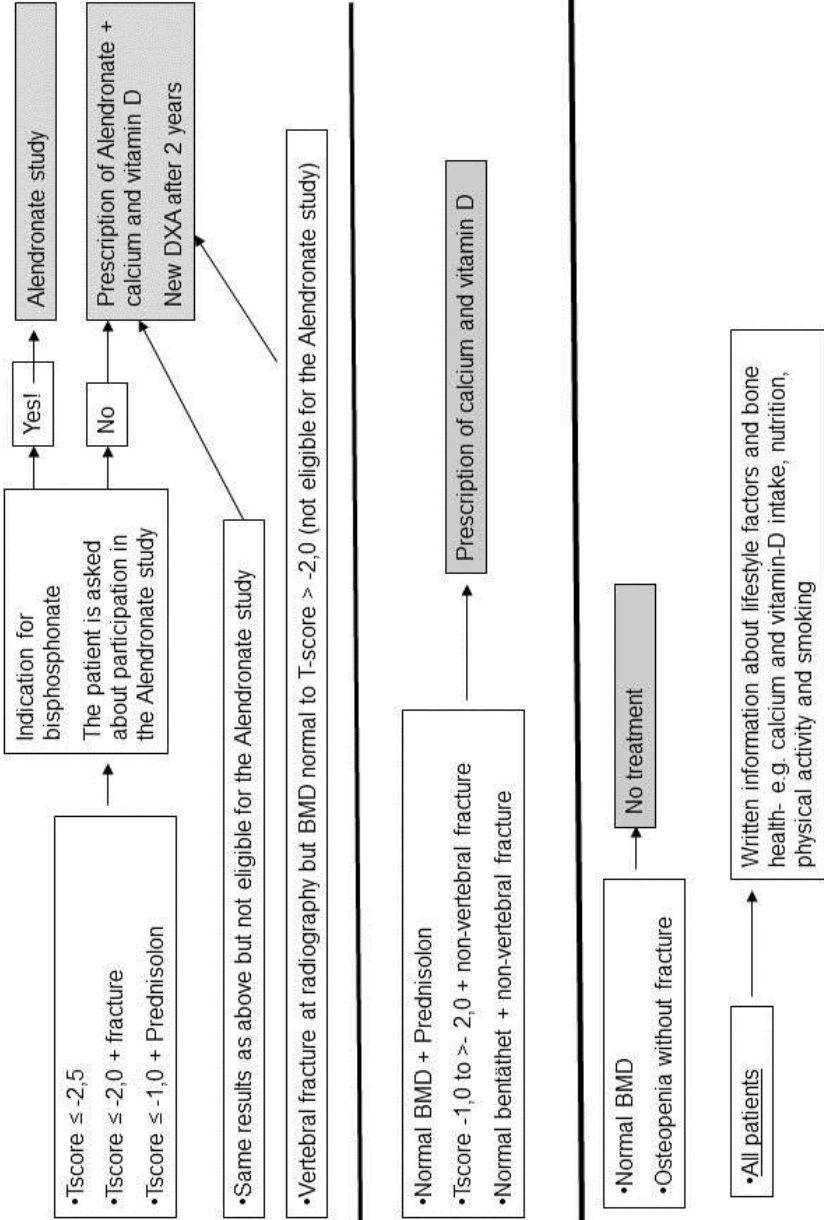
Paper V: A log transformation of fecal calprotectin and serum calprotectin was made, to convert them into normally distributed variables. Multiple linear regression analyses were performed in a stepwise method, to explore the relationship between log-transformed calprotectin and the demographic and disease-related variables, which had shown significant correlations in the first analyses.

Follow-up and treatment after the study

The results from the DXA measurements and radiography were communicated to the patients by mail. A flow-chart for the follow-up and treatment of the patients is shown in fig 9. After the study treatment with a bisphosphonate in combination with calcium/vitamin D was initiated in 30 patients (14.2%) and calcium/vitamin D alone in 28 patients (13.2%9).

The patients who had indication for bisphosphonate treatment were invited to participate in a prospective study on alendronate and calcium/vitamin D during 24 months. The objectives of the Alendronate study were to study the treatment effects on BMD, bone microarchitecture, osteoproliferation, disease activity and biomarkers for bone metabolism.

Figure 9: Flow-chart for treatment and follow up of the AS patients after the study

- Tscore ≤ -2,5
- Tscore ≤ -2,0 + fracture
- Tscore ≤ -1,0 + Prednisolon

→ Indication for bisphosphonate

The patient is asked about participation in the Alendronate study

- Yes! → Alendronate study
- No → Prescription of Alendronate + calcium and vitamin D

New DXA after 2 years

- Same results as above but not eligible for the Alendronate study
- Vertebral fracture at radiography but BMD normal to T-score > -2,0 (not eligible for the Alendronate study)

- Normal BMD + Prednisolon
- Tscore -1,0 to >- 2,0 + non-vertebral fracture
- Normal bentäthet + non-vertebral fracture

→ Prescription of calcium and vitamin D

- Normal BMD
- Osteopenia without fracture

→ No treatment

- All patients

→ Written information about lifestyle factors and bone health- e.g. calcium and vitamin-D intake, nutrition, physical activity and smoking

MAIN FINDINGS OF THE THESIS

Paper I: Osteoporosis in ankylosing spondylitis - prevalence, risk factors and methods of assessment.

This study showed that osteoporosis was common, but often undiagnosed and untreated in AS. Osteoporosis was found in 20.8% and osteopenia in 43.6% of patients 50 years or older. BMD below expected range for age was found in 5% of patients younger than 50. Low BMD was found both in the axial and the peripheral skeleton. A total of 36% of women, but only 8% of the men had ever done a BMD measurement before the study. Most cases of low BMD were undiagnosed and untreated.

Men had significantly higher aBMD at all measurement sites (p < 0.001 for every location), but there was no significant difference in lumbar vBMD between the sexes

Low BMD was associated with several disease-related parameters, such as long disease duration, high BASMI, high mSASSS, history of coxitis, high lifetime use of prednisolone, low hemoglobin and elevated inflammatory parameters (mean ESR during 2004-2008, CRP, WBC and PLT). The most important factors associated with low BMD in multiple linear regression analyses were old age, long disease duration, high BASMI, low BMI and menopause.

The study also showed that lateral lumbar DXA was more sensitive than AP lumbar DXA in diagnosing low BMD in AS. Significantly more women had a lumbar spine BMD T-score ≤ -2.5 SD (age ≥ 50) or Z-score ≤ -2.0 SD (age < 50) when measured with lateral DXA than when measured with AP DXA (totally 23, 26% vs. totally 14, 16%; p = < 0.001). T-scores and Z-scores for lateral lumbar DXA were not available for the men, due to the lack of a male reference data base.

In addition the results from the study indicated that lateral and volumetric lumbar BMD were less affected by syndesmophyte formation compared with AP lumbar BMD. mSASSS was negatively correlated with lateral and volumetric lumbar BMD and with BMD of the hip and radius, whereas AP lumbar BMD had a tendency to increase with increasing mSASSS. Lumbar BMD (g/cm^2) was also significantly lower when measured in lateral projection compared with AP projection (p<0.001).The difference between AP and lateral lumbar BMD increased with increasing mSASSS (r_S=0.389; p<0.001) and BASMI (r_S=0.296; p=0.001) in the men, but not in the women.

Paper II: Vertebral fractures in ankylosing spondylitis are associated with lower bone mineral density in both central and peripheral skeleton.

This study showed that the diagnosis of a vertebral fracture in AS is often missed in the clinical practice. A total of 42 vertebral fractures were found by radiography (Th4-L4) in 24 patients (11.8%). In 21 of these patients the fractures had not come to clinical attention before the study. In addition totally 5 cervical fractures were found in 3 patients. In contrast only a few non-vertebral low trauma fractures were noted, including one hip fracture. The prevalence of vertebral fractures was equal between the sexes, but the men with fractures were significantly younger than the women (53.2 ± 10.2 vs. 63.4 ± 5.8 years; p = 0.012), the youngest man being 31 years, indicating an increased risk for vertebral fractures in younger male patients.

The most important determinants for vertebral fractures were high age, long disease duration, impairment of back mobility, advanced syndesmophyte formation, smoking, menopause and low BMD in both the axial and peripheral skeleton.

Lateral and volumetric lumbar BMD showed stronger correlation with number of vertebral fractures (Genant score) than AP lumbar BMD. Low lumbar volumetric BMD and femoral neck BMD together with low self-estimated general health (BAS-G2) were the strongest determinants for a vertebral fracture in age-adjusted logistic regression.

Paper III: Biomarkers of bone metabolism in ankylosing spondylitis in relation to osteoproliferation and osteoporosis

The following biomarkers of bone metabolism were measured in serum: Wnt3a, Wnt5a, Dkk-1, sclerostin, sRANKL and OPG.

This study showed that in comparison with healthy age- and sex-matched blood donor controls the AS patients had significantly higher serum levels of Wnt3a (3.72 ± 0.99 ng/mL vs. 2.88 ± 0.84; p<0.001) and lower serum levels of sclerostin (35.33 ± 21.54 pmol/L vs. 38.33 ± 13.96; p=0.014) and sRANKL (223.98 ± 254.03 pmol/L vs. 274.64 ± 270.44; p=0.047).

BMD of femoral neck was negatively correlated with Wnt3a and OPG and positively correlated with sRANKL in the univariate analyses.

The serum levels of Wnt3a were positively correlated with mSASSS (r_S=0.196; p=0.005) and BASMI (r_S=0.219; p=0.002), but not with age, indicating that Wnt3a could be a marker for the osteoproliferative process in AS.

OPG and sclerostin were also positively associated with mSASSS and BASMI, but this could be attributable to their association with age. CRP was negatively correlated with both sclerostin (r_S= - 0.208; p=0.003) and Dkk-1 (r_S= -0.140; p=0.045), but no other significant associations were found between the biomarkers and disease activity parameters.

The study also showed that tobacco smoking was associated with osteoproliferation, impairment of back mobility and physical performance in AS. A total of 12% of the patients were current tobacco smokers and 46% were ever-smokers, defined as being a current smoker or having smoked during at least 6 months previously. The ever-smokers had significantly higher mSASSS (mean±SD, 20±22 vs. 9±15; p<0.001), BASMI (3.6±1.6 vs. 2.6±1.4; p<0.001), BASFI (3.2±2.2 vs. 2.2±1.9; p=0.001) and age (53±12 vs. 47±13; p=0.001) than the never-smokers.

In addition the study showed that the men had significantly higher mSASSS than the women (median 8, range 0-72 vs. median 2, range 0-46; p<0.001).

Multiple linear regression analyses were run with BASMI, BASFI, mSASSS and BMD of femoral neck as outcome. In these analyses BASMI was independently associated with high age, male sex, high smoking pack years, high CRP and elevated serum levels of Wnt-3a, whereas BASFI was associated with high age and high smoking pack years. High mSASSS was independently associated with high age, male sex, high CRP and elevated serum levels of Wnt3a. Low BMD of femoral neck was associated with high age, female sex, high mSASSS and low serum levels of sclerostin.

Paper IV: Bone microarchitecture in ankylosing spondylitis and the association with bone mineral density, fractures and syndesmophyte formation.

This study on 69 male AS patients indicated that the patients had lower vBMD in the peripheral skeleton than healthy age-matched controls. The AS patients had significantly lower vBMD in cortical bone of ultra-distal radius (850±55 mg/cm^3 vs. 875±41; p=0.004) and in trabecular bone of ultra-distal tibia (187±35 mg/cm^3 vs. 201±41; p=0.033) when measured with HRpQCT.

Furthermore the results from the study showed that trabecular bone loss in axial and peripheral skeleton were associated events in AS. Strong correlations were found between lumbar trabecular vBMD measured with QCT and peripheral trabecular vBMD measured with HRpQCT in ultra-distal radius (r_S=0.762; p<0.001) and tibia (r_S=0.712; p<0.001). Low lumbar trabecular vBMD was in addition associated with deterioration of the microarchitecture of peripheral bone; thinner and fewer trabeculae, thinner cortex, lower cortical vBMD and increased cortical porosity.

The study also showed that vertebral fractures were associated with lower vBMD in both the axial and peripheral skeleton and in addition coupled with poorer peripheral bone microarchitecture. The AS patients with a vertebral fracture had, in comparison with age-matched AS controls, lower cortical vBMD in lumbar spine, lower trabecular and cortical vBMD in ultradistal radius and in addition reduced cortical cross-sectional area and thinner cortical bone and trabeculae in both ultra-distal radius and tibia. We also found that

syndesmophyte formation was associated with decreasing trabecular but increasing cortical vBMD in lumbar spine after adjusting for age in logistic regression. The increasing cortical vBMD was presumably reflecting hyperostosis in the cortical part of the vertebral bodies.

Furthermore the study showed that lumbar QCT was more sensitive in detecting low BMD than AP lumbar DXA. QCT revealed significantly more cases with T-score $\leq$ - 2.5 SD (n=26; 38%) and T-score < - 1.0 SD (n=21; 30%) than AP DXA, showing considerably less osteoporosis (n=4; 6%) and osteopenia (n=13; 19%), (p<0.001). Estimated lumbar vBMD by DXA correlated well with vBMD measured with QCT (r_S=0.636; p<0.001).

<u>Paper V: Calprotectin in ankylosing spondylitis - frequently elevated in feces, but normal in serum.</u>

This study showed that elevation of fecal calprotectin was common in AS, although not associated with gastrointestinal symptoms. A total of 68% (n=140) of the patients had fecal calprotectin above the threshold value 50mg/kg, whereof 62 patients had levels above 200 mg/kg, which is considered clinically more significant. The percentage of patients with elevated fecal calprotectin was not significantly different between users and non-users of NSAIDs (71% vs. 63%), but the NSAID users had higher fecal calprotectin levels (median, range 109, 0-1229 mg/kg vs. 63, 10-510; p=0.003). Furthermore the elevation of fecal calprotectin in NSAID-users followed a dose dependent pattern. Fecal calprotectin was also higher in users of proton pump inhibitors (PPI), for unexplained reasons. Conversely users of methotrexate and infliximab displayed lower levels of fecal calprotectin than non-users, which is consistent with their effect on intestinal inflammation in IBD.

A small series of ileo-colonoscopies were performed in patients with fecal calprotectin >500 mg/ml, showing macroscopic or histologic signs of chronic inflammation in 5 out of 8 patients.

Fecal calprotectin showed weak correlations with disease activity parameters, ESR, CRP and ASDAS, but was not associated with chronic AS related changes in the spine or disease manifestations, such as uveitis or swollen joint count.

Furthermore unpublished data showed that the levels of fecal calprotectin were not convincingly associated with osteoporosis. Although fecal calprotectin was weakly correlated with vBMD of lumbar spine (r_S= - 0.160; p=0.025), no significant correlations were found with AP or lateral lumbar BMD or BMD measured in the hip or radius. The data is arguing against the hypothesis of intestinal inflammation as an important contributor to the osteoporosis in AS.

The study also showed that the serum levels of calprotectin were normal in 98% of the patients and not different from the levels of healthy controls. Serum calprotectin was positively associated with WBC, ESR, CRP, fecal calprotectin and ASDAS.

DISCUSSION

This thesis on osteoporosis and osteoproliferation in AS is based on a cross-sectional study with 210 included patients recruited from three rheumatology clinics in West Sweden; the Sahlgrenska University Hospital and the Departments of Rheumatology at the Hospitals of Borås and Alingsås.

Generalizability of the results

Were the patients representative for other AS patients followed at the three study centers? To analyze this we compared the medical records of the included patients (n=210) with the AS patients who were eligible for inclusion but declined participation or did not respond to invitation (n=132). (Fig 3) The patients who declined participation or did not respond to invitation were significantly younger than the included patients (46±13 years vs. 50±13 years; p=0.007) and had shorter disease duration (12±10 years vs. 15±11 years; p=0.035), but there was no significant difference in sex distribution or medication between included and not included patients. Our conclusion was that apart from the differences in age the included patients were representative for other AS patients followed at three study centers.

Possible reasons for the age difference could be that younger patients declined participation more often due to lack of time or lower interest in having their BMD measured compared with older patients, but this is only speculation.

Can the results regarding prevalence and risk factors for osteoporosis and vertebral fractures be generalized to encompass all Swedish AS patients, including patients followed in the primary health care? Not automatically. Patients followed at rheumatology clinics in Sweden usually have a more severe disease, with increased need of disease modifying anti-rheumatic drugs (DMARDs), compared with patients followed in the primary health care. The patients in the study reported a relatively high frequency of different disease manifestations such as uveitis (50%), arthritis (59%) and coxitis (8%) at present or in the past, but in many patients the disease activity was moderate (mean±SD BASDAI 3.6±2.1, ASDAS 2.4±0.9, BASFI 2.7±2.1) and inflammatory parameters were often normal (mean±SD CRP 9±10, ESR 15±14, mean ESR during 2004-2008 19±15). At radiography 27.5% of the patients had a disease restricted to the sacroiliacal joints and 72.5 % had any detectable chronic AS related change in cervical or lumbar spine. A total of 47% had syndesmophytes. Impairment of back-mobility and advanced ankylosis are however not indications to start treatment with DMARDs at present; hence many patients with advanced ankylosis but absence of high inflammatory activity are referred to the primary health care. Consequently patients who suffer from

osteoproliferation at high risk of osteoporosis and vertebral fractures are found in the primary health care as well.

Prevalence and risk factors for osteoporosis and vertebral fractures – clinical implications

One of the principal findings of this thesis was that both osteoporosis and vertebral fractures were common, but very often not diagnosed or treated in AS patients followed at rheumatology clinics in West Sweden. The first reports regarding osteoporosis in AS came already in 1970'Th, but the knowledge seems not to have been implemented in clinical practice. A total of 36% of the women, but only 8% of the men included in the study had ever done a BMD measurement before the study. Eight patients were on a bisphosphonate before the study, but treatment was initiated in additionally 30 patients after the study. Thus 14.2% of the study population had a low BMD which was insufficiently treated. Accordingly there is room for improvement in the diagnosing and management of osteoporosis in AS.

High age and long disease duration were important risk factors for vertebral fractures and low BMD, but vertebral fractures and low BMD were found in patients aged 30-50 years too. The results from this thesis show that osteoporosis and vertebral fractures should be suspected in patients of both sexes with signs of high disease burden, such as impaired back-mobility, advanced syndesmophyte formation or ankylosis, elevated ESR and CRP, hip involvement or high lifetime use of glucocorticoids.

Vertebral fractures are important to diagnose in AS for several reasons. Firstly, it's important to distinguish fractures from disease flares caused by inflammatory activity, since this has major implications on the choice of treatment. Secondly, vertebral fractures can increase the kyphosis and contribute to the impairment of back-mobility in AS.[189] Thirdly, spinal fractures can be complicated by neurologic injuries.[176] Fourthly, the presence of vertebral fractures can be an indication for osteoporosis treatment regardless of BMD.[190] Fifthly, because studies on the general population have shown that a prevalent vertebral fracture is a strong indicator for the risk of a subsequent one and that vertebral fractures are associated with increased morbidity and mortality.[191-193]

Which AS patients should be evaluated for osteoporosis?

Based on the results from this thesis it's recommendable to measure BMD regularly in all female and male AS patients from the age of 50. If BMD is normal the assessment should be repeated within five or ten years, since BMD is

likely to decrease by time. BMD assessment should be considered also in younger patients with impaired back-mobility, advanced AS related changes in the spine or high inflammatory activity.

Could fracture risk assessment (FRAX®) be used in selecting AS patients for BMD evaluation? We compared the FRAX® values for patients with and without a vertebral fracture and found that both FRAX® for the % ten-year probability of major osteoporotic and hip fracture were significantly higher in the patients with a vertebral fracture. The FRAX® tool is however not based on individuals with AS and the questionnaire does not contain questions concerning disease activity, back-mobility and osteoproliferation, which are relevant for AS. In the FRAX® tool female sex is a risk factor for fracture. In AS however, male patients have been shown to have the same or greater risk for osteoporosis and fractures than women. Consequently the FRAX® tool is not very well suited for the evaluation of AS patients.

Which AS patients should be evaluated for vertebral fractures?

The presence of a vertebral fracture should also be investigated at more regular intervals. The risk of a vertebral fracture should be considered in patients with impaired back-mobility, increasing tragus to wall distance, loss of body height of more than 3 cm, increased back pain and in patients who have been subject to trauma, even minor injury. Vertebral fractures can be assessed with lateral radiographs of the cervical, thoracic or lumbar spine. Vertebral fracture assessment (VFA), which is done using images obtained during the DXA procedure, could be another alternative in the screening for vertebral fractures.[194, 195] VFA gives minimal radiation doses and has the advantage of being done in conjunction with the DXA assessment. The method needs however further evaluation in AS. VFA usually includes vertebra Th4-L4, but not the entire columna.
In patients with persistent back pain after any type of trauma an evaluation with CT or MRI should be considered if the initial plain radiograph is negative. This is imperative if the patient has any neurological symptoms.

Assessment methods for BMD - what could be recommended?

We used only conventional methods to identify the patients in whom bisphosphonate treatment was initiated after the study; that is ordinary DXA assessment in combination with spinal radiography applying the WHO-definition of osteoporosis and using national guidelines for treatment.
Thus, by using ordinary assessment methods which are well accessible it's possible to find many AS patients with need of osteoporosis treatment.

There are however some problems in the diagnosing of osteoporosis in AS. Lumbar BMD measured with AP lumbar DXA can be difficult to interpret, since the osteoproliferative process causes falsely elevated BMD values. In the present thesis we show that lateral lumbar DXA is more sensitive in detecting low BMD, is less affected by the osteoproliferation in AS and more closely correlated with vertebral fractures

Limitations of lateral lumbar DXA are however that reference values for men are missing and that the reference data base for women is based on a limited number of individuals at present. In addition the current WHO definition of osteoporosis, with the threshold set at a T-score of -2.5 SD using AP lumbar DXA, cannot be applied to lateral DXA directly, since this could risk over diagnosing of osteoporosis and that substantially more individuals would get the diagnose of osteoporosis.[196] At present there is no evidence supporting osteoporosis treatment in patients with low lateral lumbar BMD if AP lumbar BMD and BMD of the hip is normal.

It's advisable to use BMD of both total hip and femoral neck in the assessment of AS patients. In patients with advanced ankylosis, where AP lumbar spine measurements cannot be interpreted, measurements of the forearm should also be used.[133] At present lateral lumbar DXA and estimated lumbar vBMD can only be used for follow-up of treatment. Estimated vBMD is the first to decline when bone is lost and the first to improve following osteoporosis treatment. Measuring lumbar vBMD with QCT can be considered in a patient with bilateral hip prosthesis or in patients with advanced ankylosis of the spine. Lumbar QCT measurements can be used in therapeutic decisions in conjunction with clinical risk factors, according to the official standpoints of ISCD.[133]

Treatment of osteoporosis in AS

Bisphosphonates are used for treatment of osteoporosis in AS, but there is a lack of prospective studies on the effects of bisphosphonates on BMD and osteoproliferation. Therefore the patients with indication for bisphosphonate treatment in the current study were invited to participate in a prospective interventional trial with alendronate and calcium/vitamin D during 24 months.

There is one retrospective study, were AS patients with different treatments for osteoporosis were followed during a mean time of 15 months. The patients treated with bisphosphonates showed a significant improvement of BMD in lumbar spine, but not in the hip. The follow up time was however too short to demonstrate a difference. It was concluded that the best effect on BMD was found in patients treated with a bisphosphonate in combination with a TNF-inhibitor.[197] Treatment with zoledronic acid in DBA/1 mice has also been shown to improve BMD, without affecting arthritis or ankylosis.[198]

Bisphosphonates have anti-inflammatory effects. Pamidronate has been reported to have effect on disease activity in AS.[199, 200] A randomized placebo controlled trial on alendronate in AS could however not show any effects on disease activity as presented in an abstract in 2011.[201] Both pamidronate and zoledronic acid have shown beneficial effects on symptoms, without increased osteoproliferation, in synovitis, pustulosis, hyperostosis and osteitis (SAPHO) syndrome, which like AS is a disease characterized by new bone formation.[202, 203]

It is also noteworthy that several open label studies have shown that TNFα – blocking agents have positive effects on BMD in AS.[159, 163] The effect is however not sufficient to merit the use of a TNFα-blocker in mono-therapy for osteoporosis.

In the current study we noted that many AS patients had a low intake of dairy products. Totally 63% of the patients had a calcium intake of less than the recommended 800 mg daily, based on their reported intake of dairy products and calcium substitution. AS patients may thus need more information about calcium intake, since they are at high risk of developing osteoporosis.

Trabecular bone loss affects the whole skeleton in AS, but fractures mainly occur in the spine- why?

The findings in this thesis indicate that osteoporosis in AS is a systemic process and that loss of trabecular bone in axial and peripheral bone is coupled.

We found almost as many patients with peripheral as axial osteoporosis or osteopenia. Furthermore trabecular vBMD in lumbar spine and ultra-distal radius and tibia were strongly correlated. Vertebral fractures, low lumbar vBMD and chronic AS related changes in the spine were in addition associated with a deterioration of the peripheral bone microarchitecture.

There are several factors which influence bone tissue in the whole skeleton, such as heredity, ageing, hormonal deficiency, smoking, vitamin D deficiency, physical activity, alterations of the calcium homeostasis and malnutrition. The increased expression of inflammatory mediators in AS, such as TNFα, IL-1, IL-6 and IL-17 also exerts effects on all bone tissue.

Nevertheless, elevated frequencies of vertebral fractures, but not non-vertebral fractures have been observed in AS. This has led to the view that the osteoporosis mainly affects the axial skeleton. In our study there was also a discrepancy between the number of vertebral fractures (n=42) and hip fractures (n=1). How could this be explained if osteoporosis is a systemic process?

Hip fractures occur late in life. In Sweden the mean age at time of a hip fracture is 80 years for women and 76 years for men.[136] The patients in the current study were younger, with a mean age of 50. In addition the data on the prevalence of peripheral fractures in the study was based on questionnaires filled in by the patients and there is a risk that peripheral fractures were underreported.

Reasons for the increased prevalence of vertebral fractures may be sought in mechanical and inflammatory factors in the local environment of the spine in AS. The biomechanical properties of the spine are altered in several aspects in AS. The growth of syndesmophytes between the vertebras and the apposition of cortical bone can result in an unloading of the trabecular bone and further enhancement of trabecular bone loss in the vertebral bodies. We found that vBMD of trabecular and cortical bone was correlated in ultra-distal radius and tibia, but not in lumbar spine in accordance with the above mentioned hypothesis. After adjusting for age, trabecular bone loss was in fact associated with increasing density in cortical bone in lumbar spine.

Furthermore the degeneration of the intervertebral discs during the course of the disease can reduce their shock-absorbing capacity resulting in increased risk of fractures. In addition bridging syndesmophytes and ankylosis makes the spine react more like a stiff long-bone rather than a flexible vertebral column upon trauma; instead of absorbing force by bending the tissues brake. It has also been hypothesized that the rigidity in the SI-joints and spine would result in a disturbance of the muscle tonicity in AS, which also could increase the propensity to fracture.

It is also possible that trabecular bone loss is enhanced in the vertebras due to the proximity to inflammation, with locally elevated levels of inflammatory mediators.

Biomarkers for osteoporosis and osteoproliferation - clinical utility

In the cross-sectional setting of this study we found that Wnt-3a was elevated in AS in comparison with healthy controls and associated with poor back-mobility and advanced chronic AS related changes in the spine. We also found that low serum levels of sclerostin were associated with low BMD of femoral neck. Does this make Wnt-3a and sclerostin ideal biomarkers for osteoproliferation and osteoporosis respectively in AS?

The outcome measures in rheumatology (OMERACT), an independent organization of health professionals which strives to improve health outcome measures in rheumatic diseases, has developed a filter by which new outcome measures can be validated. The filter includes questions of truth, discrimination and feasibility. The following questions should be posed to apply the OMERACT filter on our results regarding Wnt-3a and sclerostin:

I) Is the measure truthful; does it measure what it intends to measure?

Bone modeling, remodeling and osteoproliferation are governed by paracrine and autocrine signals from several regulatory mediators which build up concentration gradients in the local extracellular milieu. The serum levels of these mediators may not reflect the concentrations in the periphery. Wnts are in addition hydrophobic molecules due to palmitoylation and difficult to dissolve.[204] It has been speculated that the hydrophobic properties of the molecule helps building up a concentration gradient towards cell membranes. Wnt-3a was the first Wnt to be isolated in an active form and characterized.[112] Wnt-3a can be secreted in a soluble active form into cell suspensions by cells overexpressing Wnt-3a.[205] Wnt-3a harvested from such cell media has been used in a large number of experiments. Sclerostin is predominantly expressed in osteocytes and the level of sclerostin in serum has been measured in many studies.

We cannot prove that the elevated serum levels of Wnt-3a we found in the AS patients emanate from bone tissue and osteoproliferation. We only report the association between serum levels of Wnt-3a and chronic AS related changes in the spine and reduction of back-mobility. Coherently we cannot prove if lower levels of sclerostin are associated with osteoproliferation or osteoporosis in AS.

II) Does the measure discriminate between situations that are of interest?

It is of great interest to find biomarkers for osteoporosis and osteoproliferation in AS. Such biomarkers could be used to study the pathogenesis of the disease, to help in the finding of new treatments against osteoporosis and osteoproliferation, to prognosticate and select patients for medication and to follow up the therapy. Wnts and sclerostin are interesting to study in AS due to their effects on osteoblasts.

The results regarding Wnt-3a and sclerostin have to be repeated in a setting where the serum levels are studied in relation to radiographic progression and progression of osteoporosis in AS over time.

III) Can the measure be applied easily, given constrains of time, money and interpretability?

Even if the levels in serum may not reflect what is happening locally at the sites of inflammation and osteoproliferation, useful biomarkers in AS have to be measurable in blood samples. Biopsy material is particularly difficult to obtain in this disease characterized by inflammation in SI-joints and vertebras and would not be a feasible alternative. Measuring levels of Wnt-3a and sclerostin with ELISA in serum are easy and inexpensive methods. Serum levels of sclerostin can be difficult to interpret since sclerostin is positively associated with age. It is too early to give an opinion on interpretability of Wnt3a.

Factors associated with spinal osteoproliferation in AS

In the current thesis we found that impairment of back mobility and chronic AS related changes in the spine were associated with male sex, long disease duration, smoking and elevated levels of CRP and Wnt-3a.

AS is a disease which affects more men than women, with a prevalence ratio of 2-3:1. Male sex is also a risk factor for radiographic severity, for reasons incompletely understood. It has been shown that women with AS have more peripheral arthritis and are more often HLA-B27 negative, but no gender difference in functional impairment has been shown.[206] There is evidence of differences in the way women and men inherit the disease, but no linkage between the X chromosome and susceptibility to AS has been found.[207] Men with AS have been shown to have higher levels of DHEA, but no other differences regarding levels of sex hormones have been found between those with and without AS within each sex.[208]

A problem of the currently most used score for back mobility, BASMI, is that the reference values are not adjusted for sex, age or body height. Men are usually taller than women and there is a risk for overestimating the back mobility in taller individuals.

Several studies have shown that smoking is associated with deterioration of function and back-mobility in AS.[209-211] In part this may be explained by smokers doing less exercise and having more comorbid conditions. Smokers have on the other hand been shown to have more physically demanding jobs than non-smokers in general.[212] Smoking has also been associated with radiologic progression in AS.[211, 213] A recent study reported that smoking was associated with earlier onset of IBP, higher disease activity and increased axial inflammation on MRI in early axial Spa.[214]
Tobacco smoke contains a mixture of substances which may trigger immune responses. It is not known by which mechanisms smoking is linked to inflammation and osteoproliferation in AS.
Smoking can induce citrullination of proteins in the lung and is a risk factor for anti-citrulline positive RA. Tobacco use has furthermore been associated with radiographic progression and poorer response to biologics in RA.[215]In contrast to RA, synovial biopsies from AS patients have not been positive to staining for citrullinated proteins.[216] Anti-citrullin antibodies are however occasionally seen in AS (4%) and were in a recent study reported to be associated with peripheral arthritis.[217] In another recent study serum levels of citrullinated and degraded fragments of vimentin (VICM) were shown to be associated with radiographic progression in AS.[218] Tobacco use has also been

associated with the development of anti-dsDNA antibodies in SLE.[219] Smoking causes opposing effects on ulcerative colitis and Crohn's disease. Whereas smoking is protective for ulcerative colitis, smokers with Crohn's disease have a more aggressive course of the illness. The cessation of smoking reduces the risk of relapse in Crohn's in similar magnitude to what is obtained with immunosuppressives.[220]

Smoking is a risk factor for osteoproliferation and cardiovascular diseases which can be influenced, hence AS patients should be encouraged to stop smoking. In the present study 11.8% (n=24) were current smokers, but 33.8% (n=69) answered that they had smoked during at least 6 months before and that they now had stopped! In 2009, 10.2 % of the Swedish population was daily smokers. (www.sorad.su.se)

Calprotectin - clinical aspects

We found that 68% of the AS patients had elevated fecal calprotectin using the clinical standard assay of the time of the study and that levels of fecal calprotectin were associated with NSAID use, ESR and CRP.

Fecal calprotectin can be used to help distinguish between functional and organic gut disease in AS patients with gastrointestinal symptoms. A normal fecal calprotectin value speaks strongly against IBD as the cause of the symptoms. Elevated levels in the range of 150 to 200 mg/kg should lead to further examinations, often by colonoscopy. NSAIDs should if possible be stopped before the assessment.

Assessing fecal calprotectin in AS patients who lack gastrointestinal symptoms can however cause some trouble of mind in clinical practice. In many cases the fecal calprotectin levels will be elevated, caused by NSAIDs and/or subclinical gut inflammation and there are no clear recommendations how this should be dealt with.

AS patients with subclinical intestinal inflammation verified by biopsy have an increased risk of developing overt IBD.[22, 23] There is however at present no evidence in how to treat the subclinical gut inflammation and how to prevent the evolvement of IBD. In the current study treatment with methotrexate and TNFα-blockers where associated with lower levels of fecal calprotectin. It would be of interest to further study the effects of DMARDs on fecal calprotectin and gut inflammation in AS.

Daily NSAID use in AS - Pros and Cons

NSAIDs have good effect on inflammatory back pain and morning stiffness in AS.[87] Three different studies have in addition shown that daily or high use of

NSAIDs is associated with retardation of radiologic progression in AS. [88-90] Low dose or on-demand regime of NSAIDs has less effect on syndesmophyte formation. The effect of NSAIDs on bone formation may be mediated via hampering of PGE_2 expression in osteocytes in response to mechanical load. [91]

On the other hand we demonstrate that daily NSAID use is associated with significantly increased levels of fecal calprotectin, as compared with on-demand use of NSAIDs or no use. The elevation of fecal calprotectin could reflect either intestinal inflammation or gastrointestinal pathology caused by the NSAIDs. A study with capsule enteroscopy, before and after two weeks medication with diclofenac 150 mg daily, revealed new mucosal pathology and rise in fecal calprotectin in 68% of healthy volunteers.[221] NSAIDs are also known to cause disease flares in IBD and to cause side effects in oesophagus and ventricle, such as esophagitis, gastritis and ulcers.[222] It has been hypothesised that intestinal inflammation in HLA-B27 positive individuals can trigger spondyloarthritis. In this perspective daily use of NSAIDs could even be deleterious in AS.
Furthermore NSAID are associated with hypertension, renal insufficiency and cardiovascular morbidity and mortality.

So which patients should be put on continuous NSAID treatment? It has been shown that the presence of an existing syndesmophyte on radiography is a strong predictor for the development of new syndesmophytes.[213, 223] Patients with elevated CRP or ESR have been shown to benefit more from daily NSAID-use than patients with normal inflammatory parameters .[89]
A reasonable approach could be to recommend continuous daily NSAID treatment to younger and middle-age AS patients with radiographic syndesmophytes or elevated CRP or ESR and to avoid continuous NSAID in non-radiographic spondylarthritis, in elderly and in patients with increased cardiovascular risk or traits of IBD.

FUTURE PERSPECTIVES

On-going projects in association with the current study are:

- The treatment study with Alendronate in AS.

- A study on presence and risk factors for cardiac involvement in AS using electrocardiograms (ECG) and echocardiograms.

- A genetic project on single nucleotide polymorphisms (SNP) in the CARD8 and NLRP3 regions in AS.

The continuation of the research project

In 2014 we are planning to do a five-year follow-up, in which all patients included in the current study will be invited to participate. A new PhD student will be involved in this investigation. Radiography and BMD assessments will be repeated to study progression of osteoporosis and osteoproliferation in relation to variables of importance observed in 2009, biomarkers, medication, disease activity and life-style related factors during the five year follow-up period.

Other topics that would be interesting to study are:

- Vertebral fracture assessment with radiography compared with DXA VFA in AS.
- The effects of TNF-inhibition on biomarkers for bone metabolism prospectively.
- Gut inflammation in AS verified by colonoscopy in relation to fecal calprotectin.
- The effect of DMARDs on gut inflammation in AS.
- The progress of aortic valve insufficiency in AS over time in relation to disease activity, biomarkers and medication.
- Serum levels of vitamin D in relation to osteoporosis and disease activity in AS.
- HLA-B27 subtypes in relation to osteoporosis, osteoproliferation and disease manifestations.
- Genetic aspects of Wnts and Wnt-inhibitors in AS.
- Pathogenic effects of smoking in AS.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

BAKGRUND

Ankyloserande spondylit (AS) är en relativt vanlig kronisk reumatisk sjukdom som återfinns hos cirka 0.16-0.5 % av befolkningen i Sverige. Sjukdomen är två till tre gånger vanligare hos män än hos kvinnor. Insjuknandet sker oftast runt 25 års ålder och nästan aldrig efter 45 års ålder. Första symptom på sjukdomen brukar vara ryggsmärta och stelhet som orsakas av inflammation. Sjukdomen leder till en ökad förbeningstendens, med utväxt av bennabbar mellan kotor och i leder. I uttalade fall kan bäckenlederna förbenas helt och växa ihop, drabbas av "ankylos". Många patienter får problem med nedsatt rörlighet i ryggen, dels beroende förbeningstendensen, men också på grund av smärta och muskelspänningar.

Sjukdomen kan också ge inflammation och svullnad av leder, såsom knäleder, fotleder och höftleder. Inflammation i muskel-, ligaments- och senfästen är vanligt förkommande. Andra organ utanför ledapparaten som också kan drabbas är ögon och hjärta.

Trots att sjukdomen ger ökad bennybildning har patienter med AS en ökad risk för benskörhet (osteoporos) och kotfrakturer. Kotfrakturer vid AS kan vara instabila och medföra risk för skador på ryggmärg och nerver. Vanlig bentäthetsmätning med dual energy x-ray absorptiometry (DXA) i ländrygg är inte tillförlitlig vid svår AS, eftersom bennybildningen utanför kotkropparna gör att bentätheten blir falskt för hög. Vid konventionell DXA mäts bentäthet i ländryggen framifrån. Om man mäter bentätheten i kotkropparna från sidan kommer mindre av bennybildningen med i beräkningen. Bentäthet kan också mätas med datortomografi, Quantitative Computed Tomography (QCT). Högupplösande perifer kvantitativ datortomografi (HRpQCT) är en ny röntgenmetod för bentäthetsmätning och avbildning av skelettdelar i tre dimensioner. Vid HRpQCT röntgas skelettet i mycket tunna skikt, mer än 100 snitt per cm, vilka därefter sammanställs med digital teknik så att man får en detaljerad avbildning av skelettdelen.

Sjukdomen AS har många gemensamma drag med inflammatorisk tarmsjukdom, dvs. med ulcerös colit och Crohns sjukdom. Patienter med inflammatorisk tarmsjukdom kan utveckla en bild som liknar AS och vid tarmundersökningar (koloskopier) av AS patienter har man funnit att många har en tyst låggradig inflammation i tarmen. Calprotectin är ett ämne som finns i den vanligaste typen av vita blodkroppar, s.k. neutrofila granulocyter, som ansamlas i tarmväggen vid tarminflammation. Calprotectin mätt i avföring, s.k. fekalt calprotectin, används för att upptäcka tarminflammation och mäta hur aktiv och omfattande tarminflammationen är.

SYFTEN

Huvudsyften med studien var att undersöka förekomsten av benpåverkan, såväl osteoporos som bennybildning, hos patienter med AS och att studera samband mellan benpåverkan och sjukdomsaktivitet, sjukdomsmanifestationer och livsstilsfaktorer.

Specifika syften var

A. Att undersöka förekomsten är av osteoporos och kotfrakturer hos AS-patienter i Västra Götalandsregionen

B. Att identifiera faktorer av betydelse för utveckling av osteoporos, kotfrakturer och bennybildning vid AS.

C. Att jämföra olika metoder för mätning av bentäthet i ländryggen; DXA mätt framifrån eller från sidan och QCT

D. Att mäta nivåer av kemiska markörer, s.k. biomarkörer, för osteoporos och bennybildning i blodet hos AS patienter jämfört med friska kontrollpersoner och studera samband mellan biomarkörer och sjukdomsaktivitet, ryggrörlighet, bentäthet och bennybildning.

E. Att med hjälp av HRpQCT jämföra bentäthet i perifert skelett hos AS patienter och friska kontrollpersoner och att studera samband mellan mikroarkitektur i perifert skelett och kotfrakturer, bennybildning samt bentäthet i ländrygg mätt med DXA and QCT.

F. Att, med provtagning av fekalt calprotectin, indirekt studera förekomst av tarminflammation hos AS patienter i relation till sjukdomsaktivitet, sjukdomsmanifestationer och bentäthet.

METOD

Alla patienter med AS som var registrerade vid Reumatologkliniken på Sahlgrenska Universitetssjukhuset och Reumatologmottagningarna i Borås och Alingsås erbjöds att delta i studien.

Patienterna fyllde i enkäter och lämnade prover. De undersöktes och ryggrörligheten mättes. Röntgenundersökning gjordes av nack-, bröst- och ländrygg för bedömning av förbeningstendens och förekomst av kotfrakturer. Bentäthetsmätning med DXA gjordes i vänster underarm och höft och i ländryggen både framifrån och från sidan.

Totalt 69 män blev slumpmässigt utvalda för att även göra bentäthetsmätning med QCT i ländryggen och HRpQCT i vänster underarm och underben. HRpQCT resultaten från AS patienterna jämfördes med värden från friska kontroller undersökta med samma typ av HRpQCT apparat på Mayokliniken i Rochester Minnesota USA.

Blodprover från 80 friska blodgivare insamlades för att kunna jämföra koncentrationer av olika biomarkörer i blodet hos AS patienter och friska.

RESULTAT

I studien inkluderades totalt 210 patienter, 43 % kvinnor och 57 % män, med ålder från 17 till 78 år. Medelåldern hos patienterna var 50 år. I medeltal hade det gått 24 år sedan insjuknandet och 15 år sedan diagnosen AS ställdes.

Hos patienter som var 50 år eller äldre diagnosticerades osteoporos hos 21 % och nedsatt bentäthet (osteopeni) hos ytterligare 44 %. Totalt 5 % av de patienter som var yngre än 50 år hade bentäthet som var lägre än förväntat för åldern.

Totalt 24 patienter (12 %) hade kotfrakturer som upptäcktes vid röntgen. Hos 21 av dessa patienter var kotfrakturerna inte kända sedan tidigare. I hela gruppen upptäcktes 42 frakturerade kotor, eftersom vissa patienter hade flera kotfrakturer. Förekomsten av kotfrakturer var lika mellan könen, men män med kotfraktur var yngre än kvinnor med kotfraktur.

Totalt 36 % av kvinnorna hade gjort en bentäthetsmätning tidigare, men bland männen var motsvarande siffra bara 8 %. Efter studien startades osteoporosbehandling med en s.k. bisfosfonat i kombination med calcium och D-vitamin hos 30 patienter (14 %) och ytterligare 28 patienter (13 %) fick behandling med enbart calcium och D-vitamin.

Faktorer associerade med osteoporos var hög ålder, lång sjukdomsduration, dålig ryggrörlighet, hög förekomst av förbeningar vid ryggröntgen, hög sänka, kortisonanvändning, rökning, låg body mass index (BMI) och menopaus. Faktorer associerade med kotfrakturer var hög ålder, lång sjukdomsduration, dålig ryggrörlighet, hög förekomst av förbeningar vid ryggröntgen, dålig självskattad allmän hälsa, rökning, menopaus och låg bentäthet.

Vid jämförelser av bentäthet mätt med DXA framifrån och från sidan fann vi att DXA mätt från sidan var en känsligare metod för att upptäcka låg bentäthet, påverkades mindre av bennybildningen vid AS och hade ett högre samband med kotfrakturer.

Det viktigaste fyndet i studien av biomarkörer för bennybildning och osteoporos var resultat talande för att biomarkören Wnt-3a kan ha samband med bennybildning vid AS. Wnt-3a tillhör en grupp proteiner som kallas Wingless (Wnt), vilka har betydelse för utveckling och celldelning hos benceller (osteoblaster). Vi fann att AS patienterna hade signifikant högre koncentration av Wnt-3a i blodet än kontrollpersonerna. Höga nivåer av Wnt-3a i blodet var kopplat till sämre ryggrörlighet och mer uttalad bennybildning i ryggen. Låg bentäthet i lårbenshalsen hade samband med låga nivåer sclerostin.

Undersökningarna med HRpQCT i underarm och underben, samt QCT i ländrygg visade starka samband mellan bentätheten i det trabekulära benet i underarm, underben och ländrygg, talande för en koppling mellan benförlust i kotor och i perifert skelett.

Låg bentäthet i ländrygg, kotkompressioner och ökad bennybildning i ryggen var också associerat till försämring av mikroarkitekturen i perifert skelett, med färre och tunnare trabekler, större avstånd mellan trabekler och tunnare skikt av kompakt ben. Vid ökad förbeningstendens i ryggen såg man minskande bentäthet i det trabekulära benet i kotornas mitt, men ökad bentäthet i det kompakta benet i kotkropparnas periferi.

Totalt 68 % av AS patienterna hade förhöjt fekalt calprotectin som indirekt tecken på tarminflammation eller läkemedelspåverkan, men det fanns inget samband mellan nivåerna av fekalt calprotectin och tarmsymptom. Högre värden av fekalt calprotectin sågs hos patienter som medicinerade med inflammationsdämpande smärtstillande (antiflogistika/NSAID-preparat) och lägre värden sågs hos dem som medicinerade med metotrexat eller TNF-hämmare. Det fanns inget samband mellan fekalt calprotectin och bentäthet.

SLUTSATSER

Osteoporos och kotfrakturer är vanligt förekommande, men ofta odiagnostiserade och obehandlade hos patienter med AS som följs på reumatologkliniker i Västra Götaland. Viktiga riskfaktorer för osteoporos och kotfrakturer är hög ålder, lång sjukdomsduration, förbeningar vid ryggröntgen och dålig ryggrörlighet.

Bentäthet mätt med DXA i ländryggen från sidan kan vara ett bättre alternativ än mätning med DXA framifrån vid AS, men metoden behöver utvärderas mer och normalvärden för både kvinnor och män behöver utarbetas.

Patienter med AS har högre värden av Wnt-3a i serum än friska kontrollpersoner. Nivåer av Wnt-3a kan ha samband med bennybildning vid AS.

Osteoporos vid AS är en systemisk/utbredd process som drabbar både kotpelare och perifert skelett. Osteoporos, bennybildning och frakturer i ryggen är kopplat till försämring av mikroarkitekturen i det perifera skelettet.

Två tredjedelar av patienter med AS har förhöjt värde av fekalt calprotectin, talande för tyst tarminflammation eller läkemedelsbiverkan av inflammationsdämpande smärtstillande mediciner.

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