

A prospective cohort study on bone formation and bone loss in ankylosing spondylitis

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To John, Julia and David

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ABSTRACT

Background and objectives: Patients with ankylosing spondylitis (AS) have an increased risk of bone loss with development of osteoporosis and vertebral fractures (VFs) but also spinal new bone formation with growth of bony spurs (syndesmophytes) between the vertebrae. Measurements of spinal bone mineral density (BMD) by the routine method dual-energy x-ray absorptiometry (DXA) in anteroposterior (AP) projection can be difficult to interpret due to the spinal new bone formation. The general aims of this thesis were to study the development of bone loss and new bone formation over 5 years in patients with AS and to assess factors associated with the changes.

Methods: The studies included in this thesis are based on a cohort of patients with AS according to the modified New York criteria recruited from three rheumatology clinics in western Sweden. Patients completed the same protocol at baseline and at the 5-year follow-up with assessment of BMD with DXA at the hip (femoral neck, total hip), the spine (AP, lateral) and total radius and spinal radiographs for grading of AS related spinal alterations and VFs. A group of men were randomized in an age-adjusted algorithm to undergo high-resolution peripheral quantitative computed tomography (HRpQCT) at the ultra-distal radius and tibia for assessment of volumetric BMD (vBMD), cortical area and microarchitecture. Serum hepatocyte growth factor (s-HGF) was analyzed with enzyme-linked immunosorbent assay (ELISA) in the total cohort.

Results: Over 5 years, there were significant decreases in femoral neck BMD and tibia vBMD. Decreases were associated with signs of inflammation. In contrast, BMD at the total hip and the spine AP and lateral projections

increased. Use of bisphosphonates was associated with increases in BMD at all measured sites except tibia. Use of tumor necrosis factor inhibitors (TNFi) was associated with increases in BMD at AP spine and tibia. Only three patients developed new VFs. AS related spinal alterations increased significantly with higher increases in men compared to women. New predictors identified for spinal radiographic progression were obesity in both sexes and use of bisphosphonates and impaired mobility in women. Among previously known predictors, baseline AS related spinal alterations was shared by sexes, whereas baseline elevated CRP and smoking were predictors in men. The biomarker s-HGF was identified as a novel independent predictor of spinal radiographic progression in men.

Conclusion: The studies in this thesis suggest that the best site to assess bone loss in patients with longstanding AS is at the femoral neck and that inflammation has a negative impact on bone loss and development of AS related spinal alterations and thus is an important treatment target. The studies give further reasons to counsel the patients to stop smoking and to encourage obese patients to weight loss. Treatments with bisphosphonates and TNFi had a positive impact on BMD. Further studies are suggested regarding the role of bisphosphonates in relation to spinal radiographic progression and whether s-HGF can be useful as a predictor for spinal radiographic progression.

Keywords: Ankylosing spondylitis, bone mineral density, spinal new bone formation, longitudinal cohort study

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SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte: Hos patienter med den kroniska, reumatiska sjukdomen ankyloserande spondylit (AS) kan två olika typer av skelettpåverkan förekomma. Dels har patienter med AS en ökad risk för benförlust med utveckling av osteoporos och kotfrakturer och dels en ökad risk för bennybildning i ryggen med tillväxt av överbroande förbeningar mellan kotorna, s.k. syndesmofyter. Standardmetoden för att mäta bentäthet (BMD) är dual-energy x-ray absorptiometry (DXA) och vid mätning av BMD i ryggen används normalt anteroposterior projektion (AP), dvs. rakt framifrån. Hos patienter med AS kan resultaten från mätning med denna projektion vara svårvärderade då förbeningen i ryggen kan ge ett falskt högt värde. De övergripande syftena med denna avhandling var att studera utvecklingen av benförlust och bennybildning över fem års tid hos patienter med AS och att undersöka vilka faktorer som hade samband med förändringarna.

Metoder: Studierna som ingår i denna avhandling baseras på en kohort av patienter med AS som rekryterades från reumatologklinikerna på Sahlgrenska Universitetssjukhuset, Södra Älvsborgs sjukhus och Alingsås Lasarett. Patienterna genomgick samma undersökningar vid baslinjen och femårsuppföljningen med mätning av BMD med DXA i höften (lårbenshalsen och totala höften), ryggen (AP och lateral mätning från sidan) och underarmen (totala radius) samt röntgen av ryggen för gradering av förbeningar i ryggen och gradering av kotfrakturer. En andel av männen randomiserades till undersökning med högupplöst perifer kvantitativ datortomografi (HRpQCT) av underarm och underben för mätning av volumetrisk BMD, kortikal area och mikroarkitektur. I hela patientgruppen togs blodprover och nivån av hepatocyte growth factor i serum (s-HGF) analyserades med enzyme-linked immunosorbent assay (ELISA).

Resultat: Över fem år minskade BMD signifikant i lårbenshalsen och underbenet. Dessa minskningar var associerade till tecken på inflammation. I totala höften och ryggen, både AP och lateral mätning, hade däremot BMD ökat. Användning av bisfosfonater var förenat med ökning av BMD på alla mätlokaler utom underbenet. Användning av läkemedel som hämmar cytokinet tumörnekrosfaktor alfa (TNF- α) var relaterat till ökning av BMD i ryggen (AP) och underbenet. Endast tre patienter utvecklade ny kotfraktur. Förbeningen i ryggen ökade signifikant mer uttalat hos männen jämfört med kvinnorna. Nya riskfaktorer som identifierades för utveckling av förbening i ryggen var obesitas för både män och kvinnor och användning av bisfosfonater och nedsatt rygg- och höftförlighet vid baslinjen för kvinnorna.

Bland tidigare kända riskfaktorer för utveckling av förbening i ryggen var förekomst av förbening i ryggen vid baslinjen gemensam riskfaktor för både män och kvinnor medan högt CRP vid baslinjen och rökning predikterade förbening i ryggen hos männen i vår kohort. En högre nivå av biomarkören s-HGF var en oberoende riskfaktor för ökad förbening i ryggen hos männen.

Konklusion: Studierna i denna avhandling tyder på att bästa lokalen att mäta bentäthet hos patienter med långvarig AS är lårbenshalsen och att systemisk inflammation bidrar till en ökad benförlust och också utveckling av förbening i ryggen. Därmed är hämning av inflammation ett viktigt behandlingsmål. Studierna ger ytterligare anledning att råda patienterna till rökstopp, hjälpa dem med rökavvänjning samt att stötta patienter med obesitas till viktnedgång. Behandling med TNF-hämmare och bisfosfonater hade en positiv effekt på bentätheten. Det behövs ytterligare studier för att klarlägga bisfosfonaternas roll när det gäller förbeningen i ryggen och om HGF kan vara användbar som prediktor för utveckling av förbening i ryggen.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. **Deminger A**, Klingberg E, Lorentzon M, Geijer M, Göthlin J, Hedberg M, Rehnberg E, Carlsten H, Jacobsson LT, Forsblad-d'Elia H. Which measuring site in ankylosing spondylitis is best to detect bone loss and what predicts the decline: results from a 5-year prospective study. *Arthritis Research & Therapy* 2017; 19: 273.
- II. **Deminger A**, Klingberg E, Geijer M, Göthlin J, Hedberg M, Rehnberg E, Carlsten H, Jacobsson LT, Forsblad-d'Elia H. A five-year prospective study of spinal radiographic progression and its predictors in men and women with ankylosing spondylitis. *Arthritis Research & Therapy*, 2018; 20: 162.
- III. **Deminger A**, Klingberg E, Lorentzon M, Hedberg M, Carlsten H, Jacobsson LT, Forsblad-d'Elia H. Factors associated with changes in volumetric bone mineral density and cortical area in men with ankylosing spondylitis. A five-year prospective study using HRpQCT. *Submitted*.
- IV. **Deminger A**, Klingberg E, Nurkkala M, Geijer M, Carlsten H, Jacobsson LT, Forsblad-d'Elia H. Elevated serum level of hepatocyte growth factor predicts development of new syndesmophytes in men with ankylosing spondylitis. Results from a five-year prospective study. *Manuscript*.

CONTENT

ABBREVIATIONS	IV
1 ANKYLOSING SPONDYLITIS	1
1.1 Introduction.....	1
1.2 Epidemiology	1
1.3 Clinical presentation	2
1.4 Classification and diagnosis.....	3
1.5 Pathogenesis.....	6
1.6 Management.....	7
2 BONE	8
2.1 Bone physiology	8
2.2 Bone cells.....	8
2.3 Bone formation and remodeling.....	9
2.4 Regulation of bone cells.....	9
2.5 New bone formation in ankylosing spondylitis	11
2.6 Osteoporosis.....	17
2.7 Fractures.....	17
2.8 Measurement of bone mineral density	19
2.9 Osteoporosis and ankylosing spondylitis.....	20
2.10 Measurement of bone mineral density in patients with ankylosing spondylitis.....	21
2.11 Factors associated with changes in bone mineral density in patients with ankylosing spondylitis	22
2.12 Fractures in patients with ankylosing spondylitis	23
3 AIMS	26
4 PATIENTS AND METHODS.....	27
4.1 Patients.....	27
4.2 Controls.....	28
4.3 Questionnaires.....	28

4.4	Review of medical records.....	29
4.5	Blood samples.....	29
4.6	Radiography.....	30
4.7	Bone mineral density.....	32
4.8	Ethical considerations.....	33
4.9	Statistics.....	33
4.10	Follow-up after the study.....	35
5	RESULTS AND DISCUSSION.....	36
5.1	Paper I.....	36
5.2	Paper II.....	39
5.3	Paper III.....	42
5.4	Paper IV.....	45
6	CONCLUDING DISCUSSION AND FUTURE PERSPECTIVES.....	48
7	CONCLUSION.....	51
	ACKNOWLEDGEMENT.....	52
	REFERENCES.....	54

ABBREVIATIONS

aBMD	Areal bone mineral density
AI	Aortic insufficiency
AP	Anteroposterior
AS	Ankylosing spondylitis
ASAS	Assessment of SpondyloArthritis International Society
ASDAS_CRP	Ankylosing Spondylitis Disease Activity Score based on C-reactive protein
AU	Anterior uveitis
AUC	Area under the curve
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BASFI	Bath Ankylosing Spondylitis Functional Index
BAS_G	Bath Ankylosing Spondylitis Patient Global score
BASMI	Bath Ankylosing Spondylitis Metrology Index
bDMARD	Biological disease modifying anti-rheumatic drug
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMP	Bone morphogenetic protein
cMET	Cellular MET receptor
COX	Cyclooxygenase
CRP	C-reactive protein

csDMARD	Conventional synthetic disease modifying anti-rheumatic drug
CTSS	Computed Tomography Syndesmophyte Score
DKK	Dickkopf
DXA	Dual-energy x-ray absorptiometry
EAM	Extra articular manifestation
ELISA	Enzyme-linked immunosorbent assay
ERAP	Endoplasmic reticulum aminopeptidase
ESR	Erythrocyte sedimentation rate
ESSG	European Spondylarthropathy Study Group
EULAR	European League Against Rheumatism
FRAX [®]	Fracture Risk Assessment Tool
GWAS	Genome-wide association study
HGF	Hepatocyte growth factor
HLA	Human leucocyte antigen
HMW-APN	High molecular weight adiponectin
HRpQCT	High-resolution peripheral quantitative computed tomography
IBD	Inflammatory bowel disease
IBP	Inflammatory back pain
ICC	Intraclass correlation coefficient
IGF-1	Insulin-like growth factor 1
IL	Interleukin

LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
LSC	Least significant change
M-CSF	Macrophage colony-stimulating factor
MHC	Major histocompatibility complex
MIF	Macrophage migration inhibitory factor
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
mSASSS	Modified Stoke Ankylosing Spondylitis Spine Score
Nr-axial SpA	Non-radiographic axial spondyloarthritis
NSAID	Non-steroidal anti-inflammatory drug
OPG	Osteoprotegerin
PGE2	Prostaglandin E2
PsA	Psoriatic arthritis
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RA	Rheumatoid arthritis
RANKL	Receptor activator of NF- κ B ligand
RCT	Randomized controlled trial
ReA	Reactive arthritis
ROC	Receiver operating characteristic
SASSS	Stoke Ankylosing Spondylitis Spine Score

SD	Standard deviation
SDC	Smallest detectable change
S-HGF	Serum hepatocyte growth factor
SI	Sacroiliac
SLE	Systemic lupus erythematosus
SpA	Spondyloarthritis
SSRI	Selective serotonin reuptake inhibitor
TBS	Trabecular bone score
TGF- β	Transforming growth factor β
TNF α	Tumor necrosis factor α
TNFi	Tumor necrosis factor inhibitor
US	Ultrasound
vBMD	Volumetric bone mineral density
VEGF	Vascular endothelial growth factor
VF	Vertebral fracture
VICM	Citrullinated and matrix metalloproteinase-degraded fragment of vimentin
WBC	White blood-cell count
WHO	World Health Organization

1 ANKYLOSING SPONDYLITIS

1.1 INTRODUCTION

Ankylosing spondylitis (AS) is a chronic, inflammatory disease that mainly affects the axial skeleton. AS is the major subtype of the family of related diseases called spondyloarthritis (SpA) that share common clinical and genetic characteristics. Psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease (IBD), reactive arthritis (ReA) and undifferentiated SpA are also part of the SpA-family. Depending on the clinical manifestations that predominate, SpA can be classified as axial SpA with symptoms mainly from the spine and sacroiliac (SI) joints or as peripheral SpA with symptoms mainly from peripheral joints and entheses. [1] Axial SpA includes both patients with radiographic findings of sacroiliitis in the SI-joints (radiographic axial SpA or AS) and patients without radiographic sacroiliitis (non-radiographic axial SpA (nr-axial SpA)). [2]

The name ankylosing spondylitis derives from the Greek word “ankylosis” meaning stiffness, “spondylos” meaning vertebra, and the suffix “-itis” which denotes inflammation. Ankylosing spondylitis is also known as Bechterew’s disease.

1.2 EPIDEMIOLOGY

AS typically starts in the third decade of life with an average disease or symptom onset of 25 years. [3] Studies have estimated the ratio of man to woman with AS to approach 2-3:1. [4-6] The prevalence of the disease varies between ethnical populations and geographical regions, and correlates strongly to the prevalences of human leucocyte antigen (HLA) B27 positivity. [7] The prevalence of HLA-B27 in blood donors in northern Sweden has been shown to be 16.6% [8], whereas the prevalence in southern Sweden was 10 %. [9] In line with this, a Swedish study from 2015 on the prevalence of AS found a prevalence of 0.24 % in northern Sweden compared to 0.16 % in southern Sweden. Total prevalence of AS in Sweden was 0.18 %. [10] There are methodological differences that can make comparisons between different prevalence studies difficult. Nonetheless, in two systematic reviews, the prevalence of AS in Europe was reported to be 0.23 % and 0.25 % respectively. [11, 12]

1.3 CLINICAL PRESENTATION

The main initial clinical feature of AS is chronic back pain. The definition of chronic is duration of more than three months. The pain that often starts at the pelvis and the lower back is caused by sacroiliitis. However, inflammation can affect all parts of the spine. [2] The pain typical for AS is characterized by alternating gluteal pain, insidious onset, improvement with exercise but not with rest, pain at night and early mornings, and accompanied by morning stiffness. There are several sets of criteria for classification of this inflammatory back pain (IBP), partly overlapping (Table 1.). [13-15] The sensitivity and specificity for the criteria are around 70-80 %, meaning not all patients have this type of back pain, and that other causes of chronic back pain can present this way as well.

Table 1. Inflammatory back pain according to various criteria

Calin criteria [13]	Berlin criteria [14]	ASAS criteria [15]
	To be applied if duration of back pain > 3 months and if age at onset < 50 years	To be applied if duration of back pain > 3 months
Age at onset < 40 years	Morning stiffness > 30 minutes	Age at onset < 40 years
Duration of back pain > 3 months	Awakening at second half of the night because of back pain	Pain at night
Insidious onset	Alternating buttock pain	Insidious onset
Morning stiffness	Improvement with exercise but not with rest	No improvement with rest
Improvement with exercise		Improvement with exercise
IBP if 4/5 are present	IBP if 2/4 are present	IBP if 4/5 are present

IBP; inflammatory back pain, ASAS; Assessment of SpondyloArthritis International Society

In the spine, pathological new bone formation commonly develop in AS, and together with inflammation and pain contributes to the limited mobility and impaired physical function that often affect these patients. [16-18] In the advanced stages of new bone formation, complete ankylosis of the spine can develop, often referred to as a “bamboo spine”. [19, 20]

Non-axial musculoskeletal manifestations of the disease are peripheral arthritis, usually an asymmetric oligoarthritis, and enthesitis, both typically engaging the lower limbs. [2]. Enthesitis is inflammation at the insertion of tendons, ligaments and joint capsules to the skeleton. The heel is the most frequently affected enthesial site with inflammation engaging the insertions of the Achilles and the plantar fascia. [21] The pooled prevalences of arthritis

and enthesitis in patients with AS was around 30 % respectively reported in a meta-analysis by de Winter. [22] Peripheral enthesitis is usually diagnosed by clinical examination assessing tenderness at the enthesial site, a method that lacks specificity and objectivity. An imaging tool that can be useful in the evaluation of enthesitis is ultrasound (US), which can detect both active inflammation and chronic changes at the entheses. [21] Limitations with US are discordant data about the ability to differentiate between SpA and other conditions and healthy controls, and until recently there was no clear agreement on which components to assess and how to define enthesitis. A proposed score is under evaluation. [23]

There are also extra-articular manifestations (EAMs) associated with AS. The three most common EAMs are anterior uveitis (AU), IBD defined as Crohn's disease or ulcerative colitis, and psoriasis. AU is inflammation involving the iris or ciliary body of the eye. The reported prevalences in AS for AU are 20-30%, for psoriasis 10-25 % and for IBD 5-10 %. [22, 24-26] More common than IBD in patients with AS is the occurrence of microscopic or macroscopic subclinical inflammation in the gut, where studies have revealed such inflammation in 40-60 % of patients with AS or SpA. [27-29] The heart can also be affected in AS. The most common affections are conduction disturbances and valvular disease with prevalences ranging from 1-35 % for conduction disturbances, 0-34 % for aortic insufficiency (AI) and 5-74 % for mitral insufficiency. Higher rates of aortic valve surgery and higher use of pacemaker than controls have been reported for AS, whereas the rate of mitral valve surgery did not differ. [30] Baseline, cross-sectional reports on our cohort showed a prevalence of conduction disturbances between 10-35 % depending on if conservative or less conservative criteria were applied, [31] and a prevalence of AI of 18 %. [32] Register based studies from our group have showed Swedish patients with AS to have an increased risk compared to the general population for atrioventricular block II-III, atrial flutter and pacemaker implantation, [33] and also an increased risk of acute coronary syndrome, stroke and venous thromboembolism. [34]

1.4 CLASSIFICATION AND DIAGNOSIS

In clinical studies it is of importance to identify a homogenous, well-defined group of patients in order to be able to compare results between studies. The classification criteria are traditionally intended to have a high specificity, meaning the patients that don't have the disease will test negative. Diagnostic criteria on the other hand are aiming at high sensitivity, meaning to identify all individuals with the disease. Diagnostic criteria are generally broader and

reflect the different features of the disease and apply to the individual patient, whereas classification criteria apply to groups of patients. [35]

The modified New York criteria were developed for classification and diagnosis of AS in 1984. The first criteria for AS were specified at the Rome conference in 1963. The criteria were then revised in 1966 to the New York criteria and in 1984, the last revision was made and the currently used modified New York criteria were defined (Table 2). [36]

Table 2. The modified New York criteria for ankylosing spondylitis [36]

Clinical criteria
-Low back pain and stiffness > 3 months that improves with exercise, but is not relieved by rest
-Limitation of motion of the lumbar spine in the sagittal and frontal planes
-Limitation of chest expansion relative to normal values correlated for age and sex
Radiological criterion
-Sacroiliitis grade ≥ 2 bilaterally or grade 3-4 unilaterally
Definite AS if the radiological criterion is associated with ≥ 1 clinical criterion. Probable AS if three clinical criteria are present or if radiological criterion is present without signs or symptoms satisfying the clinical criteria.

Table 3. Grading of radiographic sacroiliitis (1966)[37]

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 sacroiliitis with one or more of erosions, evidence of sclerosis, widening, narrowing or partial ankylosis.
Grade 4:	Severe abnormality – total ankylosis

The modified New York criteria perform well in patients with established disease. However, it takes time to develop radiographic sacroiliitis, so patients with early disease cannot be classified or diagnosed with AS. In 1990 and 1991 two different classification criteria to capture patients with undifferentiated SpA were constructed: the Amor criteria and the European Spondylarthropathy Study Group (ESSG) criteria (Table 4). [37-39] The criteria cover the whole spectrum of the SpA-family and include axial, non-axial musculoskeletal symptoms and EAMs. These criteria do not distinguish between patients with radiographic sacroiliitis or not and the specificity was considered too low. [40]

Table 4. The Amor and the European Spondylarthropathy Study Group (ESSG) classification criteria for spondyloarthritis [37-39]

Amor	ESSG
Criterion (points)	
Clinical symptoms or past history: -Lumbar or dorsal pain during the night, or morning stiffness of lumbar or dorsal spine (1) -Asymmetric oligoarthritis (2) -Buttock pain (1) if affecting alternatively the right and the left buttock (2) -Sausage-like toe or digit (dactylitis) (2) -Heel pain or any other well defined enthesopathy (2) -Iritis (2) -Non-gonococcal urethritis or cervicitis accompanying, or within 1 month before, the onset of arthritis (1) -Acute diarrhea accompanying, or within 1 month before, the onset of arthritis (1) -Presence of history of psoriasis, balanitis, or IBD (2)	Inflammatory spinal pain (IBP according to Calin criteria except age of onset here < 45 years)
Radiological finding: -Sacroiliitis (grade ≥ 2 if bilateral, grade ≥ 3 if unilateral) (3)	OR
Genetic background: -Presence of HLA-B27, or familial history of AS, Reiter syndrome, uveitis, psoriasis, or IBD (2)	Synovitis (asymmetric or predominantly in the lower limb)
Response to treatment: -Good response to NSAIDs in < 48 h, or relapse of pain in < 48 h if NSAID is discontinued (2)	AND
Spondyloarthritis if sum score ≥ 6	One of the following: -Family history (first-degree or second-degree relatives with AS, psoriasis, acute uveitis, ReA, IBD) -Psoriasis, past or present diagnosed by a doctor -IBD, past or present, diagnosed by a doctor, confirmed by radiography or endoscopy -Non-gonococcal urethritis, cervicitis, or acute diarrhea < 1 month before arthritis -Buttock pain alternating between right and left gluteal areas -Enthesopathy, past or present spontaneous pain or tenderness at examination site at the insertion of the Achilles tendon or plantar fascia -Sacroiliitis. Bilateral grade 2-4, unilateral grade 3-4 according to the following radiographic grading system: 0 = normal, 1 = possible, 2 = minimal 3 = moderate, 4 = ankylosis

IBD; inflammatory bowel disease, IBP; inflammatory back pain, NSAID; non-steroidal anti-inflammatory drug, ReA; reactive arthritis

In 2009, new classification criteria for axial SpA with subdivision in radiographic axial SpA and nr-axial SpA were developed: the Assessment of SpondyloArthritis international Society (ASAS) criteria. With these criteria, patients can be classified either by an imaging arm, which includes magnetic resonance imaging (MRI) or an HLA-B27 arm (Table 5). [41] In 2011, ASAS presented classification criteria for peripheral SpA (Table 6). [42]

Table 5. The Assessment of SpondyloArthritis International Society (ASAS) criteria for classification of axial spondyloarthritis [41]

To be applied in patients with back pain \geq 3 months and age at onset $<$ 45 years		
Sacroiliitis on imaging plus \geq 1 SpA feature	OR	HLA-B27 plus \geq 2 other SpA features
SpA features:		Sacroiliitis on imaging:
-IBP	-Crohn's disease	-Active (acute inflammation) on MRI highly suggestive of sacroiliitis associated with SpA
-Arthritis	-Ulcerative colitis	-Definite radiographic sacroiliitis according to the modified New York criteria
-Enthesitis (heel)	-Good response to NSAID	
-Uveitis	-Family history for SpA	
-Dactylitis	-HLA-B27	
-Psoriasis	-Elevated CRP	

CRP; C-reactive protein, IBP, inflammatory back pain, NSAID; non-steroidal anti-inflammatory drug

Table 6. The Assessment of SpondyloArthritis International Society (ASAS) criteria for classification of peripheral spondyloarthritis [42]

Arthritis or enthesitis or dactylitis plus		
\geq 1 of	OR	\geq 2 of
-Psoriasis		-Arthritis
-Inflammatory bowel disease		-Enthesitis
-Preceding infection		-Dactylitis
-HLA-B27		-IBP in the past
-Uveitis		-Positive family history of SpA
-Sacroiliitis on imaging		

1.5 PATHOGENESIS

Knowledge about the pathogenesis of AS is limited. Genetic factors are important and the strong association between HLA-B27 and AS was discovered in the early 1970s [43, 44] The majority of genes contributing to the risk of developing the disease are still unknown. A recent large genome-wide association study (GWAS) demonstrated that HLA-B27 and related major histocompatibility complex (MHC) variants attributed to 20.4 % of the heritability of AS whereas non-MHC variants contributed with 7.4 %. The remaining 72.2 % are yet to be identified [45]

The mechanism of HLA-B27 in the pathogenesis of AS is not established, [46] HLA genes encode MHC class I proteins which present peptides to T-cells. The MHC-I molecules are synthesized, folded and loaded with peptides

in the endoplasmic reticulum. The peptides are trimmed to a length preferred by MHC-I by the endoplasmic reticulum aminopeptidase (ERAP). [47] The first identified non-MHC gene with observed association with AS was ERAP1. The association is only found in HLA-B27 positive patients and the role of ERAP1 as a trimmer of peptides indicates that HLA-B27 is likely to affect AS via a mechanism that involves abnormal presentation of peptides. Genetic studies also provide evidence for the involvement of interleukin (IL) 23 and its downstream pathway with IL-17 and other pro-inflammatory cytokines in the pathogenesis of AS. [46]

Genetics alone cannot explain the onset of the disease. Disturbed barrier functions against microbes in the gut and the skin might trigger a pathogenic immune response in genetically susceptible individuals. Also, bacteria with invasive properties that can penetrate through intact mucosal barriers can trigger the immune system in susceptible individuals, as found in ReA. [48] The entheses in patients with SpA are prone to inflammation both in the spine and the peripheral skeleton and mechanical stress at the enthesis level is believed to induce and maybe also maintain inflammation in this patient group. [49]

1.6 MANAGEMENT

There are international recommendations published for the management of SpA from Europe [50] and North America. [51] National Swedish treatment guidelines are updated annually (www.svenskreumatologi.se/srfs-riktlinjer). The recommendations are similar. General principles for the management of AS and nr-axial SpA includes education about the disease, encouragement to exercise regularly and to stop smoking. Non-steroidal anti-inflammatory drugs (NSAIDs) are the first-line treatment for pain and stiffness. Local glucocorticoid injections at the site of inflammation in peripheral joints or sacroiliac joints can be used, whereas patients with axial disease should not be treated with systemic glucocorticoids. Conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) are not recommended for pure axial disease, but sulfasalazine may be considered for treatment of peripheral arthritis. When conventional treatment is not sufficient, a biologic DMARD (bDMARD) should be considered, and the recommended class of drug is tumor necrosis factor inhibitor (TNFi). If TNFi therapy fails, another TNFi or switching to an anti-IL-17A therapy should be considered.

2 BONE

2.1 BONE PHYSIOLOGY

The skeleton has several functions in the body; it gives structural support for the rest of the body, serves as attachment sites for muscles and ligaments and thereby enables movement and it protects internal organs. The skeleton also maintains metabolic homeostasis of minerals such as calcium and phosphate and harbors the bone marrow where hematopoiesis takes place. The outer shell of the bone is a compact, dens layer called cortical bone. Cortical bone surrounds the trabecular bone, which is a rigid network of mineralized bone that contains the bone marrow and is more metabolically active than the cortical bone. The composition in the skeleton is 80 % cortical bone and 20 % trabecular bone, with different ratios in different bones. The ratio cortical to trabecular bone is 25:75 in the vertebra, 50:50 in the femoral head and 95:5 in the radial diaphysis. [52]

Bone strength is determined by several different factors: tissue properties, microarchitecture and the whole bone geometry. Tissue properties are characteristics such as the degree of mineralization, the degree and type of collagen cross-linking and osteocyte density. For the microarchitecture, both trabecular and cortical microarchitecture matters. Bone geometry includes factors like the bone size, cortical thickness and geometry of the femoral neck. [53]

2.2 BONE CELLS

There are two categories of bone cells involved in bone formation and remodeling, osteoclasts and the osteoblast family. The osteoblast family consists of osteoblasts, osteocytes and bone lining cells. The osteoclasts resorb bone and are derived from monocyte/macrophage progenitor cells. [52, 54] Osteoblasts are bone forming cells originating from the mesenchymal cell-line in the bone marrow. Osteoblasts produce osteoid composed of bone matrix proteins and mediate calcification of the osteoid. They also participate in the regulation of osteoclasts. When the osteoblasts have finished the bone formation, some of them are buried in the bone matrix and become osteocytes, or they can become lining cells on the bone surface. The osteocytes and the lining cells are connected to each other with long branches and functions as mechanoreceptors and can regulate osteoblasts and osteoclasts [52, 54]

2.3 BONE FORMATION AND REMODELING

There are two types of physiological bone formation that takes place during embryonic development and postnatal growth: endochondral ossification and intramembranous ossification. In endochondral ossification, a cartilage template is gradually replaced by bone, whereas in intramembranous ossification bone is formed directly on a mesenchymal growth plate without cartilage intermediate. In both cases, bone matrix is synthesized by osteoblasts while osteoclasts degrade the tissue. Most bones are formed by endochondral ossification. [55, 56]

Throughout life, the bones undergo modeling and remodeling. Modeling is the process where bones change the overall shape in response to for example mechanical forces. Remodeling is more frequent than modeling and is a mechanism to preserve the bone strength by replacing older micro-damaged bone with new healthier bone, but also to maintain the homeostasis of calcium and phosphate. The remodeling cycle begins with recruitment of osteoclast precursors that binds to the bone matrix and develops to osteoclasts that start the resorption phase. When the resorption phase is finished, the osteoclasts undergo apoptosis and osteoblasts start to synthesize collagenous matrix which is gradually mineralized to form new bone. [52]

2.4 REGULATION OF BONE CELLS

Some of the major mechanisms involved in the regulation of osteoclasts and osteoblasts are hereby described (Figure 1). Osteoclast recruitment and differentiation are stimulated by macrophage colony-stimulating factor (M-CSF) and receptor activator NF- κ B (RANKL). Osteoclast activation and resorption are stimulated by RANKL. Osteoprotegrin (OPG) on the other hand inhibits RANKL signaling by acting as a decoy receptor that blocks binding of RANKL to its receptor RANK. Factors that can enhance osteoclastogenesis driven by RANKL are inflammatory mediators like IL-1, tumor necrosis factor α (TNF- α), IL-6 and prostaglandin E2 (PGE2). [57]

Osteoblast precursors are recruited by growth factors like insulin-like growth factor 1 (IGF-1) and transforming growth factor- β (TGF- β). Osteoblast differentiation and survival is stimulated by bone morphogenetic proteins (BMPs) and WNTs. WNTs are inhibited by sclerostin and dickkopf1 (DKK1) and BMPs are inhibited by noggin. Osteoblasts participate in the regulation of osteoclasts by expressing RANKL, M-CSF and OPG [58-60]

The mechanism how osteocytes control and regulates osteoblasts and osteoclasts is not fully elucidated but two important factors are RANKL and sclerostin. [58]

In the initiation of endochondral ossification, chondrocyte proliferation and hypertrophy is stimulated by proteins as Hedgehog, WNTs and BMPs. When the chondrocytes die, blood vessels invade the tissue together with osteoblasts and osteoclasts. Important stimulatory factor for the angiogenesis is vascular endothelial growth factor (VEGF) whereas RANKL promotes invasion of osteoclasts. WNTs and BMPs are stimulating the osteoblasts. [61]

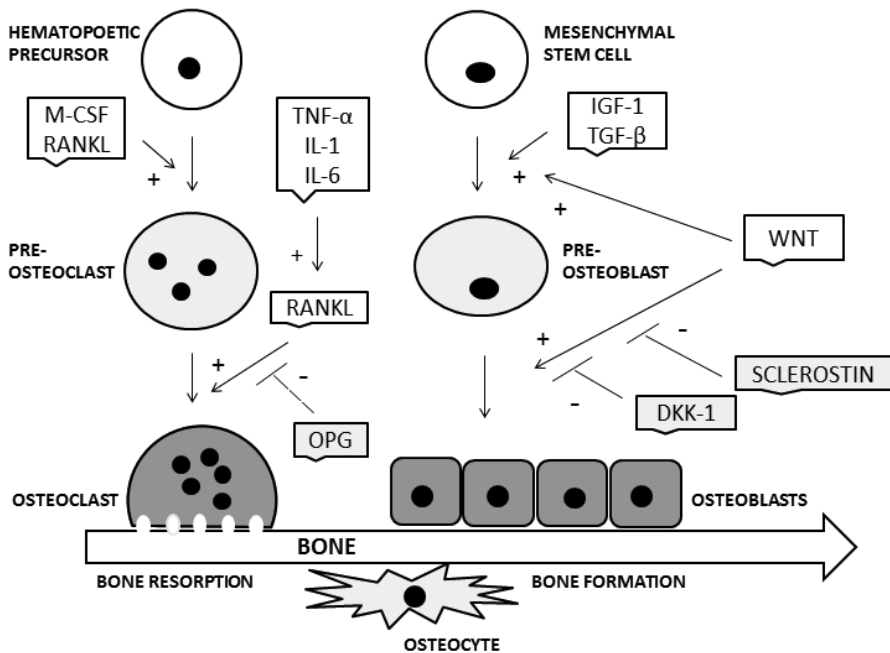


Figure 1. Some of the major mechanisms involved in the regulation of osteoclasts and osteoblasts

2.5 NEW BONE FORMATION IN ANKYLOSING SPONDYLITIS

AS is characterized by pathological spinal new bone formation with development of syndesmophytes and a risk of developing total ankylosis. The AS related spinal alterations can be visualized on plain radiographs and the preferred method to grade these changes in clinical studies is by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS). [62-64] The score ranges from 0 to 72. [65] Not all patients with AS develop AS related spinal alterations and the progression rate over time is highly variable between patients [66] but also in the same patient over time. [67] Commonly reported progression rates in different cohorts of patients with AS are progression of mean 1 mSASSS/year [66, 67] or between mean 0.8-1.5 mSASSS/2 years. [68-71] More AS related spinal alterations are found in men compared to women. [72-74]

The knowledge of the mechanisms of the pathological new bone formation in AS is limited and research in this area is hampered by the difficulties in obtaining biopsies from the affected tissues, the slow process of new bone formation and the restricted sensitivity to change for mSASSS. [75]

There are different theories about the relation between inflammation and spinal new bone formation. Some researchers have proposed that the process is always initiated with inflammation at the spine, osteitis, and is then followed by a repair mechanism that replaces the subchondral bone marrow with granulation tissue from which stimuli for new bone formation is released. [75] Other researchers have proposed the new bone formation to be at least partly uncoupled from inflammation. One such theory is that inflammation at the spine causes loss of trabecular bone which affects the microarchitecture and stability of the vertebra. As a consequence in an attempt to stabilize the spine, new bone formation is hypothesized to occur. In this theory, inflammation is thought to start with mechanical stress causing micro damage at the enthesis level. [76, 77] Both research theories consider BDNFs, WNTs and Hedgehog proteins to be of importance on the molecular level in the stimulation of new bone formation and that dysregulation of inhibitors of bone formation such as sclerostin, DKK1 and noggin also can be involved. [2, 78]

New bone formation in AS takes place in connection with existing bone but extends outside the normal shape and is a complex remodeling process. Both endochondral and intramembranous bone formation seem to contribute. [79]

2.5.1 HISTOPATHOLOGIC STUDIES

Histopathologic material from the axial skeleton has mainly been obtained from facet joints from AS patients with total ankylosis undergoing surgery due to hyperkyphosis and from biopsies from SI-joints. A process found in the facet joints, was growth of fibrous granulation tissue from the bone marrow. The fibrous granulation tissue invaded the subchondral bone plate and reached the cartilage where spots of new bone were formed. The invasion seemed to be facilitated by osteoclasts and the granulation tissue carried osteoblasts with bone forming capacities. They also found replacement of the subchondral bone marrow by fat tissue. However, fat tissue without granulation tissue was not associated with new bone formation. [80-82] A recent study aimed at specifically analyze the fatty lesions seen on MRI by immunohistological analyses of anterior vertebral edges. Fatty lesions were found to correspond to the presence of adipocytes and to have a high number of osteoblasts, whereas dominance of osteoclasts was found in the MRI inflammatory lesions. [83] Another research-group found signs of persistent inflammation with aggregates of both T-cells and B-cell and signs of neoangiogenesis in the bone marrow of facet joints despite patients having complete ankylosis. [84]

A large biopsy study on SI-joints in early axial SpA found the most common feature to be pannus formation of highly vascular granulation tissue from the synovium or bone marrow with invasion of the subchondral bone plate. They also found some signs of endochondral ossification with new bone formation at the bone-cartilage interface and some signs of enthesitis. [85] Francois et al. analyzed different stages of sacroiliitis, and summarized that synovitis and subchondral granulation tissue from the bone marrow gradually destroys and replaces the articular cartilage and subchondral bone. Some signs of enthesitis were found but were not considered important in the process. They found evidence of both endochondral and intramembranous bone formation but also an unusual form of chondroid metaplasia. [86]

2.5.2 BIOMECHANICAL FACTORS

Enthesitis has been proposed to be the primary disease location in the different SpA subtypes. [87] The entheses, especially at the spine and the lower limbs are exposed to mechanical loading and subjected to micro damage. It has been hypothesized that mechanical stress is an initial trigger for inflammation through micro damage at the enthesis. [78] In a mouse model of SpA, inflammation started at the entheses and then spread to the synovium, finally involving the whole joint. When mice were tail-suspended with hind limbs unloaded, no inflammation developed. In a second mouse

model, new bone formation developed mainly at the enthesal sites and tail suspension led to less new bone formation. [88] Recently, Cambré et al. showed in other mice models that unloaded limbs did not develop arthritis, that higher grade of loading by voluntary running led to enhanced inflammation and that inflammation developed especially at sites with high mobility and rich in attachment sites for tendons. [89] Another important mouse study by Sherlock et al. found evidence that IL-23 over-expression induced enthesitis by acting on a specific enthesal resident T-cell, identified at the entheses and the aortic root. [90] These T-cells responded to systemic expression of IL-23 and severe enthesal inflammation and enthesal new bone formation developed both at the paws and at the attachment of the spinal ligaments. Also, inflammation at the aortic root and valve developed. The resident T-cells were also shown to produce IL-17 and IL-22 after stimulation with IL-23. How these results can be applied in human disease needs further research.

There are two clinical studies on patients with AS which indicate that mechanical stress might be involved in the pathogenesis of spinal new bone formation. One cross-sectional study on patients with disease duration ≥ 20 years showed that patients whose previous occupations had required dynamic flexibility or exposure to whole body vibration had significantly more AS related alterations in the spine. [91] A longitudinal study by Ramiro et al. investigated the effect of mechanical stress on spinal radiographic progression by using type of occupation divided into blue collar (physically demanding) and white collar (sedentary) labor. The direct effect of the type of occupation on radiographic progression was weak. In an indirect analysis they showed that blue collar work amplified the effect of inflammation on new bone formation. [92]

2.5.3 MAGNETIC RESONANCE IMAGING

Researchers have used MRI to explore the relation between inflammatory lesions in the vertebrae and the association with development of new bone formation in the spine. MRI studies have shown that inflammatory lesions with bone marrow edema in the vertebral corners can predict the development of new syndesmophytes. [93, 94] Syndesmophytes also develop in vertebral corners with fatty degeneration, generally believed to represent some kind of repair tissue. [95] In patients treated with TNFi, acute inflammatory lesions resolved without sequelae while more advanced vertebral inflammatory lesions that had started to show signs of reparative changes (fatty lesions) progressed to new bone formation. [96] Baraliakos et al. found that inflammation and fatty lesions in combination had the highest risk for new syndesmophytes. However, most of the new syndesmophytes

developed without signs of pathological MRI-findings at baseline. [97] It is difficult to draw definitive conclusions from MRI studies since lesions can occur and disappear between examinations and a histopathologic study revealed that a substantial degree of bone marrow inflammation is necessary for detection on MRI. [98]

2.5.4 BIOMARKERS AND RADIOGRAPHIC PROGRESSION

One indication that inflammation is involved in the process of spinal new bone formation is studies of C-reactive protein (CRP) as a predictor of radiographic progression. There are several studies showing inflammation measured by CRP to predict spinal new bone formation in patients with AS and nr-axial SpA, both elevated baseline CRP and elevated time-averaged CRP. The association of elevated CRP and new bone formation was found despite differences in use of TNFi and disease duration in the cohorts. [68, 99-102] Other serum biomarkers of inflammation that have been shown to predict spinal radiographic progression are IL-6 and serum calprotectin. [103, 104]

Macrophage migration inhibitory factor (MIF) was found to predict spinal radiographic progression and additional experiments suggested that MIF has a direct role in enhancing mineralization by osteoblasts. [105] MIF has been studied in one cohort of patients. A not so uncommon feature of biomarker studies is the inability to reproduce the results in other cohorts. Elevated matrix metalloproteinase 3 (MMP3) and VEGF were found to predict spinal radiographic progression over 2 years, especially in patients with presence of AS related spinal alterations at baseline. [106, 107] However, VEGF lacked predictive value in patients treated with TNFi and results were not confirmed for MMP3 in another cohort. [108, 109] In two relatively small studies, low serum levels of functional DKK1 [110] and low levels of sclerostin predicted spinal radiographic progression. [111] Result for sclerostin was not reproduced. [109] Elevated levels of the adipokine visfatin as a predictor of spinal radiographic progression could not be repeated either. [112, 113] An inverse relationship between the adipokines leptin and high molecular weight adiponectin (HMW-APN) and spinal radiographic progression has been found [113] and a recent publication confirmed this relationship and also found higher levels of VEGF in patients with radiographic progression. The combination of VEGF, leptin and HMW-APN had the best predictive ability of spinal radiographic progression. However, the added value to clinical parameters was rather small. [109]. So far, no biomarker except CRP is used in clinical practice.

2.5.5 HEPATOCYTE GROWTH FACTOR

There are two studies on hepatocyte growth factor (HGF) in AS. An association was found for high levels of HGF and increased disease activity. [114] In our cohort, patients with AS had higher levels of serum HGF (s-HGF) than healthy controls, and higher s-HGF was associated with higher mSASSS in the baseline cross-sectional analysis. [115] Higher levels of s-HGF compared to controls have also been found in patients with rheumatoid arthritis (RA), IBD and systemic lupus erythematosus (SLE). [116-119] Patients with RA had higher level of HGF in synovial fluid compared to peripheral blood, [120, 121] and high plasma HGF predicted progression of erosion and joint space narrowing in the finger joints in patients with RA. [122] Whether HGF has a mechanistic role in rheumatic diseases, or if HGF is upregulated in response to pro-inflammatory cytokines is not clear. Studies have shown HGF to affect immune cells, and in different animal models HGF prevents and attenuates inflammatory diseases, [123] for example collagen induced arthritis and experimental colitis. [124, 125]

HGF can affect a variety of cells in many different organs, and can stimulate cell proliferation, survival, motility and promotes angiogenesis. [126] HGF is required for self-repair after injuries of skin, muscle and cartilage. [127] Therapeutic effects of recombinant HGF has been shown in many different animal models for diseases in organs like the liver, the kidneys, the lungs, the skin and the cardiovascular system. [128] During tissue repair, several cytokines like IL-1, IL-6 and TNF- α induce transcription of HGF and its receptor cellular MET (cMET). [129] Knowledge about the role of HGF in regulation of bone cells is limited. Both osteoclasts and osteoblasts express HGF and cMET [130-132] and HGF stimulates migration of osteoclasts. [131] There are studies indicating an osteogenic effect of HGF; HGF in combination with vitamin D or alone was shown to promote differentiation of osteoblasts and to be important for mineralization. [133, 134] In animal models, HGF improved fracture healing. [135, 136] However, there are also studies reporting that HGF inhibits osteogenic differentiation. [137]

2.5.6 FACTORS ASSOCIATED WITH SPINAL RADIOGRAPHIC PROGRESSION

Several longitudinal, observational cohort studies on patients with AS or nr-axial SpA have assessed predictors for spinal radiographic progression. The follow-up time and intervals for radiographs differ, but an interval of at least 2 years between radiographs is needed to detect changes in mSASSS. [138] The strongest and the most commonly detected predictor is presence of AS related spinal alterations at baseline, most commonly ≥ 1 syndesmophyte.

[66, 68, 74, 100, 139-141] Other reported independent predictors for spinal radiographic progression are smoking, [99, 140, 142] male sex, [67, 69, 143], history of uveitis, [143] drinking alcohol (vs not drinking), [143] and low bone mineral density (BMD). [140] Increased disease activity, especially measured by Ankylosing Spondylitis Disease Activity Score based on CRP (ASDAS_CRP) at baseline and over time has also been shown to be associated with radiographic progression. [102, 144] The effect of ASDAS_CRP on progression was higher in men than women, [102] in smokers versus non-smokers and in blue collar workers vs white collar workers. [92]

2.5.7 TREATMENTS AND SPINAL RADIOGRAPHIC PROGRESSION

There is yet no treatment proven to be very effective in halting development of AS related spinal alterations. One randomized controlled trial (RCT) comparing the effect of continuous vs on-demand treatment with NSAID found continuous use of NSAIDs to reduce the spinal radiographic progression over two years. [145] However, another RCT with the same design but with diclofenac instead of celecoxib found no such effect. [71] Whether different effect on radiographic progression is related to different cyclooxygenase (COX)-selectivity is not elucidated.

The question if TNFi have effect on spinal radiographic progression is difficult to answer since radiographic progression is slow and long term RCTs comparing treatment with TNFi versus no treatment in patients in need of such treatment would be unethical. [146] Initial studies on use of TNFi versus another historical TNFi-naïve cohort failed to prove an effect on spinal radiographic progression. [147-149] There are now some reports from observational studies that show treatment with TNFi to retard radiographic progression, especially when TNFi is used for a longer time period [69, 99, 150] and initiated early in the disease course. [99]

Quite recently, the IL-17A inhibitor secukinumab was introduced as a treatment option for AS. Data about the effect on spinal radiographic progression are limited. There is one study that compared radiographic progression in patients treated with secukinumab for two years with TNFi-naïve patients from a historic cohort treated with NSAIDs. No significant differences in progression between groups were found. [151]

2.6 OSTEOPOROSIS

Osteoporosis is defined as a systemic skeletal disease characterized by low bone mass and deterioration of the microarchitecture with a consequent increase in bone fragility and susceptibility to fractures. [152] A person's bone mass later in life is determined by the peak bone mass accumulated up to puberty and the subsequent rate of bone loss. Bone loss occurs because of an imbalance between the activity of osteoclasts and osteoblasts. Estrogen is important in normal bone remodeling. During menopause when estrogen levels decrease, bone loss occurs at a higher rate. [153] Declining levels of bioavailable levels of estrogen may also be an important factor in age-related bone loss in men and declining levels of testosterone might also contribute. [154] Other age-related mechanisms of bone loss are secondary hyperparathyroidism, declining muscle mass and reduced mechanical loading. [153]

Diagnosis of osteoporosis is made based on measurement of BMD. BMD in an individual can be expressed in relation to the mean of a reference population in standard deviations (SD). The T-score is the SD expressed in relation to a young, healthy population. The Z-score is the comparison with the same age and sex group. A definition of osteoporosis was developed by the World Health Organization (WHO) in 1994 for post-menopausal women based on T-score in comparison to young women. They established the following four categories for assessments done using dual-energy x-ray absorptiometry (DXA); normal: T-score > -1 SD, low bone mass or osteopenia: T-score < -1 SD to > -2.5 SD, osteoporosis: T-score ≤ -2.5 SD, and severe osteoporosis: T-score < -2.5 SD and ≥ 1 fragility fracture. [155] The definition is now applied also on men ≥ 50 years old and women in menopausal transition. Measurements at the total hip, femoral neck and lumbar spine are primarily used, but measurements at radius can be used for diagnosis if the other sites are not assessable (www.iscd.org/officialpositions). For premenopausal women and men < 50 years a Z-score ≤ -2 SD is defined to be below expected range for age. [156] The prevalence of osteoporosis in Sweden in the age group 50-80 years based on BMD-measurements at the femoral neck has been reported to be 6.3 % for men and 21.2 % for women. [157]

2.7 FRACTURES

Osteoporosis is a silent disease until complicated by fractures preceded by little or no trauma. The most common fractures associated with osteoporosis are the so called major osteoporotic fractures at the hip, spine (clinical),

forearm and proximal humerus, [158] but almost all types of fractures are increased. Especially hip fractures but also vertebral fractures (VFs) are associated with increased mortality, morbidity and loss of function. Both hip fractures and VFs increase the risk of subsequent fractures. [159, 160] Many VFs occur un-diagnosed and there are two types of definitions: clinical fractures and cases where radiographs show vertebral deformities. [161] Incidence of fractures varies between populations worldwide with the highest risk of hip fractures in the Nordic countries. [162] The highest incidence of morphometric VFs (based on measurements of vertebral heights using imaging) among European countries was found in Sweden. [163] The majority of osteoporotic fractures occur in elderly women. [164]

There are many conditions, diseases and medications that contribute to osteoporosis and fractures, some of them are listed in Table 7. [160, 165]

Table 7. Some lifestyle factors, diseases and medications that contribute to osteoporosis and fractures

Lifestyle factors:		
Alcohol abuse	Smoking	Low physical activity
Low calcium intake	Vitamin D insufficiency	
Genetic diseases:		
Cystic fibrosis	Ehler-Danlos	Marfan syndrome
Hypogonadal states		
Anorexia nervosa	Menopause < 40 years old	Athletic amenorrhea
Hypogonadism		
Endocrine disorders:		
Hyperparathyroidism	Cushing's syndrome	Acromegaly
Diabetes mellitus	Thyrotoxicosis	
Gastrointestinal diseases:		
Celiac disease	IBD	Gastric bypass surgery
Primary biliary cirrhosis	End stage liver disease	
Neurological diseases		
Multiple sclerosis	Epilepsy	Parkinson's disease
Rheumatic diseases:		
Rheumatoid arthritis	SLE	
Other diseases		
End-stage renal disease	Chronic obstructive lung disease	
Medications:		
Glucocorticoids	Proton pump inhibitors	SSRI
Anticoagulants (heparin)	Anticonvulsants	Loop diuretics

SSRI; selective serotonin reuptake inhibitor

2.8 MEASUREMENT OF BONE MINERAL DENSITY

Bone consists of mineral, mainly calcium hydroxyapatite, embedded in the matrix. The matrix consists of collagen type I and different proteins. Calcium absorbs much more radiation than the matrix, and the amount of x-ray energy that is absorbed by calcium reflects the bone mineral content (BMC). BMD is estimated by BMC divided by the area or volume of the bone. Areal BMD (aBMD) is the average of mineral per unit area (g/cm^2) and volumetric BMD (vBMD) is the average of mineral per defined volume of bone (g/cm^3). [166]

2.8.1 DUAL-ENERGY X-RAY ABSORPTIOMETRY

DXA is the most commonly used technique to assess BMD and is used in clinical practice for diagnosis of osteoporosis according to WHO. The hip, lumbar spine, forearm and whole body can be measured. DXA typically assesses aBMD, a measurement dependent of the size of the bone; a larger bone with the same mineral density as a smaller bone will have higher aBMD. The projection normally used for the spine is the anteroposterior (AP) projection which includes the posterior elements of the spine, the facet joints and also the abdominal aorta. Aortic calcifications and osteoarthritis in the spine can result in a false high BMD of the spine. [166] One way to overcome this is to measure BMD in the spine by the lateral projection which excludes the posterior elements and the abdominal aorta and primarily assesses the trabecular bone. [167] Studies have shown BMD by lateral DXA to be less affected by degenerative joint disease than AP DXA. [168, 169] There is no reference material with T-scores and Z-scores for the lateral projection for men. If lateral and AP projections are combined, an estimation of vBMD can be obtained. [170]

2.8.2 HIGH-RESOLUTION PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY

High-resolution peripheral quantitative computed tomography (HRpQCT) is mainly used for research purposes and is not used in clinical practice. With HRpQCT, vBMD at the distal tibia and radius can be obtained for the whole bone and separately for cortical and trabecular bone. In addition, the microarchitecture of the cortical and trabecular bone and bone geometry can be assessed. Based on HRpQCT images, bone strength can be estimated using finite element analysis. There are no reference values such as T-scores or Z-scores for HRpQCT measurements. [171]

2.8.3 OTHER METHODS OF ASSESSMENT

There are other methods of assessment of bone density that are not used in routine clinical practice in the diagnosis of osteoporosis. Quantitative computed tomography (QCT) also assesses vBMD and can separate trabecular bone from cortical bone. [172] QCT can be used for measurements both at the appendicular skeleton and the spine. The method is more useful for spinal measurements since the false increase in BMD by for example degenerative disease can be avoided. However, disadvantages compared to DXA are higher cost and higher exposure to radiation. [173] The Trabecular bone score (TBS) can be computed using the DXA image of the AP spine. It is an evaluation of grey-level texture variations in the image and gives an indirect index of the microarchitecture and an overall score is computed. TBS can be used for fracture prediction in association with the Fracture Risk Assessment Tool (FRAX[®]) and aBMD in postmenopausal women and men > 50 years. Limitations are the lack of a well-established cut-off value for defining normal values and TBS is influenced by body mass index (BMI) and body composition and can only be assessed in patients with BMI in the range of 15-37 kg/m². [174, 175] Quantitative ultrasound (QUS) does not involve any radiation but measures attenuation of ultrasound and speed of sound and gives a reflection of the bone density and structure. The method is mostly applied at the calcaneus. [176] Measurements from different scanners are not comparable to each other and there is no international consensus on how to define osteoporosis with QUS. [177]

2.9 OSTEOPOROSIS AND ANKYLOSING SPONDYLITIS

There are many cross-sectional studies on the prevalence of osteoporosis or low BMD in patients with AS or SpA and many studies show lower BMD in patients compared to age- and sex-matched reference values or controls, which could seem paradoxical considering that spinal new bone formation and ankylosis is a hallmark of the disease. The reported prevalences of low BMD differs between cohorts and ranges from 4 % to 58 %. [178] Reasons for differences in prevalences can be differences in the severity of the disease, the age and disease duration of the studied patients, the underlying diagnosis and the technique used to evaluate BMD. However, low BMD and osteoporosis is found also in patients with early disease, with ranges of prevalences of osteoporosis in 3 - 29 % and of osteopenia in 14-56 % of the patients with disease duration < 10 years. [179] The cross-sectional baseline report from our cohort showed a prevalence of osteoporosis of 21 % and osteopenia of 44 % in the patients > 50 years old, whereas a BMD below

expected range for age was found in 5 % of the patients < 50 years old. Osteoporosis was more prevalent in women (30 %) than men (14 %) whereas BMD below expected range for age was equally prevalent in women and men in patients < 50 years old. [180]

2.10 MEASUREMENT OF BONE MINERAL DENSITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS

In patients with radiographic AS related spinal alterations, BMD can be falsely high when measured at the lumbar spine AP projection. Patients with AS related spinal alterations have been shown to have higher aBMD when measured with the AP projection compared to patients without such alterations. [20, 181, 182] Patients without AS related spinal alterations were shown to have lower aBMD at the AP spine compared to healthy controls whereas aBMD for patients with radiographic changes did not differ compared to controls, [183] and spinal radiographic alterations have been shown to be positively correlated to aBMD at the spine. [184, 185] European League Against Rheumatism (EULAR) management guidelines for use of imaging in SpA have recommended that osteoporosis should be assessed by hip DXA in patients with syndesmophytes in the lumbar spine and supplemented by either spine DXA in lateral projection or by QCT of the spine. [186] The few cross-sectional studies on lateral DXA of the spine in AS have shown that lateral BMD was lower in AS patients in comparison with controls, whereas AP BMD did not differ. [185, 187, 188] One of the studies also grouped the patients in late and early disease and found in comparison with controls, lower lateral BMD in both groups whereas only the group with early disease had lower AP BMD. The only measuring site that differed between patients with and without syndesmophytes was the lateral spine. [188] In this current cohort, baseline analyses showed more women to be diagnosed with osteoporosis with lateral BMD than AP BMD. Reference values for men were lacking. [180] Studies on QCT in AS patients are also limited and three studies included ≤ 15 patients. [183, 189, 190] Studies have shown trabecular vBMD measured by QCT to be less affected by new bone formation than aBMD by AP DXA [182, 190] and higher frequency of osteoporosis/osteopenia or lower Z-score was detected by QCT in patients with syndesmophytes vs patients without syndesmophytes. [191, 192] Over 10 years, trabecular vBMD by QCT had decreased but aBMD by AP DXA increased in 15 patients with AS. In the group with more advanced radiographic spinal alterations, trabecular vBMD was lower compared to patients without AS related spinal alterations. However, in multivariate

analyses, radiographic alterations were not associated with bone loss by QCT. [189]

2.11 FACTORS ASSOCIATED WITH CHANGES IN BONE MINERAL DENSITY IN PATIENTS WITH ANKYLOSING SPONDYLITIS

Longitudinal cohort studies on changes in BMD (Δ -BMD) using DXA in patients with AS differ in follow-up time, age of the patients, duration and severity of the disease, presence of AS related spinal alterations and treatments, among other factors. There are some studies of patients without treatment with TNFi that show an association between inflammation and bone loss. Two studies had similar patient groups in age and disease duration, they also excluded patients with vertebral ankylosis and stratified patients in persistent active or inactive disease based on CRP/erythrocyte sedimentation rate (ESR). One of the studies found that patients with persistent active disease decreased in AP lumbar spine and femoral neck BMD whereas BMD in patients with inactive disease did not change over time. Elevated CRP was found to be independently associated with bone loss at the lumbar spine. [193] The other study found decreases in femoral neck BMD, with greater reduction in the active group vs the inactive group. AP spine BMD did not change in either group. [194] In patients with very early IBP without radiographic sacroiliitis, there were no changes in BMD at total hip, femoral neck, lumbar spine AP or the hand in the total group, however, in the group with persistent high CRP, femoral neck and total hip BMD decreased with significant difference from the group with normal CRP. [195]

Other studies on patients without TNFi have included patients no matter the severity of AS related spinal alterations. One such study found BMD at the AP spine, femur (probably total hip) and forearm to increase significantly over time. Elevated ESR during follow-up and hip involvement were reported to be associated with decreases in spinal and femoral neck BMD. The association between AS related spinal alterations and Δ -BMD was not investigated. [196] Another study stratified AS patients in active and inactive disease based on Bath AS Disease Activity Index (BASDAI). No differences in Δ -BMD between groups were observed in AP spine, femoral neck or total hip BMD. Overall, AP spine BMD increased, as did SASSS (Stoke AS Spine Score, which assesses only the lumbar spine) but the authors reported no significant relationship between Δ -SASSS and Δ -BMD. [197] Several studies on patients treated with TNFi, did not find a relationship between AS related spinal alterations and Δ -BMD either. [198-200] Only one longitudinal study

found such a relationship; increases in SASSS was associated with increases in AP spine BMD. Additionally, patients with combination of TNFi and bisphosphonates increased more in SASSS compared to patients treated with TNFi alone, whereas no difference in Δ -SASSS was found when comparing use of bisphosphonates or not in patients without TNFi. [201]

Most studies on changes in BMD aim at exploring the role of TNFi on BMD. One meta-analysis on the effect of TNFi on changes in spine and hip BMD in patients with AS, reported that AP lumbar spine BMD and total hip BMD had increased after 1 year of treatment and further increased the second year. Femoral neck BMD remained stable after 1 year; there were not sufficient data for analyses of 2 year changes. [202] Studies published after that meta-analysis also show increases in AP spine BMD [199, 203-205], total hip BMD [199, 205] as well as femoral neck BMD for patients treated with TNFi. [204] One study showed use of NSAID to have a protective effect on bone loss at total hip in patients with early inflammatory back pain suggestive of SpA, both in patients with and without TNFi. Additionally, in patients without TNFi, a 2 year increase in BMI was protective for bone loss. [206]

Studies on the effect of bisphosphonates on changes in BMD in AS patients are scarce. Studies vary in length from 6 months to 2 years and included between 12 to 34 patients with bisphosphonate treatment. Results are conflicting. In a 6 month study on disease activity in AS, patients were randomized to the TNFi infliximab or intravenous bisphosphonate neridronate. Patients treated with neridronate increased in AP lumbar spine BMD whereas no change was observed in patients treated with infliximab. No significant changes in femoral neck BMD or total hip BMD were observed. [207] One observational cohort study found indications of a synergistic effect of oral bisphosphonates and TNFi on increases in BMD, but only at the greater trochanter. [208] Two studies could not find an effect on BMD by bisphosphonate treatment. [201, 209] The IL-17A inhibitor has not been studied in regards of changes in BMD.

2.12 FRACTURES IN PATIENTS WITH ANKYLOSING SPONDYLITIS

2.12.1 VERTEBRAL FRACTURES

There are many studies on VFs in patients with AS and the prevalence in different studies varies from 0.9 % to 39 %. [210] The prevalence of VFs found on radiographs in this current cohort at baseline was 12 %. [192]

Patients with AS have been shown to have an increased risk of VFs compared to controls, both regarding morphometric fractures [211, 212] and clinical fractures. [213] Patients with AS are used to spinal pain, and spinal fractures can be overlooked by the patient and the doctor. Patients with a fused or partly fused spine due to AS related spinal alterations are susceptible to severe spinal fractures even after low energy trauma, mainly in the cervical spine. Due to ossification of supportive soft tissues, the fractures have the risk of being unstable and may dislocate and cause neurological deficit. [214] Plain radiographs in these patients may also fail to detect the fracture due to the ossifications. [215]

A recent meta-analysis to examine the risk of VF and non-vertebral fractures found that patients with AS had almost double the risk of VFs when compared to non-AS subjects. The risk remained the same when analyses were stratified in morphometric and clinical fractures. [210] In the meta-analysis they found prevalent VFs to be associated with lower BMD at the femoral neck and total hip, but not at the lumbar spine. BMD at the forearm was also lower in patients with VFs, however, only two studies with relatively small number of fractures assessed this measuring site and further studies are needed. Also, older age, male sex, longer disease duration, more AS related spinal alterations and IBD were risk factors for prevalent VFs. [210] Data regarding the association between NSAID-use and VFs are conflicting. [70, 213, 216-218]

There are some longitudinal studies assessing the effect of TNFi on development of new VFs, however, number of patients who develop new VFs are relatively small. Despite improvement in BMD and decreases in disease activity with TNFi, VFs continued to develop with up to 20 % of patients developing new VFs with up to 4 years of treatment. [199, 203, 205] Two studies found no significant difference in frequency of new VFs between patients with and without TNFi. [216, 219] Variables reported to be independently associated with development of new VFs over 4 years were baseline VFs and increases in CRP at the 2-year control. [216] Two studies did not conduct multivariate analyses; in univariate analyses, both studies found new or progressive VFs to be associated with older age and lower AP spine BMD. Additionally, new VFs over 2 years were associated with lower total hip BMD and less use of NSAIDs at baseline [219], whereas new VFs over 4 years were associated with longer duration of smoking, higher Bath AS Functional Index (BASFI), presence of VFs and use of anti-osteoporotic treatment at baseline. [203] One longitudinal study found no variables associated with incident VFs. [199]

2.12.2 NON-VERTEBRAL FRACTURES

Non-vertebral fractures in patients with AS are less studied than VFs. The aforementioned meta-analysis on fracture risk in patients with AS found the risk of non-vertebral fractures to be 10 % higher in AS patients compared to controls. The result was based on 3 studies. Two studies compared the frequency of hip fractures between AS and controls, and the meta-analysis showed no significant difference between AS and controls. No analyses regarding factors associated with non-vertebral fractures could be done. [210] One study has reported risks of fractures at radius/ulna for AS patients, and did not find the risk to be significantly higher in AS patients compared to controls. [213]

3 AIMS

The general aims of this thesis were to study the development of bone loss and new bone formation over 5 years in a cohort of patients with AS, as well as to assess factors associated with the changes.

The specific aims of the papers included in the thesis were:

- I. To evaluate changes in aBMD measured by DXA at the lumbar spine, both the AP and lateral projections, the total hip, femoral neck and total radius and to assess disease-related variables and medications associated with the changes in BMD.
- II. To evaluate the progression of AS related spinal alterations and to assess predictors for the progression overall and by sex.
- III. To evaluate changes in trabecular and cortical vBMD, cortical area and microarchitecture at tibia and radius measured by HRpQCT in men with AS and to assess factors associated with changes in vBMD and cortical area.
- IV. To study associations of baseline s-HGF and the average s-HGF with progression of AS related spinal alterations overall and by sex and to assess factors correlated with changes in s-HGF.

4 PATIENTS AND METHODS

This thesis is based on a longitudinal, prospective study on a cohort of patients with AS.

4.1 PATIENTS

All papers in this thesis are based on a cohort of patients recruited at baseline from the rheumatology clinics at Sahlgrenska University Hospital in Gothenburg and the hospitals at Borås and Alingsås in Sweden. The recruitment procedure has been described in detail in a previous report. [180] Medical records of all patients with AS registered in the hospitals' databases were assessed for eligibility of the study. Inclusion criterion was AS according to the modified New York criteria. [36] Exclusion criteria were psoriasis, IBD, dementia, difficulties in understanding the Swedish language and ongoing pregnancy. In total, 204 patients completed DXA, X-rays of the spine, blood samples, medical examination and questionnaires at baseline and were invited to the 5-year follow-up. Results from the follow-up are reported in paper I, II and IV. The flow chart of participation is shown in Figure 2.

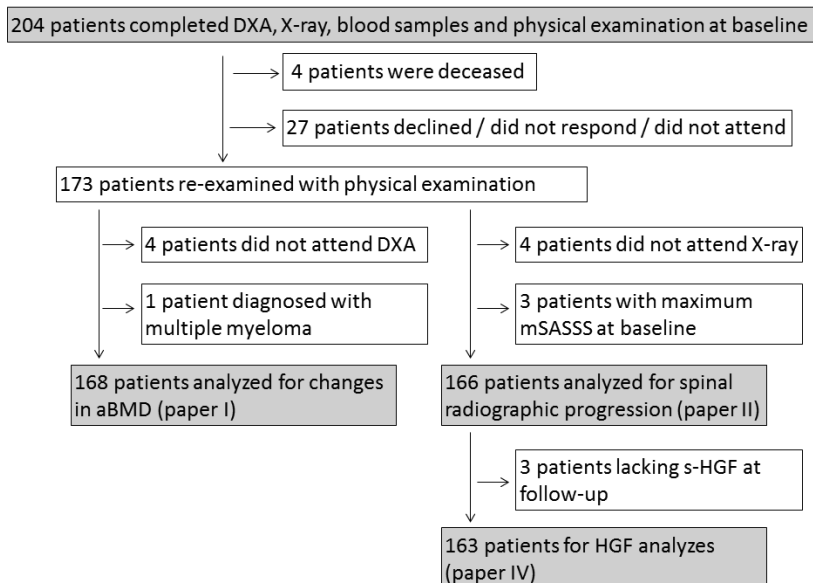


Figure 2. Flow chart of participation from baseline to the 5-year follow-up for assessment with DXA, radiography and serum HGF

Paper III. Of the 204 patients, 69 men were also randomized in an age-adjusted algorithm to undergo HRpQCT at baseline. Of these 69 men, 2 were deceased and 10 declined participation, did not respond to invitation or did not come to examination at the 5-year follow-up; thus 57 men were re-examined with HRpQCT. However, due to motion artifacts, examinations of 54 men were eligible for analyses of changes in vBMD and cortical area. Microarchitecture parameters are more sensitive to motion artefacts than vBMD and cortical area, and 45 examinations were eligible for analyses of changes in microarchitecture. [220]

The patients underwent the same physical examinations at baseline and at the 5-year follow-up performed by one physician, Eva Klingberg, at baseline and by me at the follow-up. Physical examinations included evaluation of 66/68 joint count for swollen and tender joints and the Bath AS Metrology Index (BASMI) for evaluation of spinal and hip mobility. [37] BASMI contains five clinical measurements: tragus to wall distance, lumbar flexion, cervical rotation, lateral lumbar flexion and intermalleolar distance. BASMI ranges from 0-10, with 10 being the most impaired mobility. To calibrate the examination, BASMI was practiced together with Eva Klingberg before the 5-year follow-up. Patients' height and weight were measured and BMI was calculated. In paper II BMI was categorized in three groups: 1 = normal (BMI 18.5 – 24.9 kg/m²), 2 = overweight (BMI 25.0 – 29.9 kg/m²) and 3 = obese (BMI ≥ 30 kg/m²). [221] In paper II, patients were also categorized according to type of occupation; blue collar work involving manual labor and physical tasks and white collar work requiring less physical activity and more formal education. [222]

4.2 CONTROLS

Paper IV. Healthy controls were used for comparison of baseline s-HGF. Controls were recruited among blood donors at Sahlgrenska University Hospital while giving blood. They answered a questionnaire stating they were in full health and not on any medication.

4.3 QUESTIONNAIRES

Paper I-IV. Questionnaires included lifestyle factors, risk factors for osteoporosis, medical history, AS manifestations and medications. The effect of AS on general wellbeing was evaluated with Bath AS Patient Global score (BAS-G) during the last week (BAS-G1) and the last 6 months (BAS-G2). [223] Disease activity was evaluated with BASDAI and ASDAS_CRP. [37]

BASDAI includes 6 questions and the patients grade each answer from 0-10 using a visual analogue scale. The questions include 1) the level of fatigue/tiredness, 2) the overall level of neck, back or hip pain, 3) the level of pain/swelling in other joints, 4) the level of discomfort from areas tender to touch or pressure, 5) the level of morning stiffness and 6) the duration of morning stiffness. An overall score is then calculated, ranging from 0-10. ADSAS_CRP is calculated by a specific formula which includes question number 2, 3 and 6 from BASDAI, BAS-G1 and CRP. At the 5-year follow-up, data about NSAID consumption during the last 5 years was collected and quantified according to the recommendations of the ASAS recommendations. [224] The NSAID-index is calculated based on the type of NSAID, the dose and the number of days taking NSAID during the period of interest. The index ranges from 0-100.

4.4 REVIEW OF MEDICAL RECORDS

Information about the duration of treatments with TNFi and bisphosphonates and dose of glucocorticoids during the follow-up time was extracted from the medical records at the 5-year follow-up. The dose of glucocorticoids was converted into milligrams of prednisolone. In paper I, use of TNFi or bisphosphonates was calculated by dividing the number of months of exposure to either medication with the follow-up time in months for each patient, resulting in a value between 0 and 1. In paper II-IV treatment during follow-up time with TNFi or bisphosphonates was dichotomized in exposure to the treatment during follow-up or not. Additionally in paper III, patients were also dichotomized in having used TNFi ≥ 4 years or not during follow-up. This was done to have a more homogenous group of patients with similar length of exposure to TNFi in this study with fewer participants. Of the 16 patients exposed to TNFi during follow-up, only 4 patients had used TNFi for < 4 years, and had a short time of exposure of median 10.5 months. In paper III, the dose of prednisolone was dichotomized in having used $<$ or ≥ 450 mg prednisolone during follow-up, a dose equivalent of 5 mg prednisolone/day during 3 months.

4.5 BLOOD SAMPLES

Paper I-IV. Blood samples for analyses of CRP, ESR and white blood cell count (WBC) were analyzed consecutively by standard laboratory techniques at baseline and at the 5-year follow-up. At both occasions, serum and plasma samples were obtained and stored, first at -20°C and then at -80°C until further analyses were done.

Time-averaged CRP and ESR for the follow-up time were calculated at the 5-year follow-up. The first recorded CRP/ESR in the medical records each year was used unless the patient had an infection; in that case the subsequent test was used.

Paper IV. S-HGF was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (Quantikine® ELISA, R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instruction after baseline and the 5-year follow-up. Absorbance was read at 450 nm in the spectrophotometer SpectraMax® 340PC³⁸⁴ (Molecular Devices, San Jose, CA, USA). The software SoftMax® Pro 5.2 (Molecular Devices) was used for calculating the concentration of HGF.

4.6 RADIOGRAPHY

Conventional radiographs of the cervical, thoracic and lumbar spine were obtained at baseline and at the 5-year follow-up. All radiographs were scored simultaneously by the same musculoskeletal radiologist blinded to the clinical data but with known chronological order.

4.6.1 AS RELATED SPINAL ALTERATIONS

Paper I-IV. AS related spinal alterations were scored according to the mSASSS. With mSASSS, each anterior corner of the vertebrae in cervical (from the lower corner of C2 to the upper corner of T1) and lumbar spine (from the lower corner of T12 to the upper corner of S1) is graded on lateral radiographs with a score between 0 and 3: 0 = normal, 1 = erosion, squaring or sclerosis, 2 = syndesmophyte and 3 = total bony bridging between upper and lower vertebral corners (ankylosis). The total score ranges from 0 to 72 [65] (Figure 3). Spinal radiographic progression was defined as either an increase of ≥ 2 mSASSS over 5 years or development of ≥ 1 new syndesmophyte over 5 years.

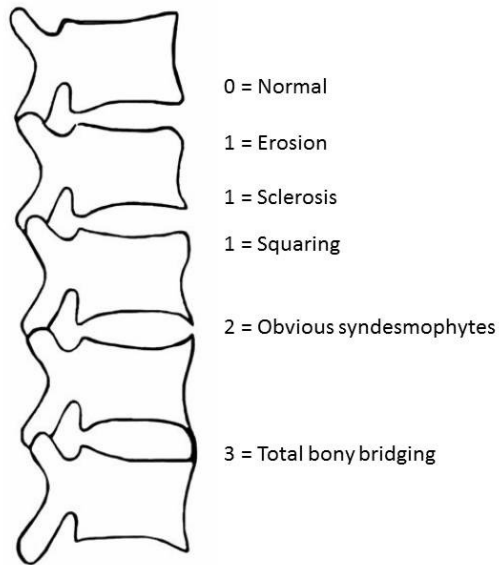


Figure 3. With mSASSS, each anterior corner of vertebrae lower C2 to upper T1 and lower T12 to upper S1 are graded from 0 to 3. The total score ranges from 0 to 72 [65]

4.6.2 VERTEBRAL FRACTURES

Paper I. Vertebral fractures were evaluated by the semiquantitative method Genant score. With Genant score, vertebrae T4 to L4 are assessed for reductions in height of the anterior, middle and/or posterior vertebral body. Each vertebra is graded 0 = normal, 1 = mild (20 - 25 % reduction in height), 2 = moderate (26 – 40 % reduction in height) or 3 = severe (> 40 % reduction in height). [225] Progression in Genant score was defined as development of a fracture in a previously normal vertebra or worsening of at least 1 point in Genant score (Figure 4).

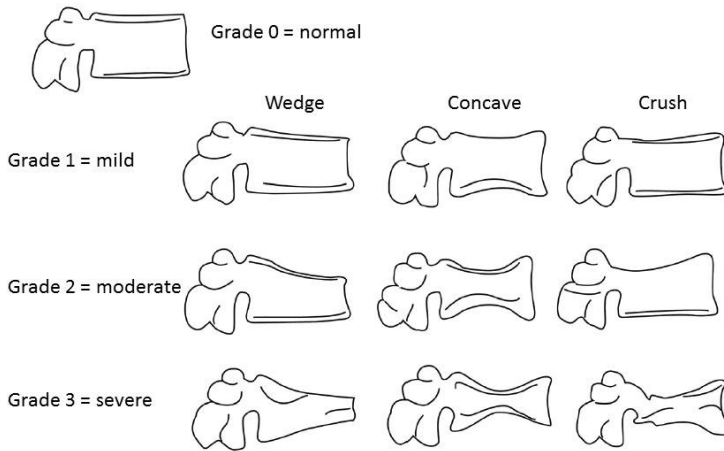


Figure 4. Grading of vertebral fractures according to Genant score [225]

4.7 BONE MINERAL DENSITY

4.7.1 DUAL ENERGY X-RAY ABSORPTIOMETRY

Paper I and III. With DXA, aBMD (g/cm^2) was assessed at the left hip (total hip and femoral neck), the lumbar spine AP projection (vertebrae L1-L4) and lateral projection (vertebrae L2-L4), and the non-dominant forearm (total radius). By combining the AP and lateral projection, an estimation of lumbar vBMD (g/cm^3) was obtained from the DXA machine. Measurements were done using the same DXA scanner (Hologic Discovery A, Hologic Inc., Bedford, MA USA) at baseline and at the 5-year follow-up.

4.7.2 HIGH RESOLUTION PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY

Paper III. With HRpQCT, trabecular and cortical vBMD (mg/cm^3), cortical area (mm^2) and microarchitecture (trabecular number (per mm), trabecular separation (mm), trabecular thickness (mm)) were assessed in the non-dominant ultra-distal radius and tibia using the same machine at baseline and the 5-year follow-up (Xtreme CT, Scanco Medical AG, Brüttisellen, Switzerland). Trabecular and cortical vBMD, cortical area and trabecular number were measured directly and the other parameters were derived. [226]

4.8 ETHICAL CONSIDERATIONS

All patients were exposed to radiation through the spinal radiographs and the DXA, with estimated effective radiation dose of 1.6 mSv for the radiographs and 0.025 mSv for the DXA, both at baseline and at the 5-year follow-up. The men that were randomized to HRpQCT also underwent QCT and had an additional exposure of 0.61 mSv at each occasion. For comparison, the approximate effective annual radiation dose in Sweden from sources like cosmic radiation, the ground, the body and food is 1.5 mSv (www.stralsakerhetsmyndigheten.se/contentassets/27621960d345484694bcd4f0e734b4bb/200702e-radiation-environment-in-sweden-summary). All data were collected in a database with the personal data coded, and only authorized investigators had access to the database and the code. The patients were free to withdraw from the study at any point. Approvals of the regional ethics committee in Gothenburg and the local committee of radiation protection in Gothenburg were obtained at baseline and at the 5-year follow-up. The study was carried out in accordance with the principles of the Declaration of Helsinki. All patients and healthy controls gave their informed written consent.

4.9 STATISTICS

Paper I-IV. All statistical analyses were performed using IBM SPSS Statistics 22 (IBM, Armonk, NY, USA), except calculation of HGF cut-off point in paper IV where SAS software version 9.4 (SAS, Cary, NC, USA) was used.

Descriptive statistics are presented as number and percentage, mean and standard deviation (SD) or median and 25th (Q1) and 75th (Q3) percentile. To compare continuous data between different groups, the *t*-test was used for normally distributed data and the Mann Whitney U-test for variables not normally distributed. The Chi-square test or Fischer's exact test were used for comparison of categorical variables. For repeated measurements, the paired *t*-test was used for normally distributed data, the Wilcoxon signed rank test for not normally distributed data and McNemar's test for categorical variables. Bivariate correlations were calculated using Spearman's rank correlation (r_s). Δ -values were calculated by subtracting the baseline value from the follow-up value. All tests were two-tailed and $p \leq 0.05$ was considered statistically significant.

Paper I. The one-sided *t*-test was used to compare the Z-score of the patients to the test value 0. Standard multivariable linear regression analyses were

conducted with Δ -BMD at the different measuring sites as a dependent variable. Covariates entered in the models were demographic variables known to affect BMD and disease related variables as well as medications that were hypothesized to influence Δ -BMD.

Paper II. For calculation of reliability data, 40 randomly selected radiographs were re-scored by the same radiologist. Intra-reader agreement for status scores and change scores were assessed by an intraclass correlation coefficient (ICC) two-way mixed-effect model, with single measurement and absolute agreement. [227] The smallest detectable change (SDC) was calculated as proposed by Bruynesteyn et al. [228] Univariate and multivariable (backward method) binary logistic regression analyses were conducted with progression ≥ 2 mSASSS/5 years or development of ≥ 1 new syndesmophyte over 5 years as a dependent variable. Yes was coded 1 and no was coded 0. Analyses were conducted in the total group and stratified by sex. Covariates considered for the multivariable models were variables with p -value < 0.2 in the univariate analyses for the total group and p -value < 0.1 for the sex stratified analyses. Propensity scores for the probabilities of being exposed to bisphosphonates, being exposed to TNFi or being treated with TNFi ≥ 2 years were calculated and used as a covariate together with the treatment variable in standard binary logistic regression analyses.

Paper III. Univariate and standard multivariable linear regression analyses with Δ -cortical vBMD, Δ -trabecular vBMD or Δ -cortical area as a dependent variable were conducted. Covariates considered for the multivariable models were variables with a p -value ≤ 0.1 in the univariate analyses.

Paper IV. The average s-HGF was calculated. For men, a receiver operating characteristic (ROC) curve was plotted with baseline s-HGF as the test variable and development of ≥ 1 new syndesmophyte over 5 years as the categorical state variable. Youden's index was then used to identify an optimal cut-off point for a predictive s-HGF value. For the s-HGF cut-off point, calculations of positive (LR+) and negative (LR-) likelihood ratio for developing ≥ 1 new syndesmophyte were performed. Univariate binary logistic regression analyses with progression of ≥ 2 mSASSS over 5 years or development of ≥ 1 new syndesmophyte as dependent variable and baseline s-HGF, HGF cut-off point or the average s-HGF as a covariate were conducted. If the univariate p -value was ≤ 0.05 , multivariable logistic regression analyses (backward method) were conducted and adjusted for covariates significant for spinal radiographic progression in paper II.

4.10 FOLLOW-UP AFTER THE STUDY

The results from radiography and the DXA examinations at the 5-year follow-up were communicated to the patients by mail. All patients also got written information about life-style factors that can influence bone health. Patients were prescribed treatment with bisphosphonates and/or substitution with calcium and vitamin D according to the algorithm shown in Table 8.

Table 8. Algorithm for prescription of bisphosphonates and/or calcium + vitamin D after DXA and radiography at the 5-year follow-up

Patients were prescribed alendronate and calcium + vitamin D if:	
<ul style="list-style-type: none"> -Vertebral fracture on radiography or hip fracture -T-score < -3 SD -T-score \leq -2.5 SD and 1 major or 2 minor risk factors 	
<p style="text-align: center;">Major risk factors</p> <ul style="list-style-type: none"> -Previous low-energy fracture in wrist, upper arm, pelvis (VF and hip fracture) -Treatment with prednisolone >3 months -Hip fracture or VF in parent 	<p style="text-align: center;">Minor risk factors</p> <ul style="list-style-type: none"> -Age of menopause before 45 years old -Smoking -Inactivity -BMI < 20 kg/m² -High risk of falling
<ul style="list-style-type: none"> -T-score \leq -2 SD and > -2.5 SD + FRAX[®] \geq 15 % + previous fracture -T-score < -1 SD + Prednisolone 	
Patients were prescribed calcium + vitamin D in monotherapy if:	
Normal BMD + Prednisolone	
Decision was based on BMD at femoral neck, total hip and lumbar spine AP.	
FRAX [®] ; Fracture Risk Assessment Tool	

Treatment with bisphosphonates and calcium + vitamin D was prescribed to 17 patients, 9 women and 8 men, with median (Q1, Q3) age of 64 (54.5, 72) years old. All women except one were menopausal at the 5-year follow-up. Monotherapy with calcium + vitamin D was not initiated in anyone.

5 RESULTS AND DISCUSSION

5.1 PAPER I

Which measuring site in ankylosing spondylitis is best to detect bone loss and what predicts the decline: results from a 5-year prospective study.

In this study we investigated how BMD changed over five years in patients with AS and factors associated with the changes. Several measuring sites were assessed: the total hip, the femoral neck, the total radius and the spine, both AP and lateral projection. For the first time, the lateral projection of the spine was assessed longitudinally in patients with AS. There were 168 patients that were examined at both baseline and at the 5-year follow-up, 92 men and 76 women. At baseline, the mean (SD) age was 50 (13) years old and the mean (SD) symptom duration was 24 (13) years.

5.1.1 MAIN RESULTS

- BMD decreased significantly at the femoral neck and total radius.
- BMD increased significantly at the total hip and the lumbar spine, both the AP and lateral projections.
- Elevated time-averaged CRP was associated with decreases in femoral neck BMD.
- Use of bisphosphonates was associated with increases in BMD at all sites except total radius.
- Use of TNFi was associated with increases in AP spine BMD.

5.1.2 CONCLUSION

This longitudinal study that assessed changes in BMD at five different measuring sites including the lateral spine, suggests that the best measurement site for assessment of bone loss over time in patients with long standing AS is at the femoral neck. This study also suggest that systemic inflammation has a negative effect on BMD at all measured sites except total radius and that use of bisphosphonates and TNFi has a positive effect on BMD in patients with AS.

5.1.3 DISCUSSION

BMD and T-score decreased during follow-up at the femoral neck, in both sexes, and persistent elevated CRP was independently associated with the

decreases. These findings are in line with some previous studies on patients without TNFi. In patients with early AS or IBP, decreases in femoral neck BMD were found to be more pronounced in patients with persistent systemic inflammation, [194] or decreases only found in patients with active disease. [193, 195] One study found no significant change in femoral neck BMD regardless of disease activity. [197] Also in an older patient group, all with active disease and low BMD at baseline, femoral neck decreased. [204] The patients in these studies were younger than in the current study, and we found older age to be an independent predictor of femoral neck bone loss in this current cohort. Also, in comparison with the reference population, the femoral neck BMD in the current study did not differ significantly from the age and sex-matched controls. However, if age was excluded from the multivariable regression model and the change in adjusted R^2 was assessed, age explained a rather small part of 4 % of the variation in Δ -BMD. Use of bisphosphonates but not TNFi was independently associated with increases in femoral neck BMD. The effect of TNFi on femoral neck BMD is not so well studied, and previous studies have shown increases in BMD [204] as well as no significant change. [229]

The AS patients also decreased in BMD and T-score at the total radius. However, the only variable found to be independently associated with changes in BMD was older age. Also, compared to the age- and sex-matched reference values, the patients differed in BMD neither at baseline nor at the follow-up. Hence, the decreases in BMD at radius do not seem to be related to the disease.

In contrast to femoral neck and total radius, BMD at the total hip and lumbar spine AP and lateral projection increased. In sex stratified analyses, significant increases were found in men, and male sex was independently predicting increases in BMD at the total hip with a trend at the AP spine. However, in sex stratified analyses, men and women with AS did not differ in BMD at baseline compared to the age- and sex-matched reference group, whereas BMD at the follow-up at the total hip and AP spine were higher in the AS patients. This would imply an increase in BMD or less bone loss than in the general population for both sexes. Reference values for the lateral projection were only available for women and women with AS showed higher lateral BMD than the reference population at the follow-up also at this site.

Increasing ESR from baseline to follow-up was associated with decreases in BMD at the total hip, spine AP and lateral spine, whereas use of bisphosphonates were independently associated with increases. Use of TNFi

was independently associated with increases in AP spine BMD. When patients with bisphosphonates and TNFi were excluded from analyses, BMD did not change significantly at these sites and high time-averaged ESR was associated with decreases in BMD, except at AP spine. Previous reports on changes in BMD in AS patients without treatment with bisphosphonates and TNFi are not consistent. BMD at the spine AP has been shown to be stable, [194, 195] to increase, [196, 197] and also to decrease in patients with active disease. [193] Methods for evaluation of AS related spinal alterations differed between the studies and this parameter is difficult to compare between the studies. Changes previously reported for total hip BMD are decreases in patients with active disease, [195] and high ESR during follow-up was shown to be associated with decreases in total hip BMD and also AP spine BMD in another cohort. [196] To further analyze the impact of bisphosphonates and TNFi on the increases in BMD at the spine and total hip in the current cohort, the treatments were removed from the multivariable regression models, and the change in adjusted R^2 was assessed. For the total hip and the lateral spine, bisphosphonates and TNFi together explained 10 % of the variation in Δ -BMD, and for the AP spine it was as much as 20 % (data not in the article). These treatments have an impact, but other factors are also contributing to the changes in BMD. No independent association between AS related alterations in the spine and changes in spinal BMD were found.

The AP spine was the site with the highest adjusted R^2 , with 44 % of the variation in Δ -BMD explained by the independent variables. Hence, many factors associated with changes in BMD were not identified in this current study. There are factors not included in the regression models that can affect BMD, such as use of proton pump inhibitors, selective serotonin reuptake inhibitors, anti-diabetics, alcohol abuse, and serum levels of vitamin D, calcium or parathyroid hormone. However, the number of participants in the study restricted the number of variables possible in the multivariate regression models.

Prevalences of osteoporosis did not change significantly between baseline and the 5-year follow-up. If patients with osteoporosis/BMD below expected range for age at baseline were excluded, 12 of 129 patients were found to develop osteoporosis/BMD below expected range for age during follow-up. All had osteopenia at baseline, but patients were too few for analyses of independent predictors for development of osteoporosis. The measured sites with newly developed osteoporosis were femoral neck ($n = 5$), radius ($n=4$), AP spine ($n=3$) and lateral spine ($n=1$). Of the 39 patients with osteoporosis/BMD below expected range for age at baseline, 6 patients did not have osteoporosis any longer at the 5-year follow-up. All 6 patients had

been exposed to bisphosphonates, and one patient was exposed to TNFi during follow-up (data not in article).

Very few patients developed VFs during follow-up; only 3 patients (1.8 %) developed new VFs of which one patient also worsened in previous VFs. Despite treatment with TNFi and increases in BMD, previous studies have reported AS patients to continue to develop new VFs in up to 20 % of the participants. [199, 203, 205] We can only speculate if the low occurrence of new VFs in patients in this current cohort is related to initiation of bisphosphonates after the baseline measurements in patients at higher risk of developing VFs.

5.2 PAPER II

A five-year prospective study of spinal radiographic progression and its predictors in men and women with ankylosing spondylitis.

In this study we assessed spinal radiographic progression over five years and predictors for the progression in the total group and in men and women separately. Definite spinal radiographic progression was defined as either progression of ≥ 2 mSASSS/5 years or development of ≥ 1 new syndesmophyte/5 years. There were 166 patients examined at baseline and at the 5-year follow-up who also had a baseline mSASSS < 72 .

5.2.1 MAIN RESULTS

- The total group had a progression of mean (SD) 1.6 (3.3) mSASSS over five years ($p < 0.001$), 47 patients (28 %) had a progression of ≥ 2 mSASSS/5 years and 36 patients (22 %) developed ≥ 1 new syndesmophyte.
- Men increased more in mSASSS compared to women, and more men than women had a progression of ≥ 2 mSASSS and development of ≥ 1 new syndesmophyte.
- In the total group, radiographic progression was associated with baseline syndesmophytes, elevated baseline CRP, male sex, older age, obesity and exposure to bisphosphonates during follow-up.
- Factors associated with radiographic progression only found in men were elevated baseline CRP and smoking.
- Factors associated with radiographic progression only found in women were exposure to bisphosphonates during follow-up and a high BASMI.

5.2.2 CONCLUSION

Over five years, men had more radiographic progression than women. The study suggests that predictors for spinal radiographic progression may partly differ between sexes. New predictors identified in this study were obesity in both sexes and impaired spinal mobility and exposure to bisphosphonates during follow-up in women. Among previously known predictors, baseline AS related spinal alterations was shared by sexes whereas elevated baseline CRP and smoking were predictors in men. The role of bisphosphonates in spinal radiographic progression needs to be further studied.

5.2.3 DISCUSSION

Men had higher baseline mSASSS and higher progression of mSASSS than women, more men had definite radiographic progression compared to women and male sex was an independent predictor for progression of ≥ 2 mSASSS in this current study. Previous studies have also reported more AS related spinal alterations in men [72-74], faster progression and more men with development of new syndesmophytes compared to women [67, 74] and male sex to predict spinal radiographic progression. [69, 143] The reason for this difference is not known. Reports on disease related sex-differences are scarce and knowledge about underlying mechanisms for spinal radiographic progression is limited. This current study is the first to report factors associated with spinal radiographic progression in sex stratified analyses, except Hartl et al who studied biomarkers stratified by sex and reported low leptin and HMW-APN to predict radiographic progression in men. [113]. We had a rather even distribution of men and women in the current cohort. However, women had a low frequency of definite spinal radiographic progression which reduced the statistical power in the sex stratified analyses which need to be interpreted with caution and is a limitation with the study. Nevertheless, our results suggest that smoking has a negative effect on spinal radiographic progression in men only. Elevated CRP was a significant predictor in men, but showed a trend in women, so the sex difference might be related to few women with definite radiographic progression. Both elevated CRP and smoking have been shown to predict radiographic progression in several previous studies. [68, 99, 100, 140]

Obesity was associated with radiographic progression in both sexes. One previous study has reported overweight and obese patients to have higher baseline mSASSS compared to normal weight patients, and high BMI and $\text{BMI} \geq 25 \text{ kg/m}^2$ were predictors of spinal radiographic progression in univariate analyses. However, no independent association was found in that study. [70] In a retrospective study published as a letter (with some important

information on methodology lacking) some type of time-averaged BMI ≥ 25 kg/m² was independently associated with spinal radiographic progression, [230] whereas Molnar et al found no association between baseline BMI categories and radiographic progression. [69] There are several possible mechanisms for obesity being associated with spinal radiographic progression. It could be related to increased mechanical stress as new bone formation has been shown to be associated with mechanical loading in a mouse model of SpA, [88] and indirectly with blue collar work. [92] It could also be related to adipose tissue being an endocrine organ with production of adipokines, [231] and elevated level of the adipokine serum visfatin has been shown to independently predict spinal radiographic progression. [112] Additionally, obesity has been shown to be independently associated with higher disease activity and worse physical function in patients with axial SpA [232] and AS patients with overweight and obesity had a more negative perception of benefits of exercise compared to those with normal BMI. [233] Also, overweight and obese axial-SpA patients had worse treatment response to TNFi compared to patients with normal BMI. [234, 235]

Exposure to bisphosphonates during follow-up was found to be associated with radiographic progression in women. This novel finding is intriguing, and has resulted in a letter by Orsolini et al. [236] They hypothesized that the association found was due to more severe bone loss in patients with radiographic progression. This hypothesis is plausible. Kim et al. recently reported low BMD at any measured site (spine AP, total hip and femoral neck) to be an independent predictor for spinal radiographic progression in patients with axial SpA. [140] A very recent publication reported low BMD at the lateral spine to be an independent predictor of radiographic progression. [237] Analyses in our current study show no univariate association with radiographic progression for baseline BMD (AP, lateral, total hip, femoral neck and total radius) or baseline osteoporosis/BMD below expected range for age at any measured site (data not in article). Mechanistically, bisphosphonates bind to hydroxyl apatite crystals in the skeleton, especially at sites with high bone turnover. They mainly have an anti-resorptive effect and inhibit the osteoclasts maturation, proliferation and activity and promote apoptosis of the osteoclasts. Bisphosphonates are also suggested to have a direct positive effect on osteoblasts but this role is less clear. [238] Results from experiments on the effect of bisphosphonates on osteoblasts are contrasting, but overall, the observations suggest that bisphosphonates promote bone formation. [239] Bisphosphonates also have an anti-inflammatory effect. [240] Open-label studies on patients with SpA have shown an effect on both peripheral and axial disease with the intravenously administered bisphosphonate pamidronate, [241] whereas

orally administered alendronate did not have such an effect in a randomized, placebo controlled study. [242] The role of bisphosphonates in spinal radiographic progression needs indeed to be further investigated since it is an important medication for osteoporosis and prevention of fractures.

5.3 PAPER III

Factors associated with changes in volumetric bone mineral density and cortical area in men with ankylosing spondylitis. A five-year prospective study using HRpQCT.

In this study we assessed changes in cortical and trabecular vBMD, cortical area and microarchitecture over five years and factors associated with the changes in vBMD and cortical area using HRpQCT at the ultra-distal radius and tibia. Of the 69 men randomized to the examinations at baseline, 54 men had assessable examinations for analyses of vBMD and cortical area both at baseline and at the follow-up, whereas 45 men had assessable examination for analyses of microarchitecture. At baseline, the mean (SD) age was 48 (14) years old and the mean (SD) symptom duration was 22.5 (13) years.

5.3.1 MAIN RESULTS

- Cortical and trabecular vBMD at tibia decreased more than the least significant change (LSC).
- Worsening of the microarchitecture at tibia was found, but changes did not exceed LSC.
- An increase in ASDAS_CRP from baseline to follow-up was independently associated with decreases in cortical vBMD and cortical area at tibia.
- High time-averaged ESR was associated with decreases in cortical area at radius.
- Use of TNFi ≥ 4 years during follow-up was associated with increases in cortical vBMD and cortical area at tibia.
- Exposure to bisphosphonates during follow-up was associated with increases in cortical vBMD and cortical area at radius.

5.3.2 CONCLUSION

Over five years, the men with AS decreased significantly in cortical and trabecular vBMD at tibia. Disease related factors and medications were found to affect cortical bone. Signs of active inflammation were associated with

decreases in cortical parameters whereas treatment with TNFi and bisphosphonates were associated with increases in cortical bone.

5.3.3 DISCUSSION

This is to our knowledge the first longitudinal study on patients with AS or nr-axial SpA using HRpQCT. We found decreases in trabecular vBMD and cortical vBMD that exceeded LSC (change judged as “true” change with 95 % confidence, the change exceeding the precision error of the method) at tibia. The precision error was larger for the assessments of microarchitecture (trabecular thickness, trabecular separation and trabecular number) and the deterioration found in microarchitecture did not exceed LSC.

One of the limitations with the method is that there are no reference values for HRpQCT measurements to compare our results to. Previous cross-sectional studies on patients with AS or nr-axial SpA have compared measurements between patients and controls. Two studies included both sexes and showed patients to have impaired measurements compared to controls in cortical bone, with decreased cortical vBMD at both tibia and radius [243] and decreased cortical vBMD, area and thickness at radius (tibia not measured). [244] The baseline study on our cohort with men showed decreased cortical vBMD at tibia and decreased trabecular vBMD at radius compared to controls, [245] whereas Caparbo et al. reported decreased trabecular parameters (vBMD, thickness and separation) at tibia in men with AS. [246]

We have no control group for the longitudinal assessments, which is a limitation. There are two longitudinal studies using HRpQCT on general population that we can use for indirect comparison. In relation to these studies, our patients deteriorate in cortical vBMD at tibia at earlier age than men in the general population, whereas decrease in trabecular vBMD did not occur in the men in the general population in any age group. [247, 248]

Regression analyses regarding factors associated with changes in HRpQCT measurements were conducted for changes in cortical and trabecular vBMD and cortical area. Factors associated with changes in microarchitecture were not analyzed based on the large precision errors for these measurements and because of less assessable examinations (n = 45). In regression analyses, factors associated with changes in HRpQCT parameters were mainly identified for cortical vBMD and cortical area. For tibia, an increase in disease activity from baseline to follow-up was negative for bone health and for radius, high time-averaged ESR was negative. One of the cross sectional studies using HRpQCT in this patient group analyzed the association between

disease activity or markers of inflammation and HRpQCT parameters. They found a negative correlation between ESR and cortical and trabecular vBMD and cortical thickness at tibia. [243] Negative impact on changes in aBMD in this patient group by inflammation has been found in several longitudinal studies both at the femoral neck and spine AP. [193, 194, 196]

Exposure to TNFi during follow-up was not significantly associated with changes in HRpQCT parameters. However, four patients had a short exposure time (median 10.5 months) to TNFi. Since there were relatively few patients in the study, patients with longer TNFi treatment (≥ 4 years) were analyzed for associations with changes in HRpQCT parameters. This longer exposure to TNFi was associated with increases in cortical parameters at tibia, in line with the previously mentioned association with TNFi treatment and increases in aBMD at the femoral neck and spine. Relatively few patients were exposed to bisphosphonates during follow-up (13 %) and fewer patients were exposed to a systemic dose of prednisolone ≥ 450 mg during follow-up (7 %). Both medications are important factors for BMD and were therefore included in the analyses. Exposure to bisphosphonates was associated with increases in cortical parameters at radius and prednisolone was associated with decreases in cortical parameters at tibia. Why treatments did not affect both radius and tibia could not be elucidated, but could be related to the small sample size and few exposed patients.

Hardly any factors related to changes in trabecular vBMD were found. At tibia, only younger age was associated with decreases in trabecular vBMD, and decreases were especially found in patients < 40 years old at baseline. Further analyses could not elucidate the reason for this unexpected relationship.

Do HRpQCT parameters have any clinical implications? Fracture prediction by aBMD measured by DXA has limitations, and a large proportion of fractures occur in patients with T-score $> - 2.5$ SD, in men in a larger proportion than in women. [249] Prospective studies on incident fractures in older men using HRpQCT have shown an additive value for fracture prediction by these measurements. After adjustment of aBMD (among other things) incident fractures were significantly associated with decreased cortical parameters and trabecular bone mass at tibia [250], decreased cortical and trabecular vBMD at tibia and radius [251] and decreased trabecular vBMD, number and separation at radius. [252] There are no studies on changes in HRpQCT measurements over time and fracture risk.

5.4 PAPER IV

Elevated serum level of hepatocyte growth factor predicts development of new syndesmophytes in men with ankylosing spondylitis. Results from a five-year prospective study.

In this study we investigated if baseline s-HGF and the average s-HGF were associated with spinal radiographic progression in patients with AS overall and by sex. We also analyzed factors correlated to changes in s-HGF between baseline and follow-up. This is the first longitudinal study assessing s-HGF as a predictor of spinal radiographic progression. There were 163 patients with radiographs and s-HGF at both baseline and the 5-year follow-up who also had a baseline mSASSS < 72.

5.4.1 MAIN RESULTS

- Baseline s-HGF and the average s-HGF were significantly higher in men with spinal radiographic progression compared to men without progression. This difference was not found in women.
- Baseline s-HGF and the average s-HGF did not differ between men and women with AS.
- An optimal cut-off point for baseline s-HGF for the prediction of development of ≥ 1 new syndesmophyte was calculated for men; in the ROC analysis, the area under the curve (AUC) was 0.70.
- Baseline s-HGF, the optimal cut-off point and the average s-HGF were all independent predictors of spinal radiographic progression in men.
- Changes in s-HGF were positively correlated to changes in ESR and CRP.

5.4.2 CONCLUSION

Elevated s-HGF is an independent predictor of spinal radiographic progression in men with AS. The predictive ability is modest. Whether the predictive ability can be improved by adding other biomarkers or clinical parameters is not known.

5.4.3 DISCUSSION

In the baseline, cross-sectional report on this cohort, elevated s-HGF was found to be independently associated with higher mSASSS, so in this study we aimed to assess if s-HGF also was related to spinal radiographic

progression. The analyses showed baseline s-HGF, s-HGF \geq cut-off point and the average s-HGF to independently predict development of ≥ 1 new syndesmophyte in men with AS. Supporting the role of a prognostic biomarker for radiographic progression is the correlation between other variables previously identified as predictors, such as elevated CRP, smoking and high BMI. Besides a study showing an association between s-HGF and elevated CRP and BASDAI in patients with AS, [114] no other findings on s-HGF and AS are reported. The role of HGF in other rheumatic diseases is not clear, as previously mentioned, and studies are limited.

Possible mechanisms for HGF in relation to spinal radiographic progression include promotion of osteogenesis by an effect on bone cells or by an angiogenetic effect, [137, 253] or by HGF having an effect on the disease by the immune system. [123] Or, elevated HGF could be a response to inflammatory cytokines [129] with no direct mechanism on the disease.

The AUC of 0.70 for prediction of ≥ 1 new syndesmophyte in men in this current study was similar to the AUC reported in other studies on biomarkers for the prediction on spinal radiographic progression in AS and nr-axial SpA. The AUC previously reported ranged from 0.65 to 0.74 for elevated levels of biomarkers like VEGF, MMP3, s-Calprotectin and visfatin, [104, 106, 107, 112] and lower levels of leptin. [113] Even though elevated citrullinated and matrix metalloproteinase-degraded fragment of vimentin (VICM) was independently associated with radiographic progression, the AUC was only 0.59. [254] Lower level of HMW-APN showed an AUC of 0.62. [113] The predictive value for studied biomarkers are moderate, and as previously mentioned, not consistent in different cohorts. The most recent study on biomarkers published in this patient group added clinical parameters to a combination of biomarkers and some improvement of the prediction was found; the AUC for VEGF, leptin and HMW-APN together increased from 0.73 to 0.77 with clinical parameters. [109] Whether the prediction of spinal radiographic progression can be improved if HGF is combined with other biomarkers or with clinical parameters remains to be investigated.

S-HGF decreased over five years in the patients with AS. The decrease was associated with decreases in inflammatory parameters. No treatments were correlated with the changes. The effect of different treatments on changes in HGF can't be evaluated properly though, since the interval between the measurements are too long and patients started treatments at various starting points. The natural fluctuation of HGF over time is not known in patients with AS. The handling of the blood samples in this current cohort followed a defined protocol used for all patients and controls. Blood samples were stored

at -80°C and were not thawed and re-frozen repeatedly. The same protocol for analyses from the manufacturer was used and followed carefully at baseline and the 5-year follow-up. The manufacturer reports no changes in the ELISA kit between baseline and follow-up analyses. All s-HGF values were within the standard curve. One difference between serum samples from baseline and the 5-year follow-up was the time in the freezer and the analyte can be degraded over time. However, the baseline serum samples were stored in the freezer for approximately 10 months longer than the follow-up samples before analyses were performed and thus one would expect the baseline samples to be lower than the follow-up values if degradation was an issue. Hence, no specific explanation for the decreases in s-HGF over time can be found.

6 CONCLUDING DISCUSSION AND FUTURE PERSPECTIVES

Skeletal involvement in AS is complex. Both bone formation and bone loss are associated with the disease. Both processes contribute to the burden of the disease with impairment of mobility and function and an increased risk of vertebral fractures with the risk of neurological complications in an ankylosed spine. [16, 17, 214, 255] Knowledge about underlying mechanisms for bone loss and new bone formation is limited and it is of importance to gain further knowledge in this area to find modifiable factors in order to improve the care and the outcome for the patients with AS.

Taking the results from the studies in this thesis together, BMD at the femoral neck as well as the distal tibia decreased and mSASSS increased. Both processes were negatively affected by signs of inflammation. This supports the negative relationship between inflammation and both bone loss and spinal new bone formation, also in patients with longstanding disease and that control of inflammation is an important target.

How bone loss and new bone formation affect each other is less clear. Previous longitudinal studies have assessed changes in AS related spinal alterations in relation to changes in aBMD [197-200] in the spine and one study found an independent association; increases in SASSS was associated with increases in AP aBMD. [201] In Paper I, baseline mSASSS was investigated as a predictor for changes in aBMD; low mSASSS predicted decreases in femoral neck BMD. No independent association was found for spinal BMD and mSASSS or lumbar mSASSS. What would the results be if changes in mSASSS over 5 years were used instead? This was not analyzed in Paper I, however, Δ -mSASSS or radiographic progression by the two definitions are not associated with changes in aBMD in univariate analyses, and no further analyses regarding DXA measurements and mSASSS are planned. With QCT of the spine, trabecular and cortical vBMD can be separated, which is helpful in the spine with syndesmophytes. One small study with 15 AS patients investigated longitudinal analyses of syndesmophytes as predictors for changes in vBMD measured by QCT. They found AP BMD to increase and QCT vBMD to decrease. QCT vBMD was lower in patients with more advanced radiographic findings, but baseline syndesmophyte score by Devogelaer did not independently predict changes in vBMD. [189] Analyses in the study are hampered by a low number of participants and it would be of interest to further study factors associated with

changes in QCT vBMD in a larger cohort and with a validated scoring method for AS related spinal alterations. We have not been able to analyze our longitudinal QCT data yet due to technical issues.

What about low BMD as a predictor for spinal radiographic progression as a proposed mechanism for AS related spinal alterations? Just recently, two longitudinal studies reported low BMD to be a possible predictor for spinal radiographic progression. [140, 237] Data from our cohort regarding this question has not been published. So far, we have found no association between aBMD and spinal radiographic progression. However, analyses of baseline QCT vBMD as a predictor of spinal radiographic progression are not conducted; this can be done and such analyses could increase the knowledge about the relationship between trabecular BMD and spinal new bone formation.

Is mSASSS a method sensitive enough to detect relevant changes in AS related spinal alterations? Of the scoring methods using conventional X-ray, mSASSS is the method most sensitive to change, [62] and the scoring method still recommended in clinical trials. [63, 64] However, assessment of syndesmophyte growth by CT is more sensitive to change than radiography. [256] Recently, a new scoring method that uses low-dose CT was developed, the CT Syndesmophyte Score (CTSS). CTSS has the ability to reliably assess the thoracic spine in addition to cervical and lumbar spine. [257] With CTSS more spinal new bone formation was detected compared to mSASSS. Most progression was detected in the thoracic spine, but CTSS also detected more new syndesmophytes and especially more growth of syndesmophytes in the cervical and lumbar spine compared to mSASSS. [258] In future studies it would be of interest to evaluate factors associated with progression of spinal new bone formation using CTSS to investigate if further modifiable factors can be detected and to better evaluate the effect of treatments on progression.

Use of bisphosphonates was found to have a positive impact on changes in BMD, whereas a negative impact was found for radiographic progression. However, the number of patients using bisphosphonates in this study is too small to draw any definitive conclusions about the negative impact. A larger cohort with more patients using bisphosphonates would be needed to increase the power of the calculations. Collaboration with other research groups that have included AS patients using bisphosphonates in longitudinal studies could be a possible approach. The same applies to further studies on sex differences in predictors for radiographic progression.

In this current cohort, the association between s-HGF and AS related spinal alteration was investigated for the first time. The mechanism and the relative importance for this association are not known. In further studies, s-HGF could be assessed in combination with other biomarkers and also with clinical predictors to analyze if the predictive value can be improved. Also, the effect of treatment on changes in s-HGF and its association with radiographic progression would be of interest to study in a larger cohort.

7 CONCLUSION

The studies in this thesis suggest that the best site to assess bone loss in patients with longstanding AS is at the femoral neck and that inflammation has a negative impact on bone loss and development of AS related spinal alterations and thus is an important treatment target. The studies give further reasons to counsel the patients to stop smoking and to encourage obese patients to weight loss. Treatments with bisphosphonates and TNFi had a positive impact on BMD. Further studies are suggested regarding the role of bisphosphonates in relation to spinal radiographic progression and whether s-HGF can be useful as a predictor for spinal radiographic progression.

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