Alterations of bone mineral density, microarchitecture and strength in patients with ankylosing spondylitis: a cross-sectional study using high-resolution peripheral quantitative computerized tomography

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by

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Graduate Department of The Institute of Medical Science University of Toronto

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Abstract

Patients with ankylosing spondylitis (AS) have low BMD and high fracture risk. BMD, bone microarchitecture and strength determine bone fragility. This study aimed to analyze how AS affects bone microarchitecture and strength. Volumetric BMD (vBMD) and microarchitecture were measured using HRpQCT, and bone strength was estimated using finite element analysis. Multivariable linear regression was used to analyze the effect of AS on HRpQCT and FEA parameters. In multivariable linear regression models, AS patients (n=44) had lower volumetric BMD, cortical thickness, BV/TV and higher cortical porosity and trabecular separation when compared to non-AS subjects (n=85). FEA parameters such as bone stiffness and stress were also abnormal in AS patients. To conclude, it was found that volumetric BMD, trabecular and cortical microarchitecture as well as FEA parameters were worse in AS patients than non-AS subjects. These abnormalities might partly explain the high fracture risk in patients with AS.
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Contributions

Angela M. Cheung was involved in the initial planning of the study, designing the study, analysis of data, interpretation of results and editing of the thesis.

Robert D. Inman was involved in the initial planning of the study, obtaining the funding for the study, designing the study, recruitment of patients, analysis of data, interpretation of results and editing of the thesis.

Janet M. Raboud was involved in the designing the study, analysis of data, interpretation of results and editing of the thesis.

Eva Szabo performed the HRpQCT scans and generated the results.

Lydia Fung and Diana Yau performed the BMD scans and generated the results.
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List of Abbreviations

aBMD: Areal BMD
AS: Ankylosing Spondylitis
ASDAS: Ankylosing Spondylitis Disease Activity Score
BALP: Bone Specific Alkaline Phosphatase
BASDAI: Bath Ankylosing Spondylitis Disease Activity Index
BASFI: Bath Ankylosing Spondylitis Disease Functional Index
BASMI: Bath Ankylosing Spondylitis Metrology Index
BASRI Bath Ankylosing Spondylitis Radiology Index
BMD: Bone mineral density
BTMs: Bone turnover markers
CAROC: Canadian Association of Radiologists and Osteoporosis Canada Risk Assessment tool
CI: Confidence Interval
CRP: C-reactive protein
CTX: C-telopeptide
DXA: Dual-energy X-ray absorptiometry
1,25(OH) 2D3: 1,25-Dihydroxyvitamin D3
ESR: Erythrocyte Sedimentation rate
FEA: Finite Element Analysis
FRAX: Fracture Risk Assessment Tool
HRpQCT: High-resolution peripheral quantitative computerized tomography
IBD: Inflammatory Bowel Disease
MMP: Metalloproteinase
mSASSS: Modified Stoke Ankylosing Spondylitis Spine Score
nr-ax SpA: Nonradiographic axial SpA (nr-axSpA).
NTX-N-Telopeptide levels
NSAIDs: Nonsteroidal anti-inflammatory drugs
OC: Osteocalcin
OP: Osteoporosis
OPG: Osteoprotegrin
OR: Odds ratio
PINP: Procollagen type I N-terminal propeptide
QCT: Quantitative computerized tomography
RANKL: Receptor activator of NF-κB ligand
SAP: Serum Alkaline Phosphatase
SpA: Spondyloarthritis
TGH: Toronto General Hospital
TNF: Tumor Necrosis Factor
TNF-α: tumor necrosis factor alpha
TWH: Toronto Western Hospital
TIMP: tissue inhibitor of metalloproteinase (TIMP)
vBMD: Volumetric BMD
WHO: World Health Organization
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Chapter 1

Introduction and Literature Review
1.1 Overview of Ankylosing Spondylitis (AS)

1.1.1 Definition of AS
Ankylosing spondylitis (AS) is a chronic systemic inflammatory disease. It predominantly affects the axial skeleton and sacroiliac joints. AS is also the prototypic disorder of a spectrum of diseases called spondyloarthritis (SpA). AS is considered as axial SpA due to the presence of inflammation of the spine and sacroiliac joints. Clinically symptomatic patients with AS who lack radiographic sacroiliitis are classified as having nonradiographic axial SpA (nr-axSpA) (Rudwaleit 2009). Other diseases included under SpA are axial SpA, peripheral SpA, undifferentiated SpA, reactive arthritis, psoriatic arthritis and enteropathic arthritis/spondylitis associated with inflammatory bowel diseases (IBD). These subsets of SpA share a common genetic background and clinical features (Amor 1990, Bakland 2013 and Rudwaleit 2009). AS is the most common type of SpA (Bakland 2013).

1.1.2 Prevalence of AS
The incidence of AS ranges from 0.5-10.6 per 100,000 people (Bakland 2013). The overall global prevalence of AS is 0.1% - 10% but it is different in various geographic regions of the world (1, 2 Amor 1990, Bakland 2013). The highest prevalence of AS is reported among the Haida tribe of British Columbia, Canada (Gofton 1984). The disease is also more common in Caucasians. In a recent study linked to the NHANES survey, up to 1% of the USA population were found to be affected with SpA and this was comparable to the prevalence of rheumatoid arthritis (Reveille 2012). A recent systematic review of 36 studies concluded that AS had a mean prevalence (per 10,000 population) of 31.9 in North America, 23.8 in Europe, 16.7 in Asia, 10.2 in Latin America and 7.4 in Africa (Dean 2014). The estimated number of cases in Europe and Asia was 1.30–1.56 million and 4.63–4.98 million, respectively (Dean 2014).

1.2 Clinical manifestations of AS
1.2.1 Clinical features of AS
AS has a chronic disabling disease course and it affects multiple organ-systems. The onset of disease is predominantly in the teenage years and twenties. The onset at a young age exposes patients to a prolonged burden of disease. It is more common in men and has a male-female ratio of 3:1 (Dean 2014). There is a strong genetic component that accounts for more than 90%

1.2.2 Diagnosis of AS

The diagnosis of AS is established when the patient has a combination of relevant symptoms, physical signs, and imaging findings. The most widely used and accepted diagnostic algorithm is the modified New York criteria (van der Linden 1984). Details are presented in Table 1.

Table 1: Modified New York criteria for AS

<table>
<thead>
<tr>
<th>Clinical Criteria</th>
<th>Radiological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low back pain ≥ 3 months, improved by exercise and not relieved by rest</td>
<td>Bilateral grade 2-4 sacroiliitis OR Unilateral 3-4 sacroiliitis</td>
</tr>
<tr>
<td>Limitation of motion of lumbar spine in sagittal and frontal planes</td>
<td></td>
</tr>
<tr>
<td>Limitation of chest expansion</td>
<td></td>
</tr>
</tbody>
</table>

*Requirements: bilateral grade 2-4 or unilateral grade 3-4 sacroiliitis AND any clinical criteria

1.2.3 Disease course and monitoring of disease activity of AS

The natural course of AS is variable (Brophy 2002). Most patients have continuous disease activity with frequent clinical flares (Stone 2008, Kennedy 1993). Disease activity, functional
status of the patient and structural damage are three important parameters that determine the long-term course of the disease. Hence various scoring systems have been developed to monitor the progression and also to study the response to therapeutic agents (Calin 1999, Spoorenberg 2004, Zochling 2011).

BAS-G is a measure of the effect of AS on general well being (Jones 1996). Clinical disease and inflammatory activity are measured by scores such as Ankylosing Spondylitis Disease Activity Score (ASDAS) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (Zochling 2011, Zochling 2008). BASDAI is calculated based on information regarding five symptom domains (Zochling 2011, Zochling 2008). The symptoms analyzed are pain with swelling in joints, fatigue, spinal pain, severity and duration of morning stiffness and discomfort with peripheral entheses. The scores ranged from 0-10. The physical function of patients is monitored by Dougados Functional Index (DFI), Health Assessment Questionnaire for Spondyloarthropathies (HAQ-S) and Bath Ankylosing Spondylitis Functional Index (BASFI) (Stone 2008, Zochling 2011). Bath Ankylosing Spondylitis Mobility Index (BASMI) is an index used to reflect spinal mobility (Zochling 2011). Radiological severity of AS are measured by the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) and BASRI (Wanders 2004, Mackay 2000). mSASSS is a cumulative score obtained by quantifying the changes at the anterior corners of the lumbar and cervical spine vertebrae (Wanders 2004). Each corner is scored from 0 to 3 and the total scores range from 0 to 72 (Table 2). In addition, serum inflammatory markers such as ESR and CRP are used to monitor disease activity of AS.

Table 2: mSASSS scoring system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mSASSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Erosion, squaring, or sclerosis</td>
</tr>
<tr>
<td>2</td>
<td>Syndesmophyte</td>
</tr>
<tr>
<td>3</td>
<td>Bridging syndesmophyte</td>
</tr>
</tbody>
</table>

1.2.4 Management of AS

Treatment of AS involves multiple modalities. Physiotherapy and pharmacological measures are the mainstays. Pharmacological treatment is initiated by prescribing nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs have been shown to reduce pain and decrease
inflammation in AS (Boulos 2005 and Poddubnyy 2012). NSAIDs may also be able to delay radiographic progression of the spine but this needs to be proven in future (Haroon 2012, Poddubnyy 2012 and Kroon 2012). Not all patients achieve complete disease remission with NSAIDs. Other medical agents used in the management of AS include corticosteroids, sulfasalazine and TNF-α inhibitors (TNFi) (Baraliakos 2012, Braun 2003, van der Horst-Bruinsma 2002, Braun 2011). Currently, TNFi are the mainstay of treatment for patients who do not achieve clinical remission with NSAIDs. Further, with the advent of the role of TNFi, the use of corticosteroids and DMARDs in patients with AS has become less common (Chen 2013, Spies 2009). A recent Cochrane review concluded that no strong evidence existed to support the role of methotrexate in the management of AS (Chen 2013).

1.3 Osteoporosis in AS

1.3.1 Bone loss in AS

It is now well established that patients with AS are at high risk of developing osteoporosis (Davey-Ranasinghe 2013). The prevalence of osteoporosis and osteopenia in ankylosing spondylitis, as assessed by cross sectional studies, varies from 2-34% and 26-52% respectively (Table 3). Thus, it is evident that osteoporosis is an extra articular manifestation that has a prevalence similar to or even higher than that of other well-known extra articular manifestations of AS such as iritis, IBD and psoriasis (El Maghraoui 2011). Despite being common, bone loss is seldom suspected and investigated, because of young age and predominantly male gender of the affected cohort.

Table 3: Prevalence of bone loss in patients with AS

<table>
<thead>
<tr>
<th>Author, Year, Country</th>
<th>N</th>
<th>Duration (years)</th>
<th>Age (years)</th>
<th>Bone loss at hip</th>
<th>Bone loss at lumbar spine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maghraoui, 1999, France</td>
<td>80</td>
<td>-</td>
<td>37 ± 12</td>
<td>Femoral neck: 11 (14%) - 33 (41%)</td>
<td>OP 19% Osteopenia 31%</td>
</tr>
<tr>
<td>Singh 2013, India</td>
<td>100</td>
<td>No data</td>
<td>35 ± 8</td>
<td>OP Trochanter 4% cases &amp; 0.7% in controls</td>
<td>OP 22% of cases &amp; 2.7% in controls</td>
</tr>
<tr>
<td>van der Weijden 2011, Netherlands</td>
<td>94</td>
<td>8 ± 6</td>
<td>37 ± 9</td>
<td>31% (osteopenia)/3% (op)</td>
<td>37% osteopenia 9% OP</td>
</tr>
<tr>
<td>Muntean, 2011, Romania</td>
<td>44</td>
<td>13 ± 9</td>
<td>41 ± 10</td>
<td>Osteopenia or OP: 48% at the</td>
<td>Osteopenia or OP: 60%</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Age (median)</td>
<td>OP or Osteopenia</td>
<td>Femoral neck</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Borman 2001, Turkey</td>
<td>Turkey</td>
<td>32 ± 15</td>
<td>39±11</td>
<td>OP = 34% (vs. 6% in controls)</td>
<td></td>
</tr>
<tr>
<td>Grazio 2012, Croatia</td>
<td>Croatia</td>
<td>80 ± 10</td>
<td>52±10</td>
<td>Osteopenia, OP at Hip: 26%, Neck: 48%, Osteopenia, OP: 20, 25%</td>
<td></td>
</tr>
<tr>
<td>Kim HR, 2006, Korea</td>
<td>Korea</td>
<td>60 ± 11</td>
<td>32 ±1</td>
<td>OP &amp; osteopenia Femoral neck: 33 &amp;41%</td>
<td></td>
</tr>
<tr>
<td>Klingberg, 2012, Sweden</td>
<td>Sweden</td>
<td>87 ± 13</td>
<td>50 ±13</td>
<td>Age&gt; 50: 21% OP &amp; 44% osteopenia, Age&lt; 50, low BMD: 5%</td>
<td></td>
</tr>
<tr>
<td>Arends, 2011, Netherlands</td>
<td>Netherlands</td>
<td>128 (1-53)</td>
<td>41±11</td>
<td>OP &amp; osteopenia Hip: 2 &amp; 39%</td>
<td></td>
</tr>
<tr>
<td>Visvanathan, 2009, USA</td>
<td>USA</td>
<td>279 ± 11</td>
<td>40 ±11</td>
<td>Hip OP: 7/279 Osteopenia: 90/279</td>
<td></td>
</tr>
<tr>
<td>Durnez 2013</td>
<td></td>
<td>59 ± 11</td>
<td>40 ±11</td>
<td>OP: 15 (31%)</td>
<td></td>
</tr>
<tr>
<td>Baek, 2005 Korea</td>
<td>Korea</td>
<td>76 ± 5</td>
<td>28 ±8</td>
<td>Hip OP: 1% Hip osteopenia: 14%</td>
<td></td>
</tr>
<tr>
<td>Vasdev, 2011, India</td>
<td>India</td>
<td>80 ± 6</td>
<td>33 ±11</td>
<td>OP at femur neck = 11% (controls: 1%, p &lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>Kang, 2011, Korea</td>
<td>Korea</td>
<td>90 ± 5</td>
<td>31 ±11</td>
<td>33 patients had OP L-spine or femoral neck.</td>
<td></td>
</tr>
<tr>
<td>Dischereit, 2012, Germany</td>
<td>Germany</td>
<td>16 ± 48</td>
<td>48 (27–74)</td>
<td>8 osteopenia, 1 osteoporosis</td>
<td></td>
</tr>
<tr>
<td>Briot, 2008, France</td>
<td>France</td>
<td>106 ±16</td>
<td>38 (11)</td>
<td>28% OP 23% osteopenia at spine/femur</td>
<td></td>
</tr>
</tbody>
</table>

Data explaining the prevalence of bone loss in AS should be interpreted with caution as bone loss in AS is influenced by many factors. First, many studies have overestimated the prevalence rates as a consequence of applying WHO definition to define osteoporosis and
osteopenia. However, it is now clear that the WHO definition cannot be used to define bone loss in young males and pre-menopausal women. This is because using the WHO criteria in young subjects overestimates the prevalence of bone loss. Thus, the ISCD recommends using Z scores to define bone loss in men aged less than 50 and premenopausal women. A cross sectional study done by Vasdev and colleagues addressed this issue (Vasdev 2011). The authors tried to analyze the prevalence of bone loss in AS patients who were younger and had shorter duration of disease than the prior studies. The patients were aged 33±8 years and had had AS for 8±6 years. In this case control study conducted in India, osteoporosis at the spine and femur neck was present in 29% (controls: 2%, p < 0.001) and 11% (controls: 1%, p < 0.001), respectively. The observed prevalence was lower when bone loss was defined using Z scores: low bone density in spine and femur was seen in 24% (controls: 1%, p < 0.0001) and 5% (controls: 0%, p < 0.01), respectively. Second, the prevalence rates of bone loss depend based on the site of measurement of BMD, as the extent of bone loss at the lumbar spine and hip are different. Osteoporosis and osteopenia at the spine have been reported in 13-34% and 20-44% of patients respectively. Similarly at the hip, 2-33% and 26-52% of AS patients have been found to have osteoporosis and osteopenia at total hip or femoral neck. Third, the prevalence rates of bone loss also differ based on the technique used for DXA imaging. For instance, lateral spine BMD is likely to be more accurate than anteroposterior spine BMD in AS (Borman 2008, Klingberg 2012, Ulu 2013, Bronson 1998). In a study done by Borman and colleagues, DXA of lateral spine was used to study bone loss (Borman 2008). In this cross sectional case-control study, there were 32 AS patients with mean disease duration of 15 years and osteoporosis was observed in 11 (34%) AS patients (vs. 6% in controls). The prevalence of bone loss in AS is also influenced by gender. Osteoporosis in AS affects both men and women but bone loss is less common in women with AS compared to men. Men are thought to have more severe disease than women. The gender differences in bone loss may be due to the protective hormonal effects in premenopausal women or may be due to women having less severe disease (van der Weijden 2011). In women, menopause is an important accelerating factor for bone loss. Thus, Muntean and colleagues decided to analyze the prevalence of bone loss in men alone so that confounding effects of gender was eliminated (Muntean 2011). Men with AS (n=44) who were aged less than 60 were eligible to participate in this cross sectional study. The subjects had a mean age of 41±10 years and disease duration of 13±9 years. BMD at femoral neck and total hip (.90 vs. 1.03 in controls and .94 vs. 1.05 in controls) were lower
in men with AS compared to controls (both $p = 0.001$). Lumbar spine BMD was also lower in patients compared to controls, but the difference did not reach significance (1.16 vs. 1.06, $p = 0.06$). This study observed a greater prevalence of bone loss than was previously reported. Osteopenia or osteoporosis was found in 60% of AS patients at the lumbar spine and in 48% at the femoral neck. Finally, the nature of bony involvement of the spine in AS may also influence the prevalence of bone loss. Lumbar spine DXA is likely to document normal values in patients with bony changes at spine and consequently underestimate bone loss (Arends 2011). Karberg and colleagues demonstrated this by categorizing patients based on their disease duration. Osteoporosis at the hip and spine were found by DEXA in 11% and 15%, respectively, in 27 patients with early AS (duration < 5 years). Lumbar spine DXA was abnormal in only 4% of patients who had prolonged disease (> 10 years) as opposed to 11% in those who had shorter disease duration. More patients were found to have osteoporosis at the hip (29%) with long standing disease. Further, in this study, spinal DEQCT measurements suggested that 18% of those with long standing disease had osteoporosis and this confirms that lumbar spine DXA measurements are not reliable to document bone loss in patients with long standing disease. Thus, it is evident that the differences in prevalence rates of bone loss noted in AS are influenced by the confounding effects of age, gender, severity, type of lumbar spine projection (anteroposterior vs. lateral) studied, site of DXA (lumbar spine vs. hip) and definitions used to define bone loss (WHO vs. ISCD). Further, data on duration of disease can be misinterpreted since AS can remain undiagnosed for up to ten years after the onset of back pain.

1.3.2 Data on longitudinal bone loss in AS
Not much is known about whether bone loss in AS occurs in a progressive manner. Most of the existing evidence on bone loss in AS is based on data obtained from cross sectional studies. This limits our ability to make conclusions regarding a causal association. Thus far, only three studies have assessed longitudinal changes of BMD in AS patients who were treated with conservative treatment (Kang 2011, Maillefert 2001, Kaya 2009). The maximum duration of follow up in these studies was two years. Longitudinal data suggest that after two years of follow up, BMD at lumbar spine either remained stable or increased. Conversely, BMD at the hip either remained stable or declined over a period of two years. In a retrospective longitudinal study conducted in Korea, the lumbar spine BMD increased from 1.17+ 0.15 to
1.20+0.16, p=0.01 (baseline vs. year 1), but femoral neck BMD did not change significantly (0.98+ 0.15 vs. 1.00+ 0.15, p=0.34 (Kang 2011). The subset of patients analyzed had been treated with conventional measures and NSAIDs. However, the sample size was very small. It is also likely that patients, who were treated with NSAIDs alone, had milder disease and bone loss at hip might take longer than one year to manifest. In another longitudinal study conducted in Turkey (n=55, mean age =36 ±11 years, duration= 11± 8 years) the lumbar spine BMD was noted to increase by 3.4% over 24 months. In contrast, BMD at femoral neck and total hip decreased by 0.9% and 0.25% after 24 months. In the sub group analysis, percentage changes in BMD measurements were not significantly different between patients with active (n = 10) or inactive (n = 11) disease. In another study, Maillefert and colleagues examined the longitudinal changes in BMD over two years (Maillefert 2001). Fifty-four patients aged 37 ± 11 years (disease duration: 12.4 ± 8.6 years) were followed up for two years. After 2 years, BMD at the lumbar spine did not change (+0.75% ±3.5, p = 0.23). But significant bone loss was demonstrated at the femoral neck (−1.6%±4, p = 0.006). QCT was used by Korkosz and colleagues to demonstrate longitudinal bone loss in AS (Korkosz 2011). In this small study of 15 patients, the authors found that trabecular bone mineral content at the spine measured by QCT, decreased significantly after ten years (change ± SD: 18 ± 7 mg/cm³, p=0.001). DXA at the spine revealed a significant increase of BMD (change ± SD: -0.15 ± 0.14 g/cm²) in contrast to the results of QCT. So far, no other study has attempted to replicate these results.

1.3.3 Risk factors of bone loss in patients with AS

Bone loss in AS is multifactorial in origin. The causes of bone loss in AS have long been speculated, if not always understood. The major pathogenetic factors of bone loss are systemic inflammation and decreased mobility of the spine.

Potential causes of osteoporosis in AS

1. Systemic inflammation
2. Poor spinal mobility
3. Low peak bone mass
4. Low physical activity
5. Life style factors such as smoking and alcoholism
6. Low BMI
7. Hypogonadism
8. Abnormal vitamin D metabolism
9. Malabsorption
10. Genetic factors
11. Abnormal bone turnover
12. Drugs: corticosteroids
13. Co morbid illnesses such as inflammatory bowel disease and psoriasis
14. Decreased skeletal loading

Systemic inflammation is likely the major contributing factor of bone loss in AS. This is because patients with AS have low BMD at both lumbar spine and the hip and spinal immobility cannot explain bone loss at the hip. Moreover, even patients with mild disease who are ambulatory and have normal spinal mobility develop low bone density (Bhalla 1992). The fact that even patients with early AS experience bone loss further suggests that systemic inflammation is the most likely explanation for bone loss in AS. Will and colleagues were the first to report that even patients with normal or mild involvement of the spine, normal mobility and early AS had low bone density (Will 1989). This notion has been confirmed by several recent studies (van der Weijden 2011, Maillefert 2001). A systematic review conducted by van der Weijden and colleagues reported that even patients within ten years of onset of AS developed bone loss (van der Weijden 2011). This review included seven studies with a total of 482 patients with AS. The subjects were relatively young with a mean age of 35 years and had disease duration of 8 years. The prevalence of osteopenia vs. osteoporosis for lumbar spine was 39 vs. 16 % and for femoral neck, 38 vs. 13 % and this was similar to the prevalence rates in those with long-standing AS patients. Recently, a prospective study showed that bone loss was clearly due to inflammatory activity in early AS and not due to poor vertebral mobility and impaired daily physical activity (Gratacoa 1999). Further, the results of Mitra and colleagues suggest that bone loss is due to inflammation, because the cohort did not have advanced spinal disease, syndesmophytes limiting spinal mobility (Mitra 2000). Maillifert et al observed that patients with persistent systemic inflammation experienced significant bone loss over two years suggesting that inflammation is an important etiology of bone loss (Mallifert 2001). In this longitudinal study, the bone loss at femoral neck was related to persistent systemic inflammation, defined using ESR (mean percentage change $-4.1\% \pm 5.7$ and $-1.2\% \pm 3.9$ in
patients with and without persistent inflammation; respectively; \( p = 0.007 \)). Data on the adverse effect of inflammation on BMD in patients with AS is fairly consistent (Grazio 2012, Arends 2011, Gratacoal 1999, Ghozlani 2009, Lange 2005, Toussirot 1999). This argument is further supported by the various recent longitudinal studies that have noted that treatment with TNFi likely improve BMD at lumbar spine for up to six years by reducing inflammation (Durnez 2013, Kang 2011, Saad 2011, Kang 2013). TNFi inhibitors have also been shown to stabilize or improve BMD at the hip. The longitudinal gain in BMD noted at the lumbar spine was found to persist even in those without radiological changes in the spine proposing that the change in BMD reflected a true gain (Kang 2013). In a prospective longitudinal study conducted by Kang et al. in 26 patients, treatment with TNFi resulted in consistent gain in BMD at the lumbar spine and total proximal femur over two years compared to 37 patients not receiving TNFi (\( p < 0.01 \) and \( p = 0.02 \)) (Kang 2013). Though mSASSS score, an indicator of radiological severity and progression had increased in those receiving TNFi, development of syndesmophytes was not different between the two groups. The authors also observed that treatment with TNFi inhibitors and the increase in SASSS were independently associated with increase in BMD at the lumbar spine BMD (\( p = 0.009 \) and \( p < 0.001 \)). This further strengthens the observation that control of systemic inflammation likely improves BMD at lumbar spine and hip.

Studies have reported that bone loss in AS is linked partly to poor spinal mobility. Grazio and colleagues observed a significant negative correlation of BMD with serum markers of inflammation such as CRP (13+ 18 mg/L) and ESR (22+ 21 mm/h) (Grazio 2012). The negative association was more pronounced at hip than at the lumbar spine. In this study, BASFI (\( p=0.014 \)), BASDAI (\( p = 0.011 \)), and spinal pain (\( p = 0.002 \)) were higher in subjects who had osteoporosis when compared to those who had osteopenia or normal BMD. The study cohort however was slightly older (age= 52± 10 years) and had prolonged disease duration (disease duration= 22± 10 years). Age is an important confounder of bone loss and might have influenced their results. Nevertheless, the results of Grazio et al. indicate that both inflammation and poor spinal mobility contribute to bone loss in AS (Grazio 2012). In a cross sectional study done by Klingberg et al. in 2012, including 204 patients (mean age =50 ± 13 years), high BASMI was identified as a significant predictor of low BMD (Klingberg 2012).
Thus, it is evident that factors such as poor mobility as indicated by high BASMI contribute to bone loss in AS.

Physical factors such as low body weight and lack of physical exercise may contribute to low BMD. El Maghraoui and Klingberg et al. have also observed the negative effect of low BMI on bone health in AS (El Maghraoui 1999, Klingberg 2012). Lack of exercise or regular physical activity is a frequent problem in patients with AS (Passalent 2010). A questionnaire-based study on 61 patients with AS, documented that walking and stretching were the most commonly followed types of exercise. However, these were reported only in 35.0% and 33% of patients respectively and most patients did not report doing exercise on a regular basis. Presence of persistent pain, fatigue, stiffness of the back and spinal immobility limits physical activity. Progressive rigidity of the spine may cause changes in gait and stooped position and consequently impairing skeletal loading and BMD.

Systemic bone loss is also mediated by abnormalities in endocrine pathways. Hypogonadism is an important risk factor for bone loss. Gonadal status may mediate bone loss in AS. Mitra et al. first studied the effect of hypogonadism on BMD in 1999 (Mitra 2000). In this study of 56 male patients with mild AS and 52 controls without AS, serum total testosterone and SHBG were significantly lower in patients (16.0 ± 5.0 vs. 21.0 ± 6.2 nmol/l vs. controls and 27.3 ± 10.6 vs. 35.0 ± 13.0 nmol/l; p < 0.001 vs. controls respectively). However, the free testosterone index, FSH and LH levels were not different. None of these serum parameters were associated with BMD and the selective inclusion of patients with milder disease limits the ability to generalize the findings. In a large cross sectional study done by Franck et al. (n=264), men with AS and osteoporosis were found to have lower free testosterone (0.15 ± 0.05 vs. 0.18 ± 0.08 µg/nmol, p < 0.05) in serum (Franck 2000). Similarly women with osteoporosis had significantly lower serum levels of estradiol (23.5 ± 34.4 vs. 80.9 ± 72.2 ng/l, p < 0.01) than those without osteoporosis. These results were replicated in a study done by Aydin and colleagues as well (Aydin 2005). In this cross sectional study of 58 young men (mean age: 38.2 years) with AS, serum DHEAS levels were low in 31% of patients with low BMD. The ratio of serum testosterone/DHEAS was higher in those with bone loss than those without (5.24 ± 3.70 vs. 3.58 ± 3.16, p = 0.026). But whether gonadal status is affected adversely in AS is still a matter of debate (73-74). Elevated DHEAS may be a response to systemic
inflammation. In a small cross sectional study conducted in 2004, male patients (n=29) with AS were found to have higher levels of serum testosterone, LH, and prolactin than controls (El Maghraoui 2005). Thus, the potential role of androgens in initiating bone loss in AS needs to be explored further.

Another crucial hormone that maintains skeletal health is vitamin D. Low vitamin D contributes to osteoporosis in AS in two different ways. First, vitamin D may have an aetiological pathogenetic role in AS and low vitamin D may worsen inflammation. This is mainly because, 1,25(OH)₂D₃, the active form of vitamin D, suppresses the proliferation of activated T cells and acts as an endogenous immune modulator (Amento 1987). Consequently, low levels of 1,25(OH)₂D₃ may perpetuate the inflammation process in AS. Systemic inflammation affects bone turnover in a negative manner and contributes to bone loss. Second, low vitamin D levels cause bone loss by causing mineralization defects. Moreover, abnormalities of vitamin D can accelerate bone loss in AS (Arends 2011). There are several possible mechanisms by which patients with AS can have low vitamin D levels in serum. Firstly, patients with AS are known to have subclinical inflammation of the gut (Ebert 2004). Gut inflammation might result in poor absorption of nutrients including vitamin D. Secondly; serum levels of TNF-α remain high in AS patients that have active disease. TNF-α is known to down-regulate the activity of 1,25-hydroxylase enzyme in the kidney thus impairing the renal production of 1,25 vitamin D (Ebert 2004). It is also known that high disease activity in AS is associated with altered vitamin D metabolism and increased bone resorption (Lange 2001). Patients with active disease were found to have low vitamin D levels in a study done by Lange and colleagues in 2001 (78). An inverse correlation was noted to exist between serum levels of 1,25(OH)₂D₃ (p<0.01) with disease activity. In addition, 1,25(OH)₂D₃ levels in serum correlated negatively with the excretion of urinary pyridinium crosslinks and positively with bone specific alkaline phosphatase (p<0.01). They also showed that AS patients with osteoporosis had significantly lower vitamin D levels compared to AS patients with normal BMD. In a previous study, patients with AS had lower mean 25-(OH) D₃ levels (21.70 ± 12.17 vs. 32.70 ± 8.77 mmol/l) than healthy controls (Mermerci 2010). Though no difference was found between patients with or without osteoporosis groups in terms of serum 25-(OH)D₃ levels, serum vitamin D levels were found to have an inverse association with serum CTX suggesting an influence on bone turnover (Mermerci 2010). Furthermore, data from a recent cross sectional study suggested that
patients with active AS (n=100) had lower levels of 25-OH-vitamin D in serum (21.7 ± 12.2 mmol/l vs. 32.7 ± 8.8 mmol/l in controls, p= 0.0001) when compared to controls (Obermayer-Pietsch 2003). In a cross sectional study done in Dutch AS patients, median 25-OH-vitamin D level was 61.4 (13.8–186) nmol and serum 25OHvitD levels were independently related to sCTX suggesting a role in mediating bone loss (Arends 2011). Therefore it is clear that high disease activity in AS is associated with an alteration in vitamin D metabolism and increased bone turnover. Finally, some authors have also reported that AS is associated with a vitamin D receptor defect in the gut. In a study by Obermayer and colleagues in male AS patients, FokI genotypes of vitamin D receptor gene (VDR) was as independent predictor of low BMD at the spine (Obermayer-Pietsch 2003). Moreover CRP, ESR values were also significantly associated with FokI genotypes (total n=104, mean age 41±12 years). Thus, the VDR gene may be a risk factor for low BMD, abnormal bone metabolism and inflammation in AS.

It is also important to recognize that bone loss occurring from systemic inflammation is likely to be worsened by multiple confounding factors. These include poor peak bone mess, genetic factors, smoking, alcoholism, IBD and malabsorption occurring secondary to clinical or subclinical inflammation of gut.

1.3.4 Bone turnover in AS

Systemic inflammation exerts negative effects on bone turnover. Specifically, inflammation is associated with increased bone resorption and there are some reports suggesting that inflammation is also linked to reduced bone formation. Inflammation causes release of TNF alpha, IL-1 and IL-6, which then stimulate osteoclasts to cause bone resorption (Park 2008). Interestingly, AS is an inflammatory disease where both excess in bone resorption and new bone formation coexist (Arends 2011, Mitra 2000, Franck 2004, Lange 2001, Acebes 1999). A cross sectional study by Arends and colleagues found that serum P1NP, CTX and OC Z-scores were related to low BMD suggesting that AS is related to high bone turnover i.e. increased bone resorption and increased bone formation (Arends 2011). In this study of 128 AS patients, most patients had active disease, BASDAI score ≥4 (89%), high ESR (74%) and CRP (77%) levels. Higher P1NP, osteocalcin and sCTX Z-scores were significantly associated with low BMD at lumbar spine and hip in the regression analysis. In an earlier study done by Maghroui
and colleagues, bone resorption markers such as urinary D-pyridinoline and C-telopeptide concentrations were increased in more than half of patients (53.9%). Also, the bone turnover markers positively correlated with CRP concentration suggesting that osteoporosis in AS occurred in parallel with inflammation (El Maghraoui 1999). Similar results were observed in another study in which serum ESR and NTX levels were significantly higher in patients with active AS (Borman 2008). Moreover, patients with active AS had significantly lower BMD and higher NTX (p < 0.05) levels (Borman 2008). In 2005, Lange and colleagues reported negative correlations between disease activity and serum levels of 1,25(OH)2D3 (p<0.01) and PTH (p<0.01) in a study conducted in 70 AS patients. Disease activity was expressed as high ESR, CRP and BASDAI (Lange 2001). In addition, they reported that the excretion of urinary pyridinium crosslinks showed a positive correlation with disease activity (p<0.01), and these results indicate that high disease activity in AS is associated with increased bone resorption (Lange 2001). Some studies have found a higher level of bone resorption markers in AS versus controls (83-86). For instance, Marhoffer et al. reported that osteocalcin concentrations were normal and similar to that of controls, but urinary pyridinium crosslinks were significantly increased in AS patients than with controls (51.2± 25.2 vs. 33.9±12.4 in controls (p < 0.001)). Serum ESR and CRP correlated only to bone resorption markers. These results were further confirmed by a study conducted by Briot and colleagues in 2005 (Briot 2005). Serum CTX levels increased and remained parallel to the increase in lumbar spine and hip BMD in patients receiving treatment with TNFi (Briot 2005). PINP levels in serum remained stable until 1 year despite an increasing until three months of treatment with TNFi (Briot 2005).

BALP, OC and PINP are the main markers of bone formation. Most studies analyzing the serum levels of markers of bone formation in AS could not detect any differences in patients and controls (Park 2008, Acebes 1999, Marhoffer 1995, Yilmaz 2000, Vosse 2008, Toussirot 1999, Allali 2003, Woo 2007). However, in a study done by Mitra and colleagues, mean serum OC levels were lower in patients with AS than controls (9.0 vs. 11.0 ng/ml, p < 0.001) (Mitra 1999) Conversely, patients with AS had higher BALP levels in serum (mean: 38.5 vs. 30.3 U/l, p < 0.001) (Mitra 1999).

Furthermore, several studies have found a correlation between bone markers such as osteocalcin, uDPD and CTX with ESR and CRP suggesting a potential role for systemic

1.3.5 Bone markers in relation to treatment with TNF alpha Inhibitors

Emerging data suggest that TNFi increase bone formation and decrease bone resorption in patients with AS. However, the data is inconsistent. Serum osteocalcin levels were found to increase at 6 weeks and then remain stable for up to 6 months after treatment with TNFi (Woo 2007). Likewise PINP levels in serum were shown to have an increasing trend at 3 months after infliximab treatment (Briot 2008). Similarly, Allali and colleagues studied 29 patients who were treated with infliximab and reported that there was an increase in serum osteocalcin levels (median: 23.8 mg/l) at 6 weeks (median change: 1.45 mg/l). However, the bone resorption marker urinary pyridinium did not change even after 6 months of treatment.

Recently, Arends et al. published a prospective cohort study of 111 AS patients who were treated with TNFi for three years and their results suggested a rise in bone-specific alkaline phosphatase after 3 months (p<0.001) and the levels remained at a higher level up to three years. Similarly the serum levels of the bone formation marker PINP was found to be increased at three months and remained stable for up to 24 months (Arends 2012). Serum CTX levels decreased and remained decreased during three years of treatment with TNFi. In another study, 26 Korean patients receiving etanercept had a significant rise in serum levels of BALP and osteocalcin after 12 weeks of treatment (p < 0.05). But serum levels of CTX did not change after treatment with etanercept (Woo 2007). Although a difference was noted in the response of bone turnover markers between infliximab and etanercept, it is still unclear if the different types of TNFi affect bone metabolism differently.

1.3.6 RANKL and OPG system in AS

The role of the signaling pathway OPG/RANK/RANKL in mediating bone loss is now supported by many studies. Bone remodeling is modulated by two groups of cells namely osteoblasts and osteoclasts (Redlich 2012, Findlay 2011). The receptor activator of nuclear factor-κB (RANK) is a cell surface receptor present on the osteoclasts. The receptor activator of nuclear factor-κB ligand (RANKL) is a crucial regulator of the formation and function of
osteoclasts and osteoprotegrin (OPG) is a negative inhibitor of osteoclastogenesis. OPG is a decoy receptor that can inhibit the interaction between RANKL and its receptor RANK. The metabolic actions of RANKL and MMP are regulated by OPG a decoy receptor and TIMP respectively. There seems to be an important role for the RANKL-OPG system in AS. The tight balance between bone formation and bone resorption is impaired by inflammation. The inflammatory cytokines such as TNF-α and IL-6 up regulate soluble RANKL, a mediator of osteoclastic bone resorption (Im 2009, Aydin 2005). In addition, inflammation leads to the release of bone and cartilage degrading enzymes such as cathepsin K and MMPs (Redlich 2012, Findlay 2011).

It is very important to better delineate how AS affects the RANK-L system. If the RANK-L system is proven to be more selectively affected in AS, targeted drugs such as denosumab can successfully be used to manage bone loss in patients with AS. Unfortunately, not much is known about how AS affects the OPG-the RANKL levels. Five studies have found that RANKL levels in serum are higher in patients with AS compared to controls (Kim 2006, Aydin 2005, Chen 2010, Taylan 2012, Rauner 2008). Existing data on this regard has shown heterogeneous results. In human studies, RANKL and RANKL/OPG ratio have been shown to be higher in AS patients with low BMD and this suggests a possible causative role of increased bone resorption in bone loss (Kim 2006, Taylan 2012). In a cross sectional study of 55 AS patients (38 had active AS) and 33 healthy controls, the RANKL/OPG ratio was higher in AS patients (.99 (0.14-22.6) vs. 0.65 (0.14-25.1), p=0.04, vs. controls). Another study found that HLA B27 transgenic rat models of SpA had higher mRNA levels of RANKL and RANKL/OPG ratio in bone tissue (Rauner 2009). However, other researchers have not replicated these results. A study conducted using surgical specimens of spinal tissue obtained from patients with AS failed to demonstrate increased sRANKL expression (Neidhart 2009). Similarly, the results of a recent cross sectional study done by Dhir and colleagues from India in 85 patients (men (mean age: 33 ± 10 years and disease duration: 11 ± 7 years) showed that sRANKL (349.2 ± 872.0, 554.7 ± 1850.1, p = ns) did not differ between cases and controls and implied that sRANKL is not overexpressed in AS (Dhir 2013). Similarly, in a small study done by Woo J and colleagues on 26 AS patients receiving treatment with etanercept, sRANKL and OPG did not change after 12 weeks (Woo 2007).
1.3.7 Osteoprotegerin levels in AS
Researchers have reported contrasting results about how OPG is affected in AS. OPG is a soluble decoy receptor for RANKL and neutralizes the ability of RANKL to bind with RANK. OPG is also a key regulator of bone remodeling and the immune system. It can initiate osteoclastogenesis and also mediate communication between T cells with dendritic cells. Some studies have suggested that serum OPG levels are high in AS patients than in controls (Dhir 2013, Grisar 2002, Golmia 2002, Vandooren 2008). Also, a high OPG expression was demonstrated in synovial lining cells and endothelial cells in a study based on immunohistochemistry of patients with spondyloarthritis (Haynes 2003). The increased production of OPG in AS could be a mechanism meant to compensate for bone loss and overproduction of RANKL. These results were not confirmed as some studies have found no difference or even lower levels of OPG in AS patients when compared to controls (Kim 2006, Taylan 2012). For instance, cross sectional data from a Korean study (n=60, 85% men and 15% premenopausal women) showed that serum levels of OPG in AS patients were not different (928 vs. 1158 pg/ml, p > 0.05) from controls (Kim 2006). Moreover, in 2004 Franck et al. observed that serum OPG levels were significantly lower in German patients with AS than controls. In this study, men (1.9 vs. 3.5 pmol/l), premenopausal women (1.85 vs. 4.4 pmol/l) and postmenopausal women (1.95 vs. 4.4 pmol/l, p < 0.001) had lower OPG levels in serum when compared to controls (71). Similarly, in a cross sectional study of 55 AS patients and 33 healthy controls, the OPG levels in serum were lower in patients with AS when compared (339 (52-1118) vs. 527 (16-1030) pg/ml, p=0.02) to controls (Rauner 2009). However, patients in these studies, except in the study by Taylan et al. were early on in their disease with a disease duration around five years (Taylan 2012). In addition, Taylan and colleagues noted that patients with active disease had higher concentrations of OPG and that OPG concentrations were significantly lower in the patients receiving TNFi compared to those receiving conventional drugs (Taylan 2012).

1.3.8 WNT pathway in AS
The Wnt-β-catenin pathway plays a crucial role in regulating bone turnover and metabolism. As shown in Figure 1, bone formation is regulated by BMPs, Wnt signaling and the Wnt inhibitors such as DKK-1 and sclerostin. The BMP family modulates the initial part of the bone remodeling process whereas WNT proteins get involved only in the later stages. TNF
alpha induced inflammation has the ability to modulate Wnt-β-catenin pathway.

Figure 1: Wnt signaling pathway

Data now suggests a possible role of the Wnt-β-catenin pathway in the pathogenesis and radiological progression of AS (Korkosz 2013). It can hence be argued that bone loss in AS is partly mediated by abnormalities of Wnt pathway. The Wnt-β-catenin pathway may be playing a dual role by mediating abnormal new bone formation and systemic osteoporosis in AS. Whether Wnt signaling is directly affected by AS or is it a consequence of systemic inflammation needs to be studied further.

1.3.9 Role of DKK-1 in AS
Sclerostin and functional Dkk1 are now being increasingly studied for their role in radiological progression in patients with AS. Serum DKK-1 levels have been shown to be high, low or unaffected in patients with AS (Taylan 2012, Korkosz 2013, Kwon 2012, Daoussis 2010). In a
study done by Daoussis and colleagues, serum Dkk-1 levels were significantly increased in patients with AS (2,730 ± 135.1 pg/ml) as compared with normal controls (p = 0.040), patients with rheumatoid arthritis (p = 0.020), and patients with psoriatic arthritis (P = 0.049) (Daoussis 2010). Also, patients with AS receiving anti-TNF alpha treatment had significantly higher serum Dkk-1 levels than patients with AS who were not receiving such treatment (p = 0.007). Interestingly, Heiland and colleagues demonstrated that serum Dkk1 levels were significantly (p=0.025) higher in patients without syndesmophytes (6.78±5.48 pg/ml) compared with those with syndesmophytes (4.13±2.10 pg/ml) (Heiland 2012).

In the study by Taylan and colleagues, serum levels of various mediators of the Wnt pathway including DKK-1, sclerostin, and sFRP1, were not different in AS patients from the healthy controls (Taylan 2012). However, some patients studied (23/55) were already receiving treatment with TNFi and this might have influenced their results. Similar to the results of Daoussis and colleagues, in the study by Taylan and colleagues, patients receiving treatment with TNFi had higher DKK-1 levels compared to those taking conventional drugs (Daoussis 2010, Taylan 2012). Conversely, two later studies showed that serum DKK-1 levels either decreased or remained the same after using TNFi for 3-6 months (Korkosz 2013, Kwon 2012). Two studies have also reported the presence of lower DKK-1 levels in AS patients than controls (Korkosz 2013, Kwon 2012). Serum DKK-1 level was lower in patients with AS (n=49) than in healthy controls (n=39) (12,321 + 6,136 vs. 20811 + 5,671 pg/ml, p<0.0001) (Kwon 2012). Furthermore, serum DKK-1 levels were increased in AS patients with low disease activity (Korkosz 2013). This may be the result of the compensatory response to the Wnt signaling being attenuated with low activity of inflammation in those with inactive disease (Korkosz 2013). Lower DKK-1 levels might promote bone formation and hence DKK-1 is currently being explored for its potential role in mediating new bone formation in AS. So far, studies have failed to demonstrate correlations between Dkk-1 and CRP (Taylan 2012, Korkosz 2013) in AS. Two studies reported no differences in serum DKK-1 levels in patients with active disease (BASDAI> 4) when compared to those with BASDAI <4 (Taylan 2012, Korkosz 2013).
1.3.10 Sclerostin abnormalities in AS

Osteocytes constitute the most abundant type of cells in bone. Sclerostin is an important product of osteocytes. It is a glycoprotein inhibitor of BMP and bone formation. In inflammatory arthritis, high TNF alpha has been found to induce skeletal expression of Dkk-1 and sclerostin production, thereby inhibiting bone formation (Saad 2012, Daoussis 2010, Heiland 2012, Heiland 2010). Low sclerostin is also thought to be a marker of syndesmophyte formation in AS (Heiland 2012). In a recent study that was done to assess the sclerostin expression and osteocyte death in joints derived from patients with AS, the sclerostin expression was noted to be suppressed in patients with AS (14.7 ± 1.1%) when compared to those with rheumatoid arthritis, osteoarthritis and healthy controls (57.7 ± 3.2%, 42.2 ± 1.0% and 53.2 ± 3.1% respectively) (Heiland 2012). Also, serum levels of sclerostin were significantly lower in patients with AS than in healthy controls (326 ± 243 pg/ml in men; 81 ± 32 pg/ml in women) than in healthy control subjects (645 ± 221 pg/ml in men; 284 ± 152 pg/ml in women). Furthermore, the serum level of sclerostin over time was significantly higher in AS patients without syndesmophytes than in AS patients with syndesmophytes (p = 0.007).

However, not all studies have reported that sclerostin levels are low in AS. In 2013, cross sectional data presented by Korkosz and colleagues (n=50) suggested that the sclerostin levels in serum were significantly greater in patients with active disease (defined as BASDAI>4) than in controls (Korkosz 2013). In the study by Korkosz et al, there was no correlation between serum sclerostin or DKK-1 with CRP suggesting that molecular mechanisms other than inflammation might also be modulating Dkk-1 and sclerostin levels in AS. Conversely, in another study, the sclerostin levels were lower in patients receiving TNFi indicating that those with active and severe disease with high inflammation have higher sclerostin levels (Saad 2012). In this longitudinal study, at baseline, patients with AS had lower sclerostin levels (60.5 ± 32.7 vs. 96.7 ± 52.9 pmol/L, p = 0.002) compared to healthy controls. There were no differences in sclerostin levels between AS patients with or without peripheral joint involvement (69.9 ± 29.6 vs. 55.7 ± 33.9 pmol/L, p = 0.269) and between AS patients with a disease duration of > 5 years or < 5 years (48.13 ± 33.50 vs. 65.76 ± 31.71 pmol/L, p = 0.180). Serum sclerostin levels showed inverse correlations with CRP (r = -0.57, p = 0.001) and ESR (r = -0.59, p < 0.001) suggesting that suppression of sclerostin occurs in the presence of
inflammation. Though the serum levels of sclerostin increased from baseline (vs. 6 months vs. 12 months) after treatment with TNFi (60.5 ± 32.7 vs. 67.1 ± 31.9 vs. 72.7 ± 32.3 pmol/L, p <0.001) respectively), they remained significantly lower in patients compared to controls (72.7 ± 32.3 vs. 96.70 ± 52.9 pmol/L, P = 0.038) at 12 months. Furthermore, serum sclerostin levels at 12 months were lower in patients with high CRP (CRP ≥ 5 mg/L) compared to those with normal levels (50.8 ± 16.6 vs. 83.6 ± 33.0 pmol/L, p = 0.021). Similarly, in a recent report serum sclerostin levels did not change with TNF alpha inhibitor treatment (Korkosz 2014). The authors attributed this to the shorter disease course of patients as WNT proteins are activated only in later stages of bone remodeling. Conversely, Taylan et al observed that there were no differences in Dkk-1 and sclerostin levels between AS patients and controls and also between AS groups with high or low BASDAI (Taylan 2012). However, the results might have been influenced by the use of TNFi in some patients (23 / 55 patients studied). Sclerostin levels have also been studied in relation to BMD in AS. Not much data exists on the link between sclerostin and BMD in AS. In the study by Saad et al, BMD at the lumbar spine increased after treatment TNFi, but no significant change was observed for hip BMD and the change in spine BMD was attributed partly to sclerostin levels remaining lower after one year of treatment with TNFi (Saad 2008). Tsui and colleagues recently demonstrated higher than normal levels of noggin and sclerostin-IgG immune complexes in AS sera (p<0.001) (Winkler 2004). This study concluded that higher levels of NOG and/or SOST-IgG immune complexes probably contribute to new bone formation in AS patients (Winkler 2004,Tsui 2013). The role of noggin and sclerostin-IgG immune complexes in mediating bone loss needs to be studied.

1.4 Fracture risk in AS

1.4.1 Prevalence of fractures

Osteoporosis has serious consequences, such as fractures. Fractures can cause severe pain, physical deformity, disability, poor quality of life and even mortality. In addition, they are sometimes associated with a need for long-term care assistance. Furthermore, fractures can be a huge financial burden to both patients and the health care system. Fragility fractures mainly occur at the spine in patients with AS. On the other hand, hip and other peripheral fractures have also been shown to occur though data in this regard is inconsistent.
Spinal fractures occur commonly in patients with AS and are associated with significant morbidity and even mortality. Patients with AS have chronic back pain, stiffness and spinal immobility due to the disease. Sustaining a vertebral fracture is likely to worsen their back pain, stiffness and immobility. Vertebral fractures also cause hyperkyphosis and spinal deformities (Ralston 1990). For example, in a prospective study done at Glasgow, by Ralston et al in 1990, patients with compression fractures were found to have greater spinal deformity (defined by the distance from wall to tragus: 24.5 cm v 12.7 cm in controls) when compared to controls. In addition, patients with fractures also had less spinal mobility (20 vs. 45.6 degrees of flexion), and chest expansion (2 cm vs. 3cm in controls). Although mostly silent, acute vertebral fractures can cause back pain, but they can be missed in diagnosis as back pain and stiffness are common symptoms in AS and a separate cause for back pain is less often sought for (Ralston 1990, Feldtkeller 2006). In fact, some vertebral fractures are difficult to diagnose because of the atypical location of vertebral fractures in AS (Wang 2005). Not all spinal fractures that occur in AS occur at the typical osteoporotic sites such as lower thoracic spine (Campagna 2009, Westerveld 2009). Some fractures occur at the cervical spine, and involve the posterior arch structures of the vertebrae (Westerveld 2009). These fractures occur at the transvertebral or transdiscal sites and may even pass through syndesmophytes. Trabecular bone loss and paravertebral calcification increases the propensity to develop transvertebral fractures. The atypical nature of these fractures leads to severe neurological involvement such as spinal cord lesions, nerve root injuries, and paravertebral hematomas and even high mortality (Westerveld 2009, Schoenfeld 2011, Sambrook 2012, Lu 2013). In addition, the fractures may heal defectively causing pseudoarthrosis and instability of the posterior arch of the vertebrae. In a systematic review conducted by Westerveld and colleagues that included 76 articles representing 345 patients with AS, found that most patients (n=227, 66%) sustained low energy trauma leading to their fractures. The most common mechanism of trauma was a fall from standing/sitting position. The sites of fractures were cervical, thoracic and lumbar spine (81.2%, 10.7% and 7.8% respectively). The rate of various complications occurring secondary to vertebral fractures was 51%. Even uncommon complications of spinal fractures such as aortic dissection, aortic pseudoaneurysm and tracheal rupture were reported (Westerveld 2009). The overall mortality within 3 months after fracture was 18% (Westerveld 2009).
Patients with AS are at high risk for low trauma fractures (Westerveld 2009, Schoenfeld 2011, Sambrook 2012, Lu 2013, Robinson 2013, Vosse D 2009, Munoz Ortego 2014, Weiss 2010). Data on fractures in AS is summarized in Table 4. High fracture risk is particularly concerning in AS given the young age of affected patients. The diagnosis of vertebral fractures in AS can be complicated given that many of these remain undetected. In a cross sectional study by Klingberg et al it was reported that majority of the vertebral fractures detected on X rays were silent and undiagnosed (Klingberg 2012). Though a total of 24/204 (12%) patients (mean age: 50 ± 13 years) were diagnosed to have vertebral fractures. But only three fractures had been previously diagnosed clinically.

The evidence for high fracture risk in AS is strengthened by data obtained from four large-scale studies (Robinson 2013, Vosse D 2009, Munoz Ortego 2014, Weiss 2010). A Swedish study based on data obtained from inpatient hospitalizations of patients with AS between 1987 and 2008, revealed that there is a rising trend in the number of vertebral fractures in AS (Robinson 2013). A significant positive linear trend was noted in the annual incidence of cervical (r = 0.82), thoracic (r = 0.85) and lumbar fractures (r = 0.56) between 1987 and 2008 (Robinson 2013). The proportion of vertebral fractures increased from 0.8% in 1987 to 11% in 2008. Similarly, in 2009, Vosse and colleagues conducted a primary care-based nested case-control study involving 231,778 patients with fractures and 231,778 controls (Vosse D 2009). They identified 758 subjects to be having AS in this cohort. Patients with AS were noted to have an increased risk for clinical vertebral fractures (OR=3.3; 95% CI: 1.5 - 7.0). In contrast, the risk of fractures at non-vertebral sites such as forearm and hip was not elevated (OR =1.2; 95% CI: 0.9 -1.7 and OR 0.8; 95% CI 0.4 - 1.4, respectively). Another key finding of this study was that the fracture risk was higher in those who had IBD and AS together (OR 2.8; 95% CI=1.1 to 7.1). Interestingly, the use of non-steroidal anti-inflammatory drugs (OR 0.7; 95% CI 0.5 to 0.8) was shown to reduce the risk of fractures. In a recent population based study from Sweden, patients with AS had a clearly higher risk of vertebral and non-vertebral fractures (Munoz Ortego 2014). In this study of 6,474 AS patients and 32,346 controls, an increased risk of clinical vertebral (hazard ratio 1.93, 95% CI 1.39 to 2.68, p < 0.001) and non-vertebral (HR 1.19, 95% CI 1.02 to 1.39, p = 0.03) fractures were shown to exist in patients with AS when compared to controls. However, the high risk was found only in those patients who were not receiving NSAIDs. The authors also reported that risk estimates were not affected by presence
of comorbid conditions such as inflammatory bowel disease and psoriasis. Similar results were observed in a large population based study conducted by Weiss et al in Sweden (Weiss 2010). The authors found that patients with AS had the highest risk for vertebral fractures compared to rheumatoid arthritis, scleroderma and psoriatic arthritis. The odds ratio of any fracture ranged from 2.6 (95% CI: 1.3-4.9) for scleroderma to 4 (95% CI: 3.4-4.6) for patients with AS. Furthermore, patients with AS had the highest odds ratio for vertebral fractures (OR =7.1, 95% CI: 6-8.4).

Both clinical and morphometric vertebral fractures have been shown to be have higher incidence in AS. In 1994, a retrospective population based study reported an increased odds ratio (OR) of 7.7 (95% CI 4.3– 12.6) for clinical vertebral fractures in AS (Cooper 1994). This shows that patients with AS have a sevenfold higher risk of vertebral fractures. Men had higher rates of fractures (OR: 10.7 versus 4.2 in women). The incidence of clinical vertebral fractures was highest (17%) 20–30 years after diagnosis. The main drawback of the study was that it had only analyzed the incidence of clinical vertebral fractures. Morphometry is a more reproducible technique to identify vertebral fractures but this study did not obtain data on the prevalence of morphometric vertebral fractures. The prevalence of morphometric vertebral fractures was estimated to be 10% (n=87) in a cross sectional study done by Donnelly et al (Donnelly 1994). The fracture rate for men was higher (14% vs. 8% in women). The prevalence of morphometric vertebral fractures was noted to be 16.7% (11/66), in a cohort of 66 men with mild AS (Mitra 2000). None of the patients had reported history of trauma. The study patients had a mean age of 41.4 years and disease duration of 12.4 years and this suggests that patients with AS develop non-traumatic vertebral fractures at a relatively young age. Again, it needs to be acknowledged that morphometric assessment is not the gold standard for assessing vertebral fractures. All deformities that are identified by morphometric methods may not be fractures. Shapes of vertebral bodies can change due to other disorders such as osteoarthritis and Scheurmann’s disease.
1.4.2 Non vertebral fractures in AS

In a recent population based study from Sweden, patients with AS had a high risk for vertebral and non-vertebral fractures when compared to controls (123). Of 6,474 patients with AS, 218 (3.4%) suffered non-vertebral fractures, compared with 861 (2.7%) matched controls. The incidence of non-vertebral fractures were higher in patients than controls (8.27 (95% CI 7.24 to 9.44) per 1000 person-years vs. 6.83 (95% CI 6.39 to 7.30) in controls (Munoz Ortego 2014). The increased risk was independent of smoking, alcohol consumption, body mass index, and use of oral corticosteroids. However, the high risk was found only in those who were not being treated with NSAIDs despite the fact that patients who were treated with NSAIDs had more severe disease. Potential mechanisms by which NSAIDs may confer protection against vertebral fractures include delay in radiographic progression, reduction in spine rigidity, relief in back pain and stiffness and improvement in physical function (Haroon 2012, Poddubnyy 2012 and Kroon 2012). The results of the study by Muñoz-Ortego and colleagues are similar to that of another large population based study conducted by Weiss et al in Sweden which suggested a high risk of hip fractures in AS (Weiss 2010). The odds ratio for hip fracture was 2.5 (95% CI: 1.9-3.1). However, two studies have documented that the risk of non-vertebral fractures is not elevated in patients with AS (Vosse 2009, Cooper 1994). In 1994, a retrospective population-based study reported the incidence of non-vertebral fractures was similar to that of controls (Cooper 1994). No increase in the risk of limb fractures was observed despite a pronounced increase in the risk of thoracolumbar compression fractures (standardized morbidity ratio = 7.6; 95% CI, 4.3-12.6) after 2,398 person-years of observation in patients with AS. Likewise, a primary care-based nested case-control study of 231,778 patients with fracture, conducted in Netherlands showed that the risk of fractures of the forearm and hip was not significantly elevated in AS patients (n=758) with AS (OR 1.21; 95% CI 0.87-1.69 and OR 0.77; 95% CI 0.43 - 1.37, respectively) (Vosse D 2009). Hip fractures occur late in life and age of occurrence of hip fracture might be a reason why some observational studies did not find an association between AS and hip fractures despite the high prevalence of osteoporosis (Vosse D 2009, Cooper 1994). Most patients recruited in the above mentioned studies were young or middle aged. High risk of non-vertebral fractures could also be underestimated by improper study design, incorrect definition of fractures or a lack of statistical power due to small sample sizes.
Table: 4: Summary of data on the prevalence and risk of fractures in patients with AS

<table>
<thead>
<tr>
<th>Author, country, year</th>
<th>Number of patients and age</th>
<th>Study design</th>
<th>Prevalence of fractures and OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ralston, U.K., 1990</td>
<td>111 patients with AS. Age: 41(19-76)</td>
<td>Prospective case-control study</td>
<td>VFs (anterior height/posterior height &lt;0.80-0.85): 16%</td>
</tr>
<tr>
<td>Cooper, USA, 1994</td>
<td>158 patients with AS</td>
<td>Retrospective population based</td>
<td>Clinical VFs: OR= 7.7 (4.3–12.6)</td>
</tr>
<tr>
<td>Mitra, U.K., 2000</td>
<td>66 patients with AS. Age: 38</td>
<td>Cross sectional</td>
<td>Morphometric VFs: 17% OR: 5.9 (1.4-23.8)</td>
</tr>
<tr>
<td>Donnelly, U.K., 1994</td>
<td>87 patients with AS. Age: 44</td>
<td>Cross sectional</td>
<td>Clinical VFs: 10%</td>
</tr>
<tr>
<td>Baek, Korea, 2005</td>
<td>Mild AS=45 Severe AS =31 Age: 28±8</td>
<td>Cross sectional</td>
<td>Morphometric VFs: 3.9%</td>
</tr>
<tr>
<td>Lange, Germany, 2005</td>
<td>84 patients with AS Age: 32-46</td>
<td>Cross sectional</td>
<td>VFs: 10.7% (9/84)</td>
</tr>
<tr>
<td>Jun, Korea, 2006</td>
<td>68 patients with AS Age: 31</td>
<td>Cross sectional</td>
<td>VFs: 16%</td>
</tr>
<tr>
<td>Feldtkeller, Netherlands, 2006</td>
<td>1071 patients with AS Age: 50± 12 years</td>
<td>Survey</td>
<td>VFs: 1.4%</td>
</tr>
<tr>
<td>Devogelaer, Belgium, 1992</td>
<td>70 patients with AS Age: 35-39</td>
<td>Cross sectional</td>
<td>VFs: 4%</td>
</tr>
</tbody>
</table>
VF = Vertebral fractures, HR = Hazard’s ratio, OR = Odds ratio

### 1.4.3 Risk factors for fractures in AS

Fractures might occur in patients with AS due to several reasons. The most common risk factors for osteoporotic fractures in the general population include low BMD, older age, use of glucocorticosteroids, family history of hip fractures, and falls. Spinal fractures occur in AS due to mechanical causes or systemic bone loss. Patients with AS have mechanical risk factors such as spinal immobility, ankylosis and syndesmophytes of the spine predisposing them to develop vertebral fractures even with minimal trauma (Donnelly 1994). There is conclusive evidence that fracture risk is increased in patients with higher mSASSS suggesting that mechanical
factors of the spine also play a role. In addition, patients with AS have high bone remodeling. Vertebral bodies have high trabecular bone content and compromised trabecular structure in AS increases the risk of fractures (Akhter 2007). High remodeling compromises bone mineralization deteriorates structure of the collagen and also causes micro-damage to the bone. No data are available on the risk of falls in AS patients.

Enough evidence now exists to suggest that fractures in AS are mainly predisposed by bone loss. Klinberg et al demonstrated that the occurrence of vertebral fractures in AS were associated with lower BMD of both the spine and hip. The strongest correlations between the Genant score and BMD were found in the hip followed by volumetric and lateral lumbar BMD. Not all studies have shown that BMD of the lumbar spine or hip is a good predictor of vertebral fractures (Akhter 2007). In 1994, Donnelly and colleagues from United Kingdom analyzed the link between BMD and vertebral fractures in patients (9/87 patients had vertebral fractures) with AS (Akhter 2007). BMD at lumbar spine or femoral neck was not significantly reduced (spine Z = -0.31 vs. -0.18 for non-fracture group, p = 0.81, femur Z= -0.81 vs. -0.80, p = 0.97) in patients who had vertebral fractures. The study had some limitations, however. For instance, many patients in this study had severe radiological disease and syndesmophytes can falsely elevate BMD of the lumbar spine. Moreover seven patients had psoriasis and IBD. Patients with IBD have additional risk factors for bone loss (Bernstein 2003, Walldorf 2013).

In another study, Mitra et al (66 men with mild AS, median age: 38 and duration: 10 years) found that there were no significant differences in the lumbar spine or femoral neck BMD in AS patients with fractures compared with those without (Mitra 2000). Ghozlani et al observed that BMD at the lumbar spine was not different between those with or without vertebral fractures (0.052 ± 0.1 vs. 0.933 ± 0.13, p=NS, total N=80) (Ghozlani 2009 ). Hip BMD was lower in patients with vertebral fractures (0.841±0.1 vs. 0.987±0.1 in those without fractures, p=0.005). In another study, though 74 vertebral fractures were detected, there were no significant differences in BMD or bone turnover markers between patients with or without vertebral fractures (Arends 2011). Specifically, the BMD T-scores at the lumbar spine (−0.70±1.33 vs. −0.71±1.51; p=0.984) and total hip regions (−0.47±1.03 vs. −0.59±1.10; p=0.591) did not differ.
1.5 Management of osteoporosis in AS

1.5.1 Management of osteoporosis in AS

No specific guidelines are available for managing bone loss in AS. Present clinical practice guidelines in Canada involve applying FRAX or CAROC based guidelines to evaluate fracture risk (Kanis 2011, Papaioannou 2010). These guidelines enable to categorize the patients into low, moderate and high fracture risk groups (Kanis 2011). Treatment decisions vary depending up on the fracture risk. However, the CAROC guidelines utilize BMD measures to define the fracture risk. In addition, both FRAX and CAROC guidelines can be applied only to people aged 50 years and above.

1.5.2 Use of anti-resorptive and anabolic agents to manage bone loss in AS

Osteoporosis in patients with AS is generally treated with conservative measures and bisphosphonates. But the use of bisphosphonates in AS raises concerns. First, if diagnosed with bone loss, young patients with AS may require prolonged use of bisphosphonates. Long-term use of bisphosphonates may be associated with atypical femoral fractures (Shane 2014). Second, the efficacy of bisphosphonates in alleviating fracture risk in patients with AS is currently unknown. Third, bisphosphonates can cross placenta and experimental studies in rats have shown that bisphosphonates accumulate in the fetal bones affecting skeletal ossification and mineralization (Patlas 1999). Hence the use of bisphosphonates is of concern given that many patients with AS are in their reproductive years. Fourth, both subclinical bowel inflammation and IBD have a strong association with AS and these conditions can cause malabsorption. Oral bisphosphonates are not absorbed well in the setting of malabsorption. Finally, bisphosphonate therapy adds to the financial liability of patients who are already receiving expensive medications such as TNFi (Schabert 2013).

Given its ability to modulate inflammation, pamidronate, an intravenous bisphosphonate, has been used in the treatment of AS for up to 6 months (Toussirot 2006, Toussirot 2007). Treatment with pamidronate resulted in mild and transient improvement in clinical and radiological features of AS. However, no long-term data exists on the use of pamidronate in AS and given the superior benefits of TNFi, pamidronate is preferred less often to treat AS. Other limitations of pamidronate are the requirement of a long infusion time and side effects
such as arthralgia, myalgia and flu-like symptoms (Olson 2007).

Teriparatide or biosynthetic human parathyroid hormone 1-34 is a bone anabolic agent that has been shown to improve BMD, bone microarchitecture, structural integrity, bone diameter, and bone strength owing to its ability to augment bone formation. In humans, intermittent use of teriparatide increases the number of osteoblasts, activates pre-existing osteoblasts and, reduces osteoblast apoptosis (Dempster 2001, Borggrefe 2010, Ito 2014, Cohen 2013). However, treatment with bisphosphonates may be needed to optimize gains in bone density after teriparatide withdrawal (Kurland 2004). Limited systemic evidence is available on the use of anabolic agent, teriparatide in patients with AS. In a small study conducted by colleagues in 2007, the safety of the combined use of teriparatide and TNFi in patients with inflammatory arthritis was studied (n=6). During the 9-month follow-up period, there was a reduction in inflammatory parameters but no differences were found in biochemical parameters. The authors did not observe any new symptomatic fractures or infections in treated patients.

The new anti-resorptive medication, denosumab, is a potential agent to treat bone loss in patients with AS (Dempster 2001). Denosumab is a human immunoglobulin G2 monoclonal antibody that acts against RANK-ligand, a crucial mediator of the formation, function, and survival of osteoclasts (Diab 2014). The potential negative consequences of adding another biological agent such as denosumab to a treatment regimen involving biological drugs such as TNFi are still unclear.

1.5.3 Role of DMARDs in managing bone loss in AS

The effect of methotrexate on BMD and fractures in AS has not been studied in detail. Methotrexate is not commonly used in the treatment of AS now (Chen 2013, Mulleman 2011). In a small RCT comparing methotrexate alone to methotrexate plus infliximab (Marzo-Ortega 2005), no significant change in hip or spine BMD was noted in the methotrexate monotherapy group when compared to those who were treated with methotrexate and infliximab after 30 weeks. A study by Sampaio-Barros PD and colleagues showed that methotrexate had no effect on BMD in AS (Marzo-Ortega 2005). No difference was noted in the lumbar spine or hip BMD between those with (n=25) or without methotrexate (n=58) treatment.
1.5.4 Effect of TNF alpha inhibitors on bone loss and fracture risk in AS

TNFi have now revolutionized the treatment of AS. Prior studies have shown that blockade of TNF alpha reduces bone resorption and causes improvement of BMD (Visvanathan 2009, Durnez 2013, Kang 2011, Dischereit 2013, Briot 2008, Allali 2003). TNFi have been consistently shown to improve lumbar spine BMD. However, the changes in total hip and femoral neck BMD were not consistent across studies. Also, limited data exists on how TNFi modify the fracture risk. A recent retrospective cohort study done by Kawai et al using administrative databases suggested that TNFi did not reduce the risk of vertebral or non-vertebral fractures (Kawai 2013). The risk of combined fractures (hip, humerus, radius, ulna, or pelvis) was comparable between TNFi and non-biologic comparators (HR 0.9, 95% CI 0.5–1.8).

1.6 The structure and composition of bone

1.6.1 Bone structure

Bone tissue is composed of bone cells such as osteoclasts, osteoblasts, and osteocytes (Clarke 2008). The osteocytes are differentiated osteoblasts. The bone cells are embedded in an osteoid matrix, which has mineral and fibrous components. The fibrous part is composed of collagen, proteoglycans and non-collagenous proteins. Mainly hydroxyapatite crystals and amorphous calcium phosphate form the rigid mineral component.

Macroscopically, whole bones are composed of cortical (compact) and trabecular (cancellous) bone tissue (Clarke 2008). Microscopically, both cortical and cancellous bone compartments are composed of small units called osteons. The relative proportion of cortical and trabecular bone differs significantly at various skeletal sites. The trabecular to cortical bone ratio is around 75: 25 in the vertebra and 50: 50 in the femoral head and 95: 5 at the ultradistal radius.

Cortical bone is abundantly present in the diaphysis or shafts of long bones and outer surfaces of the flat bones such as skull, scapula and mandible. The cortical bone appears dense and solid, constituting 80% of the skeleton. Cortical bone is not as metabolically active as the trabecular bone (Töyräs 2013, Mayhew 2005, Thomas 2009, Courtland 2013, Seeman 2003).

Trabecular bone is mainly located in the metaphyses and epiphyses. Trabecular bone is composed of interconnected rods and plates (Hildebrand 1999, Shi 2010, Stauber 2006).
Osteoporosis is a systemic disease defined as a reduction in bone mass associated with an impaired bone architecture and increased fracture risk. Thus, it is important to understand the cortical and trabecular microarchitecture.

1.6.2 Microarchitecture of the cortical and trabecular compartments of bone

Cortical bone consists of osteons. As age advances, both men and women experience thinning of the cortex and increased cortical porosity (Töyräs 2013, Mayhew 2005, Thomas 2009). Abnormal cortical remodeling leads to reduced cortical bone mass and increased cortical porosity (Courtland 2013, Seeman 2003). The cortical abnormalities associated with fragility are cortical thinning and increased cortical porosity (Zebaze 2010).

Trabecular bone has more surface than cortical bone and is composed of osteons at the microscopic level. The trabeculae are categorized into plates and rods according to their shape. Plates provide more support than rods because of their width and thickness (Shi 2010). The trabecular bone lies very near to the bone marrow cells. The healthy trabecular bone is constituted by rods and plates that form a three dimensional lattice. The interlocked lattice of trabecular bone is oriented along the lines of stress of the bone. Trabecular thickness (width of the trabeculae), trabecular separation or the distance between the trabeculae and their number (density) are important determinants of bone fragility. With loss of BMD, the plate-like trabeculae become thinner and rod-like. The trabecular abnormalities found in patients who develop fractures include more separated trabeculae, reduced bone volume, fewer plate-like trabeculae, trabecular perforation, fewer axially aligned trabeculae and poor connectivity between plates and rods (Stauber 2006).

1.6.3 Bone remodeling

Each bone repetitively undergoes modeling to adapt to varying biomechanical forces, and remodeling to replace micro damaged bone with mechanically stronger bone to help restore bone strength (Eriksen 1986, Camozzi 2007). Both cortical and trabecular compartments of the bone undergo structural remodeling in order to maintain the biomechanical properties. The aging process can cause changes in stiffness (rigidity), toughness (ability to absorb the stresses) and strength (mechanical resistance). Systemic regulation of bone remodeling is done
by hormones such as PTH, estrogen, androgen, calcitonin, thyroid hormone and growth hormone as well as 1,25 (OH)\textsubscript{2} vitamin D and corticosteroids (Camozzi 2007). Local factors that influence bone remodeling are cytokines (IL-1, TNF-alpha., IL-6,IL-11, IL-4 and OPG), colony stimulating factors, prostaglandins, leukotrienes, nitric oxide and growth factors such as IGF, TGF-β, FGF,PDGF and PTHrP (167-170 Camozzi 2007, Lorenzo 1992, Kawaguchi 1995, Inui 1998).

1.6.4 Bone modeling

Bone modeling is the ability of bones to adapt their shape and size in response to a particular mechanical load. Bone modeling also occurs during growth. Contrary to bone remodeling, bone formation is not paired with bone resorption.

1.6.5 Bone strength

Bone strength is a biomechanical property of bones and is defined as their ability to resist a fracture. It is the maximum load bones can tolerate before the structure fails. It is a function of both BMD and bone quality (NIA workshop, 1992).

1.6.6 BMD and bone strength

BMD is measured by DXA and is expressed as grams of mineral per area or volume. It accounts for about 70 percent of bone strength (Ammann 2003). However, BMD lacks sensitivity and specificity because more than 50% of women who have a fracture do not have osteoporosis (173-178 Siris 2004, Wainwright 2005, Schuit 2004, Cummings 2002, Sarkar 2002, Delmas 2004). A prior fragility fracture is a strong predictor of repeat fracture independent of BMD (Langstemo 2009). In addition, the changes in BMD over time after treatment can explain only 4–30% of the fracture risk reduction. Data from clinical trials on various therapeutic agents used in the management of osteoporosis suggest the relationship between risk reduction of vertebral fractures and increase in lumbar spine BMD is inconsistent (Black 1999).
1.6.7 Bone quality and bone strength

Bone quality is defined as the material, architectural and mechanical features of the bone (NIA workshop 1992, Black 1999, Brandi 2009, Felsenberg 2005, Augat 2006). It refers to trabecular and cortical microarchitecture; bone turnover, micro cracks, mineralization of the bone matrix and the amount of micro damage present in the bone (Table 5).

Table 5: Determinants of bone strength

<table>
<thead>
<tr>
<th>Bone volume</th>
<th>Material properties</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMD</strong></td>
<td>Hydration Tissue density Osteocyte network integrity Cellular density Mineralization degree Mineral crystallinity Degree and type of collagen cross linking Non collagenous proteins</td>
<td><strong>Bone geometry</strong></td>
</tr>
<tr>
<td></td>
<td>Size Shape Cortical thickness Moment of inertia Femoral neck geometry</td>
<td></td>
</tr>
</tbody>
</table>

1.6.8 Bone geometry

The structural characteristics that affect bone quality are bone geometry and size. Bone geometry comprises of length and cross-sectional dimensions. Major geometrical measures such as bone size and cross-sectional area or area moment of inertia may predict up to 70-80% of whole bone strength (Augat 2006). Cortical perimeter is a key parameter of bone strength, since increase in the diameter of a hollow cylinder may increase the resistance to bending and torsion. Femoral neck geometry is also relevant for bone quality. A longer hip axis length predisposes to fractures independent of BMD (Wang 2009). The incidence of hip fractures is lower in Japanese women despite having lower BMD than Caucasian women, probably
because of their better femoral neck geometry (Nakamura 1994). In a study conducted in healthy young men with prevalent fractures, men with fractures were found to have lower cortical thickness and smaller cortical bone area ($p < .005$) and also it was observed that the odds ratio for fractures increased with decreasing cortical thickness (odds ratio (OR) 1.4/SD, $p \leq .001$) and cortical area (OR 1.5/SD, $p \leq .001$) (Taes 2010). Variations in femoral shaft cross sectional area were shown to explain the differences in bone strength between African and Caucasian postmenopausal women and also between older men and women (Nelson 2004, Nelson 2000, Russo 2003, Russo 2006). Previous studies have also demonstrated that hip fractures are more frequent in women with higher femoral neck width, femoral shaft width, and longer femoral neck axis (Gnudi 2012).

1.7 Diagnosis of bone loss in AS

1.7.1 Use of DXA in AS

Currently there is no accurate measure of overall bone strength. BMD measured by DXA is utilized as a surrogate measure of bone strength. However, osteoporosis is characterized by compromised bone strength and high fracture risk. Osteoporosis and low bone density are defined using measurements of BMD. The World Health Organization (WHO) has defined bone loss using DXA based measurements of BMD (Kanis 1994). The World Health Organization defines osteoporosis as BMD 2.5 standard deviations below the mean for matched young adults. Bone loss in men less than 50 years old and premenopausal women are categorized using the ISCD definition (Schousboe 2013). BMD measurements have other advantages as well. For instance, serial BMD readings are useful to demonstrate ongoing bone loss and to monitor the effect of various treatment modalities (Marshall 1996). The three major sites used to generate BMD measurements are lumbar spine (L1 to L4), total hip and femoral neck. Though distal forearm is another important site used to measure BMD, it is recommended only when hip and/or lumbar spine cannot be used for measurements, in obese people and in patients with hyperparathyroidism. There are certain drawbacks of BMD. The main limitation of BMD is that it explains bone strength only to a certain extent (Genant 2008, Bouxsein 2008, Delmas 2004, Watts 2005). In addition, the definitions proposed by the WHO can be applied only to men older than 50 years and postmenopausal women.
1.7.2 Limitations of using DXA in patients with AS

Certain limitations exist regarding the use of DXA imaging in patients with AS. These are related to the sites of DXA used, technical difficulties in positioning of the patients and to the lack of specific data regarding the association between BMD and fracture risk in AS.

1.7.3 Limitations of spine DXA in AS

Conventional DXA machines use antero-posterior view of the lumbar spine to generate values of BMD. Spine is the most affected skeletal organ in AS and patients with AS develop bony changes at the spine as the disease progresses. This leads to false positive readings of BMD at the lumbar spine in patients with long-standing and advanced radiological disease (Ulu 2013, Gilgil 2005). BMD of the lumbar spine is also unreliable in AS to assess longitudinal bone loss since BMD tends to become normal as the disease progresses due to the potential radiological progression of the disease. Patients with advanced radiological disease have fusion and immobility of the spine and this limits their ability to lie down flat on the DXA table while undergoing the study. Improper positioning can produce erroneous results. One solution for better interpretation of lumbar spine BMD using DXA is to perform lateral DXA (Ulu 2013, Gilgil 2005). Facet joint disease, fusion of interapophyseal joints, calcification of ligaments and presence of syndesmophytes increase the miscalculation (Baek 2005, Devogelaer 1992, Masud 1993). Ulu and colleagues suggested that spine BMD can be falsely high in patients with AS (Ulu 2013). In this cross sectional study, the mean posteroanterior (PA) spinal BMD was similar in patients (n=80) and controls (n=50, p = 0.460) whereas femoral and lateral spine BMD was significantly lower in patients (p = 0.012 and p = 0.001). Moreover, the PA spinal BMD tended to be higher in patients who had vertebral fractures (28 % of patients) but the lateral spinal BMD values were significantly lower in the fracture group (p=0.004) and also in patients with syndesmophytes (p = 0.004). In summary, it is clear that BMD measurement at the lateral lumbar spine reflects bone loss and fracture risk better. DXA scan using lateral view of the lumbar spine may be better in diagnosing bone loss in patients with AS. Lateral DXA can isolate the vertebral body from the ankylosed posterior elements, the zygapophyseal joints, endplates and syndesmophytes during the measurement. It can also detect decreased BMD better than postero-anterior views of the spine (MacKay 2000). Besides, proper positioning can
be difficult in some patients who are unable to lie in the lateral decubitus position due to spinal deformities. The interpretation of images can be complicated due to the interference of ribs and iliac crests. Newer scanners are capable of measuring lateral DXA from supine positioning (Blake 1994). The main limitation with this approach is that BMD of the lateral spine has lower precision than femur neck or AP spine. Hence, lateral DXA may be unreliable for follow up.

1.7.4 Limitations of hip DXA in AS

The measurement of BMD at the hip in patients with AS is also associated with certain issues. Hip might not be a reliable site because of the pathologic involvement of hip joints in AS (Vander Cruyssen 2013, Huang 2013). Changes that happen at the hip include subchondral bone marrow edema, enthesitis, fatty infiltration of the bone marrow, bony erosions, and narrowing of the joint space. MRI is capturing more information on clinical and subclinical hip involvement than the assessments based on X-rays and clinical symptoms. For example, in a study done by Huang et al in 2013, 74% had abnormalities detected at the hip (86/116) as opposed to 21% (24/116) and 30% (35/116) detected based on radiographs or clinical symptoms (Huang 2013). Also, many patients undergo hip replacement during the course of the disease making it impossible to use hips for DXA scanning. Appropriate positioning and internal rotation of the hip are essential prerequisites for performing a good DXA study. But as stated above, patients with AS sometimes face difficulties in positioning their limbs on the DXA table. The presence of coxitis can limit the ability to internally rotate the limb during DXA scanning (Ghozlani 2009). Even minor changes in the degree of rotation of the hip can affect measurements of the DXA.

1.7.5 Limitations of DXA in determining fracture risk in AS

The ability of DXA based measurements to predict fracture risk in patients with AS is also unclear. BMD at both lumbar spine and hip have not been consistently linked to the high risk for fractures in patients with AS (Chen 2013, Mitra 2000 and Weiss 2010). For instance, in 1994, results of a study done by Donnelly et al confirmed that low femoral neck BMD was not related to the presence of osteoporotic vertebral fractures (Weiss 2010). In the cross sectional study done by Ghozlani and colleagues, lumbar spine BMD was similar in those with or without VFs (1.059 ± 0.2 vs. 1.052 ± 0.1, p=NS, n=80) (Ghozlani 2009). In the multivariable
regression model, the odds ratio of lumbar spine for a Genant grade 2 or 3 vertebral fracture was not significant (OR=12.15; 95% CI=0.41–359.7). The authors also observed that about 30% of patients with osteopenia and 20% with normal BMD were found to have vertebral fracture (grades 2 and 3). However the authors used VFA and not spine X-ray to document spine fractures. Spine radiograph is the gold standard for diagnosing vertebral fractures. Finally, there are issues related to the limitations of the DXA technique. The areal DXA measures mineral content of the bone in a two dimensional manner. It cannot account for the size and depth of the bone. The bones in men are larger; therefore corresponding DXA readings are higher in men. Also, DXA cannot distinguish between cortical and trabecular compartments of the bone. This is particularly disadvantageous in inflammatory diseases such as AS because there can be differential involvement of the cortical and trabecular compartments.

1.7.6 Assessment of bone strength in AS
Almost all the current evidence that describes bone loss in patients with AS is based on data obtained from studies done using DXA. As discussed above, DXA has limited resolving power to study the structural and strength or mechanical properties. Given the disadvantages and limitations of DXA in AS, it is crucial to identify better predictors of fracture risk. Bone strength is the main determinant of fracture risk. Information about bone strength would also help in identifying targeted treatment to treat bone loss in AS.

Monitoring bone strength also helps to identify the benefits of anti-osteoporosis or anti-TNF alpha medications on bone that may occur independent of changes in BMD. Moreover, it is likely that bisphosphonates and TNF-alpha inhibitors may exert differential effects on the cortical and trabecular compartments of the bone. However, no clear data exists in this regard. Monitoring treatment response using DXA alone might underestimate the benefits of TNF-alpha inhibitors or bisphosphonates. For instance, in addition to its ability to increase BMD, alendronate has been shown to improve parameters such as cortical thickness, trabecular BV/TV and estimated failure load (Burghardt 2010). Zoledronic acid has been shown to increase cortical thickness, cortical density and trabecular volume fraction in radius and tibia without much effect on cortical porosity (Hansen 2013). Finite element estimated bone strength was preserved, but not increased with zoledronic acid (Hansen 2013). No data exists on the
effect of TNF-alpha inhibitors on bone strength in AS. In summary, there exists an urgent need to study bone strength in AS.

1.7.7 Bone histomorphometric studies on bone structure in AS

Bone structure is studied in detail using histomorphometric of iliac crest specimens obtained from bone biopsies. Histomorphometry or quantitative histology is the gold standard for assessing bone structure (Recker). It is also helpful to study bone formation and resorption (Malluche 2007). However, bone histomorphometric studies in AS are scarce. Szejnfeld et al conducted biopsies of the iliac crest in 16 Caucasian males with AS (mean age: 34± 3 years and disease duration: 11±2 years) and reported that bone formation was affected more than bone resorption (Szejnfeld 1997). Fourteen patients presented osteopenia, ten had mineralization defects, and three patients presented with osteomalacia. Trabecular bone mass and wall thickness were found to be lower in subjects with AS than the controls. The osteoid volume, osteoid thickness and mineralization lag time were significantly greater than control values (p < 0.05) but both control subjects and patients had similar bone osteoclast interface and the eroded surface. The mineral apposition rate was less than the control group (p < 0.01). The authors concluded that decreased bone mass seen in AS was due to reduced bone formation rather than an increase in bone resorption since the resorption parameters such as bone osteoclast interface and eroded surface were similar to those of controls. However, despite being the gold standard, bone biopsy has certain limitations. Although the histomorphometric parameters are obtained from the iliac crest, the sites susceptible to fragility fractures are the load-bearing sites and vertebral bodies. Thus, it is likely that iliac crest biopsies may not accurately represent the pathological features that may predispose to fractures. Moreover, bone biopsy is an invasive procedure and for this reason, it is not practical to do biopsy in a clinical setting on a routine basis.

1.8 Bone imaging

1.8.1 Newer bone imaging techniques

With the advances in medical imaging, it is now possible to determine bone microarchitecture and strength in a precise manner (Table 6 and 7). The novel technique called HRpQCT is now
being widely used for studying bone strength and structure (Kalpakcioglu 2008, Cheung 2013). Micro-CT (µCT) of ex vivo samples is a three dimensional imaging modality used for understanding and quantifying bone microarchitecture (Bouxsein 2008, Chappard 2011).

Table 6: Anatomical hierarchy of bone*

<table>
<thead>
<tr>
<th>Nature</th>
<th>Dimensions</th>
<th>Description</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano scale</td>
<td></td>
<td>Organic phase: collagen fibers</td>
<td>Transmission electron</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mineral phase: hydroxyapatite crystals</td>
<td>microscopy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lamellar in mature bone or randomly packed collagen bundles in woven bone</td>
<td>Polarization microscopy</td>
</tr>
<tr>
<td>Texture</td>
<td>15-80 µm</td>
<td>Trabecular bone: Arch-like bone structural units in trabecular bone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortical bone: osteons centered around haversian canal</td>
<td></td>
</tr>
<tr>
<td>Structure</td>
<td>120-300 µm</td>
<td>Trabecular bone: rods and plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortical bone: compact osteons</td>
<td></td>
</tr>
<tr>
<td>Microarchitecture</td>
<td>0.2-0.4 mm</td>
<td>Trabecular bone: rods and plates</td>
<td></td>
</tr>
<tr>
<td>Macroarchitecture</td>
<td></td>
<td>Cortical bone: compact osteons</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from references Chappard 2011

Table 7: Imaging techniques for studying bone structure*

<table>
<thead>
<tr>
<th>Component</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Polarization</td>
</tr>
<tr>
<td></td>
<td>Interference profilometry</td>
</tr>
<tr>
<td></td>
<td>Fluorescence microscopy</td>
</tr>
<tr>
<td></td>
<td>Raman spectroscopy</td>
</tr>
<tr>
<td></td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td></td>
<td>Biomechanical properties by micro/nano indentation</td>
</tr>
<tr>
<td>Mineralization degree</td>
<td>Microradiography</td>
</tr>
<tr>
<td></td>
<td>Scanning electron microscopy-back scattered energy mode</td>
</tr>
<tr>
<td></td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td></td>
<td>Raman spectroscopy</td>
</tr>
<tr>
<td><strong>Toluidine blue staining</strong></td>
<td><strong>Interference profilometry</strong></td>
</tr>
<tr>
<td><strong>Biomechanical properties by finite element analysis</strong></td>
<td><strong>MicroCT/Synchrotron/NanoCT</strong></td>
</tr>
</tbody>
</table>

| **Biomechanical properties** | **Finite element analysis** |
| | **Micro/nano indentation** |
| | **Scanning acoustic microscopy** |

| **Macro architecture** | **Hip axis length** |
| | **Femoral neck axis length** |
| | **Femoral neck/shaft angle** |
| | **Cross-sectional moment of inertia** |
| | **Moment of inertia** |
| | **Moment side fall on 2D images of digitized radiographs and DXA, and on 3D imaging by CT and MRI** |

| **Microarchitecture** | **Volumetric 3D imaging based on CT and MRI** |
| **Bone remodeling** | **Histomorphometry/Histodynamic studies** |
| **Osteocyte viability** | **Apoptosis staining** |

*Adapted from references 211-216*

### 1.8.2 Overview of HRpQCT

HRpQCT is a relatively new technique used to assess bone microarchitecture. Until recently bone biopsy was the only reliable method used to study microarchitecture of the bone. The main advantage of HRpQCT over bone biopsy is that it can be done non-invasively. The exposure to radiation from HRpQCT is < 3 µSv per scan and similar to that of DXA (MacNeil 2008). HRpQCT has certain advantages over DXA (Cheung 2013). It can measure both bone volume (bone mineral density) and structural properties. HRpQCT provides data on three-dimensional bone structure and volumetric BMD as opposed to areal BMD obtained by two-dimensional imaging by DXA. The HRpQCT has better resolution than DXA and provides detailed information about cortical and trabecular compartments (MacNeil 2008). Thus, it is possible to identify whether bone loss and fractures are occurring due to cortical thinning, porosity or loss of trabecular structure. Another advantage is that HRpQCT scans take shorter time to be completed because of the peripheral nature of the sites; radius and tibia (Figures 2 & 3).
Figure 2: HRpQCT scanner located at the Osteoporosis Program, Toronto General Hospital. Shown here is how right tibia of the patient is being scanned.
1.8.3  Mechanical properties of bone and finite element analysis

The mechanical behavior of bone is a determinant of its fragility. Bone undergoes stress and strain when exposed to external forces. Bone stress is defined as the concentration of a mechanical force (Clarke 2008). Bone strain is the amount of physical deformation sustained when exposed to a mechanical force. Fractures occur when the stresses from an external force exceed the limit of the material strength of bone (Figure 4). Bone stress is mainly influenced by the bone geometry. It is now possible to estimate bone stress and strain non-invasively using HRpQCT (MacNeil 2008). A finite element analysis (FEA) of the HRpQCT images using special software helps to estimate the bone strength (MacNeil 2008). The FEA converts bone
images to a dynamic three-dimensional computer model. The method was originally used in the manufacturing of industrial products. The three-dimensional models generated by FEA are a complex network of points joined together into a mesh. The distribution of points or nodes is based on the density of the underlying image. Areas with higher stress have larger number of nodes. The FEA images may be used to reconstruct models from data obtained from MRI, HRpQCT or MicroCT. It is possible to obtain multiple simulations. The main limitation of FEA is that the mathematical modeling as well as the assumption of element properties are isotropic and linear elastic with fixed Young’s modulus and so may not truly reflect the real world scenario.

Figure 4: Load displacement curve for bone tissue

![Load displacement curve](image)

The slope: extrinsic stiffness (S); the height of the curve: ultimate force or Fu; area under the curve: work to failure (U); and total displacement: fracture is ultimate displacement (du).

1.8.4 Determining fracture susceptibility using HRpQCT and FEA

Structural parameters assessed on HRpQCT and mechanical parameters estimated from FEA are related with fragility fractures. Vilayphiou and colleagues showed that bone structure, stiffness, and failure load were associated with fractures in postmenopausal women (Vilayphiou 2010). Similarly Graeff and colleagues demonstrated that structural and mechanical parameters of bone were superior to areal BMD in discriminating men with and without vertebral fractures (Graeff 2012). Even relatively small reductions in microarchitecture and strength can relate to fracture risk. Another study by Vilayphiou et al showed that
HRpQCT and FEA parameters of radius and tibia predicted vertebral and non-vertebral fractures in men (Vilayphiou 2011). Volumetric BMD, cortical thickness, trabecular number, and separation were lower in cases than in controls, with differences ranging from -6% to 15%. The µFE-derived stiffness and failure load were 8% to 9% lower in those who had fractures.

1.9 Bone microarchitecture and structure in patients with AS

1.9.1 Previous studies on bone microarchitecture and structure in patients with AS

Not much is known about the microarchitecture of bone in AS. In 1992, Devogelaer and colleagues studied 10 patients with AS by using quantitative computed tomography (QCT) to image the lumbar spine. The results revealed that AS patients had low trabecular bone density in the vertebral bodies (Devogelaer 1992). The effect was more marked in those with severe AS, syndesmophytes and apophyseal joint fusion. In another small study (n=15) done using spine QCT, a statistically significant decline in trabecular bone mineral content (BMC) was observed (change ± SD: 18 ± 7 mg/cm3) over a period of ten years (Korkosz 2011). Patients with advanced spine involvement had significantly lower BMC both at baseline and follow-up (Korkosz 2011). However CT based measurements of the spine has certain limitations. First, CT based measurement is processed from the middle layer of the vertebra and hence may not give information about the structure of whole vertebral body. Second, the error rate because of interference from fat is high (SE: 10-30%). Third, it is also not possible to analyze a fractured vertebra. Finally, the radiation exposure is also higher (SE-QCT <100 IS).

1.9.2 Abnormal bone microarchitecture, strength and biomechanics in rat models of spondylitis

In 2009, Rauner and colleagues studied bone quality in the tibia and sixth vertebral body of HLA B27 rats and demonstrated abnormal trabecular structure in AS (Rauner 2009). The diseased rats had significantly reduced trabecular thickness and number, and increased trabecular separation. Similar results were observed in another study on transgenic rat models (Dempster 2001). In this study, transgenic HLA–B27 male rats (n=8) had lower BV/TV, trabecular thickness, and number in their vertebral bodies and distal femur compared to control littermates. Also noted was that transgenic HLA–B27 male rats had lower structural strength
(ultimate load) in the mid-shaft femur (22%, p=0.01) and femoral neck (26%, p<0.05).
Similarly, parameters such as ultimate load, stiffness and yield stress at the vertebral body were lower in transgenic HLA–B27 male rats.
Review of existing literature suggests that little has been studied about bone strength and microarchitecture in patients with AS. It is extremely important to study the bone microarchitecture and strength in patients with AS, given the high risk of osteoporosis and fragility fractures. New data suggest that BMD alone may not explain the fracture risk. Fragility is determined by bone strength, and microarchitecture in addition to BMD. Furthermore, prospective studies of the general population have shown that areal BMD identifies only 20% of men who will later sustain a fracture (Szulc 2005, Schuit 2004). Both mechanical and bone–related fractures may determine fracture risk in AS. Limited data exist as to whether fractures in AS occur due to low BMD. Most evidence that suggests that low BMD as a cause of fractures in AS is based on cross sectional studies. Therefore, the high fracture risk in AS cannot be explained by changes in BMD alone. Spine BMD is not reliable to interpret in AS due to the presence of syndesmophytes. Interestingly, not all studies have identified a strong relationship between hip BMD and fragility. Also, BMD may not be a reliable diagnostic tool in this population that comprised predominantly of young men and premenopausal women due to the fact that link between low BMD and fracture risk in such populations are not fully understood (Gourlay 2004). HRpQCT permits noninvasive in vivo assessment of cortical and trabecular bone microarchitecture and biomechanical competence. This will provide insights into compartment-specific (trabecular versus cortical) effects of inflammation in AS. This might be also significant to the explanation of fracture risk in AS.

2.1 Hypothesis

The trabecular compartment of the bone is metabolically more active than the cortical compartment making it more susceptible to insult from changes in cytokine levels and systemic inflammation. Hence, we hypothesized that patients with AS are likely to have lower trabecular volumetric BMD than healthy controls and that greater disease severity, as measured by BASDAI, is associated with greater decline in bone strength.
2.2 Primary aim
1. The primary aim of this study was to assess the association of disease severity in AS with trabecular vBMD at the radius. Specifically, this study addressed whether high BASDAI is associated with low trabecular vBMD at radius in patients with AS.

2.3 Secondary aims
1. To study the association between disease duration and trabecular vBMD
2. To study the association between elevated mSASSS and trabecular vBMD
3. To compare the parameters of bone quality between patients and healthy subjects
4. To understand gender differences in bone strength
5. To compare bone strength in patients with elevated mSASSS versus normal mSASSS
CHAPTER 3

METHODS
METHODS

3.1 Study design
This was a cross sectional study conducted at a tertiary care hospital.

3.2 Subjects

1. AS patients
The AS cohort in the study consisted of patients with AS who were recruited prospectively from the spondylitis clinic at the Toronto Western Hospital. The recruitment period started in March 2012 and ended in December 2013.

2. Non-AS subjects
The comparison group consisted of non-AS subjects. Data on non-AS subjects were obtained from the CaMos (Canadian multicenter osteoporosis study) project (223). This was a multi-center cohort study conducted in Canada. The CaMos cohort consists of a random population-based sample of non-institutionalized subjects 25 years old or more and living within 50 km. of nine Canadian cities. Subjects from the Toronto cohort were identified as non-AS control subjects for the current study. The HRpQCT data was available only from subjects aged over 40 years. Non-AS subjects with an age range of 40-70 were chosen.

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria (AS patients)
1. At least 18 years old at the screening visit
2. Provided written informed consent
3. A documented diagnosis of AS, defined by the modified New York criteria
4. Active disease, defined as BASDAI ≥ 4
Definition of AS

Modified NY criteria for AS

<table>
<thead>
<tr>
<th>Clinical Criteria</th>
<th>Radiological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low back pain ≥ 3 months, improved by exercise and not relieved by rest</td>
<td>Bilateral grade 2-4 sacroiliitis OR Unilateral 3-4 sacroiliitis</td>
</tr>
<tr>
<td>Limitation of motion of lumbar spine in sagittal and frontal planes</td>
<td></td>
</tr>
<tr>
<td>Limitation of chest expansion</td>
<td></td>
</tr>
</tbody>
</table>

Requirements: bilateral grade 2-4 or unilateral grade 3-4 sacroiliitis AND any clinical criteria

3.3.2 Exclusion criteria

1. Any condition that can impair the ability of the subject to give written informed consent and/or adhere to the study procedures
2. Pregnancy

3.4 Definitions

3.4.1 Definition of clinical severity of AS

Clinically severe AS was defined as having a BASDAI greater than or equal to 4. BASDAI was calculated from a self-administered questionnaire. BASDAI comprises of a scale that ranges from 1 to 10, one being no problem and 10 being the worst. Information about five symptom domains was collected. The symptoms analyzed were pain with swelling in other joints, fatigue, spinal pain, severity and duration of morning stiffness and discomfort with peripheral enthuses. Scores ≥ 4 indicate suboptimal control of disease. BASDAI was assessed at the same visit as the HRpQCT.

3.4.2 Definition of radiological severity of AS

Radiological severity was defined using mSASSS (Wanders 2004). In this scoring system, cervical and lumbar vertebrae are scored using pre-defined parameters. The abnormalities looked for are erosions, sclerosis, syndesmophytes and bony bridging. The presence of erosion, sclerosis or squaring is given a score of 1. Syndesmophytes and bony bridging get a score of 2 and 3 respectively. A total of 24 sites are scored, from 0 to 3 so that the composite scores range
from 0-72. Subjects with mSASSS scores above zero were considered to have elevated mSASSS. This categorization was done to compare bone microarchitecture in patients with or without radiological involvement of the spine. mSASSS was assessed at the same visit as the HRpQCT.

3.5 Clinical and laboratory data

Data regarding demographic and clinical parameters of patients with AS were obtained from the database of the Spondylitis Clinic at the Toronto Western Hospital. All patients attending the Spondylitis clinic at the Toronto Western Hospital complete a self-administered clinical questionnaire. This questionnaire has been designed to collect data on age, gender, duration of symptoms, duration since diagnosis, disease activity, history of comorbid illnesses, and use of concomitant medications. Duration of disease was defined as duration since the onset of back pain. Data was also obtained on parameters such as smoking, use of corticosteroids and bisphosphonates. Height and weight of the subjects were recorded. Height was measured by Harpenden stadiometer and weight by the balance beam scale. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m²).

Laboratory parameters

Serum alkaline phosphatase, ESR and CRP were analyzed. ESR was measured using the Westergren’s method. ELISA was used to measure CRP.

3.6 Bone imaging

3.6.1 DXA

Areal BMD was measured at the femoral neck, total hip, and L1 to L4 lumbar spine with DXA by using Hologic Discovery machine (Hologic, Bedford, Massachusetts). The scanner is part of the University Health Network Centre for Excellence in Skeletal Health Assessment, Toronto. It undergoes daily calibration with phantoms. Osteoporosis was defined using the WHO criteria (Kanis 1994). Postmenopausal women and men aged 50 years and above were considered to have osteoporosis if the T-scores at lumbar spine, total hip or femoral –neck regions were less than -2.5. Low bone mass was defined as having a T-score between -1 and -
2.5. Premenopausal women and men younger than 50 years were considered to have low bone mass if the Z-scores at lumbar spine, total hip or femoral –neck regions were less than or equal to -2.0 (Schousboe 2013).

3.6.2 HRpQCT

The high-resolution peripheral quantitative computed tomography (HRpQCT) was done at the radius and tibia using XtremeCT (Scanco Medical, Bassersdorf, Switzerland). The scanner is part of the University Health Network Centre for Excellence in Skeletal Health Assessment, Toronto. This scanner uses a two-dimensional detector array and a 0.08-mm point-focus X-ray tube enabling the concurrent acquisition of parallel slices with a resolution of 82 µm. The settings for the measurement consisted of an X-ray tube potential of 60kvp, X-ray tube current of 900 µA and an image matrix size of 1536 × 1536. The limb to be scanned was first immobilized inside a carbon fiber shell. Then an anteroposterior scout view was obtained so as to determine the exact region to be scanned. A reference line was manually placed at the end plate of the radius and tibia. The regions of interest were examined in 110 parallel slices. The first slice was obtained 9.5 mm proximal to the reference line at the radius and 22.5 mm proximal to the reference line at the tibia. At each skeletal site, a three-dimensional image of approximately 9 mm in the axial direction was obtained. Each measurement took about 3 minutes. The slices were then separated into trabecular and cortical regions by using a Gaussian filter and a threshold-based algorithm. The desired parameters were then either measured directly during the procedure or derived. For quality control, the manufacturer phantom was scanned daily. The phantom contains rods of hydroxyapatite embedded in a soft-tissue equivalent resin (QRM, Moehrendorf, Germany).

3.6.3 Measurement of trabecular and cortical parameters

The trabecular and cortical volumetric BMD are direct measures that are defined as the average bone density within the cortical or trabecular volume of interest, respectively. The BV/TV or the trabecular bone volume is calculated by assuming that a fully mineralized bone has the density of 1.2 g hydroxyapatite/ cm³.

\[
\text{BV/TV} \% = 100 \times \frac{\text{Trabecular volumetric BMD}}{1200}.
\]
The cortical thickness is a direct measure using a 3D distance transformation. The trabecular number (1/mm) is detected in the images using advanced data processing methods. The trabecular thickness is calculated using the formula (BV/TV) / Tb.N and the unit is µm. The trabecular separation is measured in µm, using the formula (1-BV/TV) / Trabecular number.

3.6.4 Finite element analysis (FEA) of HRpQCT images

HRpQCT images were used to measure mechanical parameters of bone using a finite element analysis (MacNeil 2008, Pistoia 2002). The voxel-conversion approach was used to generate linear, homogeneous finite-element meshes from the HRpQCT images (MacNeil 2008, Muller 1995, van Rietbergen 1995). The boundary conditions signified a uniaxial compression test with the nodes at the bottom surface fixed in the uniaxial testing direction. The nodes at the top and bottom surfaces were unconstrained in X and Y directions. A displacement was applied in the uniaxial testing direction that corresponded to 1% strain. A single homogeneous tissue modulus of 6829 MPa and a Poisson’s ratio of 0.3 were applied to all elements.

3.7 Consent

Informed written consent was obtained from all subjects.

3.8 Ethics

The research ethics board of University Health Network, Toronto, approved the study.

3.9 Statistical analysis

All continuous variables were tested for skewness and kurtosis. Tests for normality were conducted to analyze the distribution of different variables using Kolmogrov Smirnov test. Testing was performed at a significance level of p<0.05.

3.9.1 Descriptive statistics

Categorical variables were expressed as percentage and frequency. Continuous variables were expressed as means (±SD) if the variable had a normal distribution or medians (interquartile range) if the distribution was skewed.

3.9.2 Inter group comparisons

Characteristics of subjects with and without AS were compared using two sample t-tests for normally distributed continuous variables, Mann-Whitney U tests for skewed continuous or
ordinal variables and chi square tests for categorical variables. Paired t-tests and Wilcoxon signed rank tests were used to compare intergroup differences in the sub group that consisted of age and gender matched AS patients and non-AS subjects.

### 3.9.3 Regression analysis

Multivariable linear regression analysis was performed to study the effect of AS on volumetric BMD and bone microarchitecture. For the primary analysis, regression models were created using data obtained from both AS patients and apparently healthy non-AS subjects (table 8).

**Table 8: Multivariable linear regression analysis**

<table>
<thead>
<tr>
<th></th>
<th>AS patients</th>
<th>Non-AS subjects</th>
<th>Analysis</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary analysis</td>
<td>n=44</td>
<td>n=85</td>
<td>All AS patients (n=44) and non-AS subjects (n=85)</td>
<td>Multivariable linear regression analysis to study if AS is an independent risk factor for abnormal HRpQCT parameters</td>
</tr>
<tr>
<td></td>
<td>n=27</td>
<td>n=47</td>
<td>Sub-group consisting of AS patients and non-AS subjects in the age range of 40-65 years</td>
<td>Multivariable linear regression analysis to study if AS is an independent risk factor for abnormal HRpQCT parameters</td>
</tr>
</tbody>
</table>

Regression analysis was also done to study the effect of age, gender, BASDAI, elevated mSASSS and duration of AS on HRpQCT parameters (Table 9). Trabecular vBMD at radius was studied as the primary outcome variable for the multivariable regression analysis. The different covariates studied were age, gender, elevated mSASSS and BASDAI. Additional regression analyses were done using different dependent variables such as volumetric BMD, cortical porosity, cortical thickness, BV/TV, trabecular thickness, trabecular separation, bone
stress and stiffness.

Another set of regression analyses were performed on a subgroup comprising of AS patients and non-AS subjects that were matched by age and gender.

3.9.4 Assumptions and diagnostics of the multivariable linear regression model

Multiple linear regression models need to satisfy certain assumptions: normality, linearity, homoscedasticity and normal distribution of residuals. Tests were conducted to confirm that the assumptions were met. The distributions of variables were examined with histograms and Q-Q plots. The linearity of the relationships between outcome variables and continuous explanatory factors were examined by constructing scatter plots. Further, the possible presence of collinearity between different continuous covariates was studied by creating scatter plots. The tests of collinearity were conducted using the tolerance and variance inflation factor (Myers 1990). A value of tolerance less than 0.2 or VIF greater than 4 suggests multicollinearity. The error variance should be constant to satisfy the condition of homogeneity of variance. A well-fitted regression model shows no pattern to the residual plotted against the fitted value. Residual plots were used in this study to check the regression assumption of homogeneity of variance. Data was also examined for the presence of outliers, and influential observations. Cook’s distance was used to measure how much the residuals would have changed if the potential influential observations were deleted from the regression models (Barnett 1994).

Relationships between parameters were analyzed using Pearson or Spearman’s rank order correlation tests as appropriate.
CHAPTER 4

RESULTS
4.1 Demographic, clinical and bone health related parameters of patients with AS

4.1.1 Demographic parameters of patients with AS

Data are shown in Table 9. Most subjects were Caucasians (82%). The age range was 21 to 64 years. Only 4 subjects (three males, 1 female) were older than 60 years. 17/44 subjects (39%) were aged less than 40 years. Eight women (8/19) were postmenopausal (42%).

Table 9: Demographic, clinical and laboratory parameters of study subjects with AS

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>44</td>
<td>25 (57)</td>
<td>19 (43)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.1±11.7</td>
<td>45.2±12.1</td>
<td>42.7±11.2</td>
<td>.478</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.3±27.6</td>
<td>175.3±8.6</td>
<td>151.3±37.4</td>
<td>&lt;. 001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.1±19.0</td>
<td>85.2±16.1</td>
<td>64.7±16.2</td>
<td>&lt;. 001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (21.8-30.1)</td>
<td>27.3 (25.0-30.9)</td>
<td>23.8 (20.5-30.5)</td>
<td>.135</td>
</tr>
<tr>
<td>Current smoking</td>
<td>6 (14)</td>
<td>4 (16)</td>
<td>2 (11)</td>
<td>.157</td>
</tr>
<tr>
<td>Current steroid use</td>
<td>2 (4.5)</td>
<td>1 (4)</td>
<td>1 (5.3)</td>
<td>.186</td>
</tr>
<tr>
<td>Current bisphosphonate use</td>
<td>4 (9)</td>
<td>3 (16)</td>
<td>1 (5.3)</td>
<td>.432</td>
</tr>
<tr>
<td>Use of DMARDs</td>
<td>16 (36)</td>
<td>5 (20)</td>
<td>11 (58)</td>
<td>.676</td>
</tr>
<tr>
<td>Use of NSAIDs</td>
<td>35 (80)</td>
<td>20 (80)</td>
<td>15 (79)</td>
<td>.653</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>22.0 (9.0-28.5)</td>
<td>25.5 (13.5-30.5)</td>
<td>17.5 (3.3-24.5)</td>
<td>.044</td>
</tr>
<tr>
<td>Iritis</td>
<td>9 (20)</td>
<td>6 (24)</td>
<td>3 (16)</td>
<td>.250</td>
</tr>
<tr>
<td>IBD</td>
<td>8 (18)</td>
<td>3 (12)</td>
<td>5 (26)</td>
<td>.430</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>8 (18)</td>
<td>5 (20)</td>
<td>3 (16)</td>
<td>.508</td>
</tr>
<tr>
<td>BASDAI</td>
<td>6.7 (4.8-7.9)</td>
<td>5.8 (4.3-7.0)</td>
<td>7.5 (5.7-8.4)</td>
<td>.023</td>
</tr>
<tr>
<td>mSASSS</td>
<td>2 (0-14)</td>
<td>9 (0-31)</td>
<td>0 (0-2)</td>
<td>&lt;. 01</td>
</tr>
<tr>
<td>Elevated mSASSS **</td>
<td>24 (52)</td>
<td>17 (68)</td>
<td>7 (32)</td>
<td>&lt;. 05</td>
</tr>
<tr>
<td>HLA B27 positive</td>
<td>33(74)</td>
<td>21 (83)</td>
<td>12 (61)</td>
<td>.141</td>
</tr>
<tr>
<td>Fragility fractures</td>
<td>4 (9)</td>
<td>3 (12)</td>
<td>1 (5)</td>
<td>.121</td>
</tr>
<tr>
<td>ESR</td>
<td>22.0±23.6</td>
<td>20.1±26.1</td>
<td>22.7±19.6</td>
<td>.207</td>
</tr>
<tr>
<td>CRP</td>
<td>12.6±15.6</td>
<td>13.4±17.3</td>
<td>11.5±13.3</td>
<td>.565</td>
</tr>
<tr>
<td>SAP (IU)</td>
<td>96+43</td>
<td>95+41</td>
<td>98+47</td>
<td>.132</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD, median (IQ range) or n (% N). *p: men vs. women. ** mSASSS > zero
4.1.2 Disease related parameters of patients with AS

The duration of back pain ranged from 1-42 years (median: 22 (IQ: 9-29 years)). All patients had BASDAI scores higher than or equal to 4. The median (IQ) BASDAI was 6.7 (4.8-7.9). The mSASSS scores ranged from 0-72. The median mSASSS was 2 (interquartile range: 0-14). The mSASSS score was zero in twenty subjects. Iritis, psoriasis and inflammatory bowel disease were the common comorbidities present in patients with AS. The mean serum ESR was 22.0±23.6 (range: 2-105). The mean serum CRP and alkaline phosphatase levels were 12.6±15.6 (range: 3-71) and 96.2±43.2 IU respectively. Details are shown in Table 9.

Twenty-four subjects had elevated mSASSS (defined as mSASSS greater than zero). Patients with elevated mSASSS were significantly older (49±10 vs. 39±11 years, p=0.003) and had longer disease duration (26.5 (IQ: 15.0-33.5) vs. 16.5 (IQ: 5.0-23.0) years, p=0.032) than those with normal mSASSS (Table 9). Serum levels of inflammatory markers were also elevated in patients who had elevated mSASSS. For example, the mean ESR and CRP levels were higher in patients with elevated mSASSS (ESR: 31±28 vs. 12±13 and CRP: 19±18 vs. 6±7, p=0.003 for both, vs. AS patients with normal mSASSS). Serum alkaline phosphatase showed a trend towards being higher in those with elevated mSASSS (108±49 IU vs. 83±32 IU, p=0.057).

4.1.3 Gender differences in demographic and disease related parameters in patients with AS

Male and female patients with AS were aged similarly (45.2±12.1 vs. 42.7±11.2 years, p=0.478). Data are shown in Table 9. BMI was also similar between men and women (p=0.135). However, men had significantly higher mSASSS than women. The proportion of men who had elevated mSASSS was also greater than that of women. The disease duration was longer in men than women (p=0.044). Women had higher BASDAI scores than men (7.5 (5.7-8.4) vs., 5.8 (4.3-7.0), p=0.023). The proportion of men and women who had iritis, psoriasis and IBD were similar (Table 9).

4.1.4 Comparison of demographic characteristics in patients with elevated mSASSS compared to those with normal mSASSS

Demographic parameters were compared between patients with elevated (n=24) or normal mSASSS (n=20). Patients with elevated mSASSS were significantly older (48.2±10.0 vs.
38.7+11.3 years) and had longer disease duration (25 (9-32) vs. 14 (4-22) years) than those with normal mSASSS (Figure 5). There were significantly more men in the group with elevated mSASSS (17/24 vs. 7/20, p < .05). The two groups had similar BMI (27 (24-30) vs. 25 (21-19)).

Figure 5: Comparison of demographic characteristics in patients with elevated mSASSS compared to those with normal mSASSS

![Figure 5](image.jpg)

4.1.5 Bone health related parameters of patients with AS

Four subjects (9%) reported a history of fragility fracture (Table 9). The fractures were located at the thoracic spine (morphometric), hip (occurred from a fall), rib (occurred while coughing) and wrist (occurred from a fall). The use of corticosteroids was negligible (2/44 or 4.5%). Four subjects were on bisphosphonates. Twelve subjects were taking calcium and vitamin D supplements.

4.2 Characteristics of non-AS subjects

Eighty-five healthy subjects were included as non-AS subjects (Table 10). The non-AS subjects were significantly older than the AS patients (61.2± 7.8 vs. 44.1± 11.7 years, in non-AS subjects, p=0.000). The gender distribution was also different between AS patients and
non-AS subjects (Table 10). There were fewer men among the non-AS subjects (29% vs. 57%). However AS patients and non-AS subjects had similar BMI.

Table 10: Comparison of demographic characteristics of AS patients and non-AS subjects.

<table>
<thead>
<tr>
<th></th>
<th>AS patients</th>
<th>Non-AS subjects</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>44.1 ±11.7</td>
<td>61.2 ±7.8</td>
<td>&lt;. 001</td>
</tr>
<tr>
<td>Age range</td>
<td>21-64</td>
<td>40-70</td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>36 (82)</td>
<td>76 (89)</td>
<td>.334</td>
</tr>
<tr>
<td>Men</td>
<td>25 (57)</td>
<td>25 (29)</td>
<td>&lt;. 005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (21.8-30.1)</td>
<td>26.6 (23.9-29.4)</td>
<td>.253</td>
</tr>
</tbody>
</table>

*p: AS patients vs. non-AS subjects. Data expressed as mean±SD, median (IQ range) or n (% N).

4.3 HRpQCT parameters in patients with AS

4.3.1 Gender differences in HRpQCT parameters in patients with AS

Certain gender differences were noted in the manner in which AS affected bone microarchitecture (Table 11). While the trabecular component was affected more in women than men, men had more cortical bone abnormalities than women. The vBMD and microarchitecture of the trabecular bone at radius and tibia were impaired in women compared to men despite having similar age and BMI (Table 11). The trabecular vBMD at radius and tibia were 28 and 21% (respectively) lower in women than men (132.38+ 39 vs. 183.9+41, p=0.000 at the radius). Women had fewer (15% less) and thinner trabeculae than men. Also, women demonstrated a greater degree of trabecular separation than women. BV/TV at both radius and tibia (28 and 21% respectively) was also lower in women than men. However, cortical parameters were more abnormal in men than women. Specifically, men had lower cortical vBMD at the radius (821+56 vs. 868+49,p=0.007, % change=5%), cortical thickness at tibia (17% lower), and greater (30-52%) cortical porosity at radius and tibia. The FEA parameters such as stiffness and stress did not show any gender differences. The mean age was not different between men and women (45.2 ±12.1 vs. 42.7 ±11.2, p=0.478). Age did not influence the gender differences in bone microarchitecture on linear regression.
Table 1: Gender differences in bone microarchitecture and strength

<table>
<thead>
<tr>
<th></th>
<th>Men with AS (n=25)</th>
<th>Women with AS (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>183.99</td>
<td>41.21</td>
<td>132.38</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>821.74</td>
<td>55.91</td>
<td>868.05</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>315.08</td>
<td>64.09</td>
<td>298.47</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.153</td>
<td>.034</td>
<td>.110</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>2.11</td>
<td>.24</td>
<td>1.79</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.073</td>
<td>.012</td>
<td>.061</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.408</td>
<td>.276</td>
<td>.556</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>.737</td>
<td>.162</td>
<td>.794</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.021</td>
<td>.017-.027</td>
<td>.010</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.146</td>
<td>.034</td>
<td>.115</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>.682</td>
<td>.135</td>
<td>.820</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.060</td>
<td>.042-.078</td>
<td>.042</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.146</td>
<td>.034</td>
<td>.115</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.53</td>
<td>.348</td>
<td>1.528</td>
</tr>
<tr>
<td>Stress</td>
<td>31.67</td>
<td>8.70</td>
<td>31.56</td>
</tr>
</tbody>
</table>

4.3.2 Comparison of HRpQCT parameters between patients with elevated or normal mSASSS

Patients who had elevated mSASSS (mSASSS > zero) had worse bone microarchitecture than those who had normal mSASSS. Specifically, patients with elevated mSASSS had abnormal volumetric BMD and microarchitecture in the cortical bone. Data are shown in Table 12. The elevated -mSASSS group had lower cortical volumetric BMD. Cortical porosity at the radius was greater in those with elevated mSASSS. A similar trend was noted in cortical porosity at
the tibia. Moreover, the cortical thickness was lower in patients who had elevated mSASSS. Bone stiffness and stress also tended to be lower in patients with elevated mSASSS though the results did not reach statistical significance. On regression analysis, the negative effect of elevated mSASSS was present only in the cortical vBMD at the radius, after adjusting for age and disease duration (beta=-36.6, 95% CI: -68.0- -5.3, p= <.05).

Table 12: Comparison of bone microarchitecture and FEA parameters in patients with elevated vs. normal mSASSS

<table>
<thead>
<tr>
<th></th>
<th>Elevated mSASSS (N=24)</th>
<th>Normal mSASSS (N=20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>159.09 55.07</td>
<td>161.41 36.82</td>
<td>.874</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>815.19 59.21</td>
<td>872.60 39.50</td>
<td>&lt; .005</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>291.11 74.64</td>
<td>325.59 43.18</td>
<td>.077</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>1.92 .45</td>
<td>2.01 .24</td>
<td>.436</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.07 .01</td>
<td>.07 .012</td>
<td>.868</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.51 .27</td>
<td>.44 .07</td>
<td>.278</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.13 .05</td>
<td>.14 .03</td>
<td>.876</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>.71 .17</td>
<td>.83 .12</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Cortical porosity radius</td>
<td>.02 .018-.028</td>
<td>.014 .008-.020</td>
<td>&lt; .005</td>
</tr>
<tr>
<td>Stiffness Radius</td>
<td>1.24 .40</td>
<td>1.44 .32</td>
<td>.087</td>
</tr>
<tr>
<td>Stress Radius</td>
<td>24.35 10.03</td>
<td>29.26 8.07</td>
<td>.087</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>151.39 43.88</td>
<td>164.43 44.30</td>
<td>.339</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>827.04 82.84</td>
<td>878.87 42.59</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>278.47 60.17</td>
<td>311.61 55.25</td>
<td>.069</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>1.92 .45</td>
<td>2.01 .24</td>
<td>.421</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.07 .02</td>
<td>.07 .01</td>
<td>.976</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.55 .31</td>
<td>.47 .13</td>
<td>.312</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.13 .04</td>
<td>.14 .04</td>
<td>.343</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>.67 .18</td>
<td>.82 .17</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.06 .05-.09</td>
<td>.04 .03-.06</td>
<td>&lt; .005</td>
</tr>
<tr>
<td>Stiffness</td>
<td>1.43 .35</td>
<td>1.62 .363</td>
<td>.082</td>
</tr>
<tr>
<td>Stress</td>
<td>29.13 8.66</td>
<td>33.94 9.07</td>
<td>.083</td>
</tr>
</tbody>
</table>
4.3.3 Correlation between disease duration, age and HRpQCT parameters (AS patients alone, n=44)

An inverse correlation was present between total as well as cortical vBMD of the radius and tibia and age \((r = -.359, p = .017\) and \(r = -.543, p = .001\) respectively for total vBMD and \(r = -.370, p = .013\) and \(r = -.464, p = .002\) respectively for cortical vBMD). Data are shown in Table 13. There was no association between trabecular parameters and age. However, significant inverse correlation was noted to exist between age and cortical parameters such as cortical thickness and porosity at both radius and tibia. Age and cortical thickness showed a moderate negative correlation \((r = -.624, p = .000\) at the radius and \(r = -.567, p = .000\) at the tibia). Likewise, age had a moderate positive association with cortical porosity \((r = .584, p = .001\) at the radius and \(r = .549, p = .001\) at the tibia). Similarly, significant negative correlation existed between age and stiffness or stress at radius and tibia.

Table 13: Correlation between age and HRpQCT parameters (AS patients alone, n=44)

<table>
<thead>
<tr>
<th></th>
<th>Radius</th>
<th>Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Age</td>
</tr>
<tr>
<td>R</td>
<td>p</td>
<td>R</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>-.359</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>-.064</td>
<td>.678</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>-.543</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>BV/TV</td>
<td>-.062</td>
<td>.689</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>.102</td>
<td>.511</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>-.216</td>
<td>.158</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.006</td>
<td>.971</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>-.624</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.584</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Stiffness</td>
<td>-.422</td>
<td>&lt; .005</td>
</tr>
<tr>
<td>Stress</td>
<td>-.422</td>
<td>&lt; .005</td>
</tr>
</tbody>
</table>

4.3.4 Effect of disease duration on trabecular vBMD of radius in AS patients

On univariate linear regression analysis among AS patients, disease duration and male gender were shown to have a positive relationship with trabecular vBMD of radius \((\text{beta} = 1.521, p = <.05\) and \(\text{beta} = 51.2, p = <.001\)). However the effect of duration became statistically
insignificant after adjusting for gender (beta = 0.826, p = .143). The positive relationship between trabecular vBMD radius and disease duration was seen mainly in women (beta = 2.75, p = 0.002, figure 6) and persisted after adjusting for age.

Figure 6: Relationship between disease duration and trabecular vBMD in patients with AS (N=44)

4.3.5 Correlation between disease duration and HRpQCT parameters (AS patients alone, n=44)

Significant inverse correlation was noted to exist between disease duration and cortical parameters such as cortical thickness and porosity at both radius and tibia (Table 14). Disease duration and cortical thickness showed a moderate negative correlation (r = -.452, p = .002 at the radius and r = -.563, p = .000 at the tibia). Likewise, disease duration had a moderate positive association with cortical porosity (r = .452, p = .002 at the radius and r = .400, p = .007 at the tibia). These results suggest that patients with long standing disease had abnormal cortical porosity and thinner cortices. An inverse correlation was present between cortical vBMD of the radius and disease duration (r = -.404, p = .007). There were no associations between total and trabecular volumetric BMD and disease duration. Similarly, no significant correlation
existed between disease duration and stiffness or stress at radius and tibia.

Table 14: Correlation between disease duration and HRpQCT parameters (AS patients, n=44)

<table>
<thead>
<tr>
<th></th>
<th>Radius</th>
<th>Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease duration</td>
<td>Disease duration</td>
</tr>
<tr>
<td></td>
<td>r   p</td>
<td>r   p</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>-.080 .604</td>
<td>-.119 .442</td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>.291 &lt;. 05</td>
<td>-.069 .657</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>-.404 &lt;. 01</td>
<td>-.182 .232</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.292 &lt;. 005</td>
<td>.070 .652</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>.447 &lt;. 01</td>
<td>.350 &lt;. 05</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.073 .640</td>
<td>-.365 &lt;. 05</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>-.371 &lt;. 05</td>
<td>-.332 &lt;. 05</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>-.452 &lt;. 005</td>
<td>-.424 &lt;. 005</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.563 &lt;. 001</td>
<td>.400 &lt;. 01</td>
</tr>
<tr>
<td>Stiffness, K (kN/mm)</td>
<td>-.103 .290</td>
<td>-.200 .194</td>
</tr>
<tr>
<td>Stress</td>
<td>-.163 .291</td>
<td>-.199 .196</td>
</tr>
</tbody>
</table>

4.3.6 Correlation between ESR, CRP and HRpQCT parameters

Significant negative correlations were observed between ESR and volumetric BMD. Specifically serum ESR values showed a negative correlation with cortical vBMD at the radius, trabecular vBMD at the tibia, and total vBMD at radius and tibia (Data shown in Table 16). Statistically significant inverse correlation was also noted to exist between FEA parameters such as bone stiffness and stress and serum ESR values suggesting that inflammation is the most likely cause of bone loss. Cortical thickness at tibia also correlated negatively with ESR. High ESR levels in serum were also correlated positively to cortical porosity at the tibia. Conversely, serum CRP did not correlate with most of the HRpQCT parameters (Table 15). However, a significant inverse correlation was noted between serum CRP and bone stiffness and stress at the tibia.
Table 15: Spearman’s rank correlation between ESR, CRP and HRpQCT parameters

<table>
<thead>
<tr>
<th></th>
<th>Trabecular vBMD</th>
<th>Cortical vBMD</th>
<th>Total vBMD</th>
<th>BV/TV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trabecular Number</td>
<td>Trabecular Thickness</td>
<td>Trabecular separation</td>
<td>Cortical thickness</td>
</tr>
<tr>
<td>ESR</td>
<td>-229</td>
<td>-0.401*</td>
<td>-0.146</td>
<td>-0.234</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.052</td>
<td>-0.187</td>
<td>-0.165</td>
<td>-0.188</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>-0.114</td>
<td>-0.147</td>
<td>-0.097</td>
<td>-0.276</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0.085</td>
<td>0.207</td>
<td>-0.315*</td>
<td>-0.315*</td>
</tr>
<tr>
<td>Stress</td>
<td>0.183</td>
<td>0.229</td>
<td>-0.209</td>
<td>-0.191</td>
</tr>
</tbody>
</table>

**p<0.01, *p<0.05

4.4 BMD outcomes in patients with AS

4.4.1 Areal BMD outcomes

Data on areal BMD obtained by DXA is shown in Table 16.

Table 16: Areal BMD of study subjects with AS

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>25 (57)</td>
<td>19 (43)</td>
</tr>
<tr>
<td>Lumbar spine (L1-L4)</td>
<td>1.001±.191</td>
<td>1.050±.206</td>
<td>.936±.151*</td>
</tr>
<tr>
<td>Total hip</td>
<td>.908±.150</td>
<td>.956±.133</td>
<td>.844±.150*</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>.770±.149</td>
<td>.811±.153</td>
<td>.717±.129*</td>
</tr>
<tr>
<td>Distal 1/3 radius</td>
<td>.754±.080</td>
<td>.799±.064</td>
<td>.691±.053*</td>
</tr>
<tr>
<td>Ultradistal radius</td>
<td>.476±.082</td>
<td>.509±.085</td>
<td>.429±.050*</td>
</tr>
</tbody>
</table>

*p<0.05, men vs. women.
Almost half of the patients (n=20/44, 45%) had bone loss in the form of osteoporosis or low bone density. Four patients (9%) had osteoporosis as defined by DXA. Three out of the four patients with osteoporosis were postmenopausal women. Only one out of 25 males had osteoporosis (4%). Low bone density (defined as Z score less than two standard deviations) was present in 16 patients (36%). Women had higher prevalence of osteoporosis and low bone density (16% vs. 4% and 32% vs. 42% respectively) than men. Bone loss was more evident in women who were postmenopausal (n=8). Six of the eight postmenopausal women had either osteoporosis (n=3) or osteopenia (n=3). Not surprisingly, women had lower areal BMD at lumbar spine, total hip, femoral neck and distal radius, when compared to men.

### 4.4.2 Comparison of areal BMD between patients with normal and elevated mSASSS

Though AS patients with elevated mSASSS (n=24) were found to have abnormal bone microarchitecture when compared to those with normal mSASSS (n=20), no significant differences were noted to exist in areal BMD amongst these two groups (Figure 7).

Figure 7: Comparison of areal BMD at various sites (Elevated mSASSS vs. normal mSASSS)
4.4.3 Multivariable linear regression analysis to assess if AS is an independent predictor of abnormal areal BMD

On multivariable regression analysis, when compared to non-AS subjects (n=85), patients with AS (n=44) had lower areal BMD at the total hip and femoral neck regions, even after adjusting for differences in age and gender. Data are shown in Table 17. However, there were no differences in BMD between AS patients and non-AS subjects at lumbar spine and distal radius. In the multivariable regression model that was adjusted for mSASSS, disease duration and BASDAI, only total hip BMD was found to be lower in AS patients than non-AS subjects.

Table 17: Multivariable linear regression analysis to assess if AS is an independent predictor of abnormal BMD. AS patients (n=44) vs. non-AS subjects (n=85).

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Multivariable linear regression (adjusted for age, and gender)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta*</td>
</tr>
<tr>
<td>L1-L4 spine BMD</td>
<td>-.02</td>
</tr>
<tr>
<td>Total hip BMD</td>
<td>-.11</td>
</tr>
<tr>
<td>Femoral neck BMD</td>
<td>-.09</td>
</tr>
<tr>
<td>Distal 1/3 Radius BMD</td>
<td>.01</td>
</tr>
<tr>
<td>Ultra distal Radius BMD</td>
<td>-.02</td>
</tr>
</tbody>
</table>

* Beta represents the difference between patients with AS and subjects without AS, and subjects without AS are the reference category

4.4.4 Correlation between HRpQCT parameters and areal BMD

Statistically significant correlations were observed between volumetric BMD and areal BMD measured by DXA at the lumbar spine and hip (Table 18). The strongest correlation was noted between trabecular vBMD at the radius and areal BMD at the ultra distal radius (r=.866, p<.001). Moderate correlations existed between total hip areal BMD and trabecular vBMD, BV/TV and trabecular parameters. The correlation between cortical parameters and areal BMD were weak and significant associations existed only for select parameters.
Table 18: Correlations between areal BMD and various HRpQCT parameters

<table>
<thead>
<tr>
<th></th>
<th>Radius</th>
<th></th>
<th></th>
<th></th>
<th>Tibia</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-spine</td>
<td>Total hip</td>
<td>Fem. neck</td>
<td>Distal 1/3rd Radius</td>
<td>UD Radius</td>
<td>L-spine</td>
<td>Total hip</td>
<td>Fem. neck</td>
<td>Distal 1/3rd Radius</td>
</tr>
<tr>
<td>Tb. vBMD</td>
<td>.618**</td>
<td>.684**</td>
<td>.619**</td>
<td>.658**</td>
<td>.866**</td>
<td>.485**</td>
<td>.645**</td>
<td>.636**</td>
<td>.646**</td>
</tr>
<tr>
<td>Cort. vBMD</td>
<td>-.101</td>
<td>.114</td>
<td>.197</td>
<td>-.216</td>
<td>-.033</td>
<td>.147</td>
<td>.358*</td>
<td>.353*</td>
<td>-.106</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>.314*</td>
<td>.487**</td>
<td>.529**</td>
<td>.230</td>
<td>.561**</td>
<td>.333*</td>
<td>.574**</td>
<td>.606**</td>
<td>.243</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.617**</td>
<td>.683**</td>
<td>.618**</td>
<td>.656**</td>
<td>.865**</td>
<td>.486**</td>
<td>.645**</td>
<td>.636**</td>
<td>.647**</td>
</tr>
<tr>
<td>Tb. N</td>
<td>.556**</td>
<td>.613**</td>
<td>.464**</td>
<td>.495**</td>
<td>.590**</td>
<td>.580**</td>
<td>.682**</td>
<td>.498**</td>
<td>.538**</td>
</tr>
<tr>
<td>Tb. Th</td>
<td>.484**</td>
<td>.578**</td>
<td>.577**</td>
<td>.514**</td>
<td>.751**</td>
<td>-.085</td>
<td>-.026</td>
<td>.204</td>
<td>.338*</td>
</tr>
<tr>
<td>Tb-Sep</td>
<td>-.516**</td>
<td>-.630**</td>
<td>-.487**</td>
<td>-.507**</td>
<td>-.593**</td>
<td>-.530**</td>
<td>-.670**</td>
<td>-.509**</td>
<td>-.556**</td>
</tr>
<tr>
<td>Cort.th</td>
<td>-.127</td>
<td>.134</td>
<td>.262</td>
<td>-.163</td>
<td>-.009</td>
<td>-.130</td>
<td>.071</td>
<td>.150</td>
<td>-.338*</td>
</tr>
<tr>
<td>Cort. pt</td>
<td>.290</td>
<td>.028</td>
<td>.007</td>
<td>.280</td>
<td>.294</td>
<td>.142</td>
<td>-.161</td>
<td>-.184</td>
<td>.051</td>
</tr>
<tr>
<td>Stiffness</td>
<td>.289</td>
<td>.487**</td>
<td>.568**</td>
<td>.296</td>
<td>.588**</td>
<td>.232</td>
<td>.487**</td>
<td>.580**</td>
<td>.202</td>
</tr>
<tr>
<td>Stress</td>
<td>.289</td>
<td>.487**</td>
<td>.569**</td>
<td>.296</td>
<td>.588**</td>
<td>.233</td>
<td>.487**</td>
<td>.580**</td>
<td>.202</td>
</tr>
</tbody>
</table>


4.5 HRpQCT parameters in AS patients and non-AS subjects

4.5.1 Results of HRpQCT derived parameters of patients with AS and non-AS subjects

Data regarding the various parameters obtained from HRpQCT are shown in Table 19.
Table 19: Data on the HRpQCT parameters at radius and tibia in patients with AS and non AS subjects

<table>
<thead>
<tr>
<th></th>
<th>AS patients, n=44</th>
<th>Non AS subjects, n=85</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>161.71 ± 47.50</td>
<td>159.47 ± 40.96</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>307.91 ± 63.21</td>
<td>315.89 ± 66.01</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>841.73 ± 57.48</td>
<td>839.73 ± 64.17</td>
</tr>
<tr>
<td>Trabecular number (per mm³)</td>
<td>1.97 ± .37</td>
<td>2.03 ± .33</td>
</tr>
<tr>
<td>Trabecular thickness (mm)</td>
<td>.067 ± .013</td>
<td>.065 ± .009</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>.472 ± .199</td>
<td>.442 ± .098</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>.135 ± .040</td>
<td>.133 ± .034</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>.761 ± .155</td>
<td>.741 ± .144</td>
</tr>
<tr>
<td>Cortical porosity (%)</td>
<td>.018 (.011-.024)</td>
<td>.022 (.017-.032)</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>1.33 ± .37</td>
<td>1.43 ± .32</td>
</tr>
<tr>
<td>Stress</td>
<td>26.8 ± 9.3</td>
<td>29.2 ± 8.0</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>159.13 ± 44.93</td>
<td>165.90 ± 40.35</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>295.29 ± 59.67</td>
<td>291.96 ± 49.41</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>852.29 ± 70.98</td>
<td>831.42 ± 50.39</td>
</tr>
<tr>
<td>Trabecular number (per mm³)</td>
<td>1.873 ± .427</td>
<td>1.9031 ± .349</td>
</tr>
<tr>
<td>Trabecular thickness (mm)</td>
<td>.072 ± .014</td>
<td>.072 ± .012</td>
</tr>
<tr>
<td>Trabecular separation (mm)</td>
<td>.510 ± .242</td>
<td>.473 ± .120</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>.133 ± .038</td>
<td>.138 ± .033</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>.742 ± .185</td>
<td>.665 ± .119</td>
</tr>
<tr>
<td>Cortical porosity (%)</td>
<td>.049 (.035-.072)</td>
<td>.065 (.050-.083)</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>1.53 ± .37</td>
<td>1.48 ± .40</td>
</tr>
<tr>
<td>Stress</td>
<td>31.6 ± 9.1</td>
<td>30.6 ± 9.8</td>
</tr>
</tbody>
</table>

Data expressed as mean ±SD or median (IQ).
4.5.2 Relationship between age and various HRpQCT parameters (AS patients vs. non-AS)

A greater age related decline in volumetric BMD was seen in AS patients than non-AS subjects. The declining trend was observed both at radius and tibia (Figure 8-10). The changes in cortical thickness, porosity and BV/TV with age were also exaggerated in AS patients than non-AS subjects (Figure 9).

Figure 8: The relationship between age and volumetric BMD (AS patients vs. non-AS subjects)

Top row (radius) and bottom row (tibia)
Figure 9: The relationship between age and cortical thickness, porosity and BV/TV (AS patients vs. non-AS subjects)

<table>
<thead>
<tr>
<th>Cortical thickness</th>
<th>Cortical porosity</th>
<th>BV/TV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Diagram showing scatter plots for age vs. cortical thickness, cortical porosity, and BV/TV for controls and cases.]
Figure 10: The relationship between age and trabecular parameters and stiffness (AS patients vs. non-AS subjects)

4.6 Regression analysis

4.6.1 Multivariable regression analysis on the effect of AS on trabecular vBMD at the radius

Regression analysis was done to assess whether AS is an independent predictor of low vBMD and abnormal bone strength. Data obtained from AS patients (n=44) and non-AS subjects (n=85) were used for the regression analysis (Table 21).
Table 20: Multivariable regression analysis on the effect of AS on trabecular vBMD at the radius (44 AS patients, 85 non-AS subjects)

<table>
<thead>
<tr>
<th>Model</th>
<th>Trabecular vBMD Radius</th>
<th>Beta coefficient</th>
<th>95% CI (Lower)</th>
<th>95% CI (Upper)</th>
<th>p</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>AS patients</td>
<td>-21.02</td>
<td>-40.25</td>
<td>-1.78</td>
<td>&lt;.05</td>
<td>.187</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-.73</td>
<td>-1.46</td>
<td>0.00</td>
<td>&lt;.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender*</td>
<td>39.15</td>
<td>24.77</td>
<td>53.53</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>AS patients</td>
<td>-61.38</td>
<td>-94.68</td>
<td>-28.08</td>
<td>&lt;.005</td>
<td>.231</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.34</td>
<td>-2.16</td>
<td>-.52</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender*</td>
<td>34.74</td>
<td>20.45</td>
<td>49.04</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.54</td>
<td>.49</td>
<td>2.59</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>AS patients</td>
<td>-62.06</td>
<td>-112.98</td>
<td>-11.13</td>
<td>&lt;.05</td>
<td>.225</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.34</td>
<td>-2.16</td>
<td>-.51</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender*</td>
<td>34.80</td>
<td>20.13</td>
<td>49.45</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.54</td>
<td>.47</td>
<td>2.60</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BASDAI</td>
<td>.12</td>
<td>-6.55</td>
<td>6.79</td>
<td>.972</td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td>AS patients</td>
<td>-54.60</td>
<td>-106.25</td>
<td>-2.93</td>
<td>&lt;.05</td>
<td>.231</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.23</td>
<td>-2.06</td>
<td>-.38</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender*</td>
<td>37.24</td>
<td>22.28</td>
<td>52.18</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.73</td>
<td>.643</td>
<td>2.82</td>
<td>&lt;.005</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BASDAI</td>
<td>.16</td>
<td>-6.48</td>
<td>6.79</td>
<td>.963</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated mSASSS</td>
<td>-18.93</td>
<td>-44.28</td>
<td>6.41</td>
<td>.142</td>
<td></td>
</tr>
</tbody>
</table>

*Women as reference category

The results of the multivariable regression analysis suggest that patients with AS had lower trabecular vBMD at the radius than non-AS subjects. The negative effect on trabecular vBMD at radius persisted after adjusting for age and gender (beta=-21.02, p=.032). BASDAI was not a significant predictor of trabecular vBMD at the radius in patients with AS (beta coefficient=0.155, p=0.963) after adjusting for duration and mSASSS. Likewise, elevated


mSASSS was found to have no effect on trabecular vBMD of radius (beta=-18.93, p=.142). Advancing age was noted to have a negative effect on trabecular vBMD in the various regression models. Gender was shown to influence trabecular vBMD. Men were found to have significantly higher trabecular vBMD at radius than women (beta=37.24, p=0.000). A significant positive relationship between disease duration and trabecular vBMD at radius was also noted to be present after adjusting for BASDAI and mSASSS.

The residual plots for the multivariable regression models showed random distributions. The normality plots were also created.

**Interactions with age and gender**

Data shown on Table 21 shows that there was no interaction by either age or gender on the effect of AS on volumetric BMD at the radius.

Table 21: Multivariable linear regression analysis; volumetric BMD at the radius (44 AS patients and 85 non-AS subjects). Checking for interaction with age and gender.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Trabecular vBMD radius</th>
<th>Cortical vBMD Radius</th>
<th>Total vBMD Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS patients vs. non-AS subjects</td>
<td>Beta**</td>
<td>p</td>
<td>Beta**</td>
</tr>
<tr>
<td>-61</td>
<td>.172</td>
<td>-.34</td>
<td>.461</td>
</tr>
<tr>
<td>Age</td>
<td>-.50</td>
<td>.149</td>
<td>-.56</td>
</tr>
<tr>
<td>Men vs. women</td>
<td>.44</td>
<td>&lt;.001</td>
<td>-.14</td>
</tr>
<tr>
<td>Age* (AS patients vs. non-AS subjects)</td>
<td>.30</td>
<td>.380</td>
<td>.04</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS patients vs. non-AS subjects</td>
<td>-.62</td>
<td>&lt;.05</td>
<td>.12</td>
</tr>
<tr>
<td>Age</td>
<td>.08</td>
<td>.756</td>
<td>.24</td>
</tr>
<tr>
<td>Men vs. women</td>
<td>-.23</td>
<td>&lt;.05</td>
<td>-.51</td>
</tr>
<tr>
<td>Gender* (AS patients vs. non-AS subjects)</td>
<td>.61</td>
<td>.121</td>
<td>-.63</td>
</tr>
</tbody>
</table>

*Interaction term. **β represents the difference between patients with AS and subjects without AS, and subjects without AS are the reference category*
4.6.2 Sub group analysis (multivariable regression)

Most people achieve their peak bone mass between 20-30 years. In this study eight patients with AS were aged less than 30. Similarly, non-AS subjects in the study had a different age range than that of AS patients (40-70 years vs. 21-64 years). Hence another regression analysis was done combining AS patients (n=27) and non-AS subjects (n=47) in the age range of 40-64. The results of this analysis were similar to that of the first analysis. Data are shown in Table 22.

Table 22: Multivariable regression analysis on the effect of AS on trabecular vBMD at the radius including AS patients and non-AS subjects in the age range of 40-64 (27 AS patients and 47 non-AS subjects)

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>Beta coefficient*</th>
<th>95% CI (Lower)</th>
<th>95% CI (Upper)</th>
<th>p</th>
<th>Adjus ted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>AS patients</td>
<td>-20.02</td>
<td>-39.27</td>
<td>-76</td>
<td>&lt;. 05</td>
<td>.239</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.12</td>
<td>-2.30</td>
<td>.05</td>
<td></td>
<td>.061</td>
</tr>
<tr>
<td></td>
<td>Men vs. Women</td>
<td>41.36</td>
<td>23.15</td>
<td>59.58</td>
<td>&lt;. 001</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>AS patients</td>
<td>-56.95</td>
<td>-95.42</td>
<td>-18.48</td>
<td>&lt;. 005</td>
<td>.278</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.60</td>
<td>-2.83</td>
<td>-.37</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men vs. Women</td>
<td>37.50</td>
<td>19.41</td>
<td>55.58</td>
<td>&lt;. 001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.42</td>
<td>.12</td>
<td>2.72</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>AS patients</td>
<td>-76.28</td>
<td>-143.93</td>
<td>-8.63</td>
<td>&lt;. 05</td>
<td>.273</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.55</td>
<td>-2.79</td>
<td>-3.1</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men vs. Women</td>
<td>38.62</td>
<td>20.18</td>
<td>57.06</td>
<td>&lt;. 001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.46</td>
<td>.15</td>
<td>2.77</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BASDAI</td>
<td>2.98</td>
<td>-5.58</td>
<td>11.55</td>
<td>.490</td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td>AS patients</td>
<td>-65.75</td>
<td>-135.16</td>
<td>3.64</td>
<td>.063</td>
<td>.279</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-1.52</td>
<td>-2.76</td>
<td>-2.8</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men vs. Women</td>
<td>42.10</td>
<td>22.93</td>
<td>61.28</td>
<td>&lt;. 001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>1.60</td>
<td>.28</td>
<td>2.92</td>
<td>&lt;. 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BASDAI</td>
<td>2.90</td>
<td>-5.63</td>
<td>11.43</td>
<td>.500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elevated mSASSS</td>
<td>-20.58</td>
<td>-53.12</td>
<td>11.96</td>
<td>.211</td>
<td></td>
</tr>
</tbody>
</table>

* β represents the difference between patients with AS and subjects without AS, and subjects without AS are the reference category
4.6.3 Regression analysis to study the effect of AS on various HRpQCT parameters at the radius and tibia

Separate regression modeling was done to study the effect of AS on various HRpQCT parameters at the radius and tibia (Tables 23 and 24). Adjustments for differences in age and gender between AS patients and non-AS subjects were accounted for in the regression models. Results of this multivariable linear regression analysis suggested that patients with AS had abnormal volumetric BMD both at trabecular and cortical components of both radius and tibia. Trabecular separation was greater in AS patients than non-AS subjects at the radius. There was a trend towards trabecular separation being lower at the tibia as well. BV/TV was lower in AS patients than non-AS subjects at both tibia and radius. Similarly, AS patients had thinner cortices and increased cortical porosity at the tibia. At the tibia, cortical porosity was more in AS patients than non-AS subjects. FEA parameters such as stiffness and stress tended to be abnormal at the radius in AS patients when compared to non-AS subjects.

Table 23: Multivariable linear regression analysis to assess if AS is an independent predictor of abnormal bone microarchitecture at the radius. The model was adjusted for differences in age and gender. AS patients (n=44) vs. non-AS subjects (n=85).

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>β*</th>
<th>95% CI</th>
<th>p</th>
<th>Adjusted R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular vBMD</td>
<td>-21.0</td>
<td>-40.3 - -1.7</td>
<td>.032</td>
<td>.193</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>-38.1</td>
<td>-66.3 - -10.0</td>
<td>.008</td>
<td>.177</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>-47.9</td>
<td>-78.7 - -17.1</td>
<td>.003</td>
<td>.087</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>-.150</td>
<td>-.310 - -.013</td>
<td>.071</td>
<td>.109</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>-.004</td>
<td>-.009 - .000</td>
<td>.068</td>
<td>.219</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.072</td>
<td>.005 - .138</td>
<td>.035</td>
<td>.088</td>
</tr>
<tr>
<td>BV/TV</td>
<td>-.017</td>
<td>-.033 - -.001</td>
<td>.033</td>
<td>.192</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>-.101</td>
<td>-.167 - -.036</td>
<td>.003</td>
<td>.193</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.005</td>
<td>.001 - .010</td>
<td>.029</td>
<td>.346</td>
</tr>
<tr>
<td>Stiffness</td>
<td>-.241</td>
<td>-.406 - -.077</td>
<td>.004</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Stress</td>
<td>-6.03</td>
<td>-10.1 - -1.9</td>
<td>.004</td>
<td>.073</td>
</tr>
</tbody>
</table>

• β represents the difference between patients with AS and subjects without AS, and subjects without AS are the reference category
Table 2: Multivariable linear regression analysis to assess if AS is an independent predictor of abnormal bone microarchitecture at the tibia. The model was adjusted for differences in age and gender. AS patients (n=44) vs. non-AS subjects (n=85).

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>B *</th>
<th>95% CI</th>
<th>p</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular vBMD</td>
<td>-20.4</td>
<td>-40.3- -.589</td>
<td>&lt;. 05</td>
<td>.092</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>-30.1</td>
<td>-55.1- -5.03</td>
<td>&lt;. 05</td>
<td>.091</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>-30.4</td>
<td>-55.8- 4.99</td>
<td>&lt;. 05</td>
<td>.241</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>-.121</td>
<td>-0.3-.057</td>
<td>.182</td>
<td>.084</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>-.003</td>
<td>-.010-.003</td>
<td>.313</td>
<td>&lt;. 001</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.077</td>
<td>-.005-.159</td>
<td>.067</td>
<td>.159</td>
</tr>
<tr>
<td>BV/TV</td>
<td>-.017</td>
<td>-.034-.001</td>
<td>&lt;. 05</td>
<td>.092</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>-.055</td>
<td>-.114-.004</td>
<td>.065</td>
<td>.370</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.013</td>
<td>.002-.024</td>
<td>&lt;. 05</td>
<td>.296</td>
</tr>
<tr>
<td>Stiffness</td>
<td>-.170</td>
<td>-.356-.016</td>
<td>.073</td>
<td>.068</td>
</tr>
<tr>
<td>Stress</td>
<td>-4.24</td>
<td>-8.8-.33</td>
<td>.068</td>
<td>.068</td>
</tr>
</tbody>
</table>

* β represents the difference between patients with AS and subjects without AS, and subjects without AS are the reference category

4.7 Comparison of HRpQCT parameters and BMD between age and gender matched AS patients and non-AS subjects

4.7.1 Sub group analysis for the comparison of volumetric bone mineral density (vBMD), bone microarchitecture (HRpQCT) and strength (FEA) in AS patients and age and gender matched non-AS subjects

In the total cohort, the AS patients were younger than non-AS subjects and the gender distribution was also different between AS patients and non-AS subjects. Hence a sub-set of age and gender matched AS patients and non-AS subjects was created for inter group comparison of HRpQCT parameters. This sub-set of subjects consisted of 24 AS patients and
24 non-AS subjects and both groups of subjects were aged 40 or above. The mean age of cases was 51.5±8.3 years and the disease duration was 21.9±12.3 years. Mean BASDAI was 6.5±1.8. The median BMI was 26.2 (IQ: 23.8-28.1). Both AS patients and non-AS subjects had similar BMI.

The subgroup of AS patients (n=24) that were included in the regression analysis was significantly older than the unmatched AS patients (n=20) in age (51.5±8.3 vs. 35.3±8.6 years, p=0.000). BMI was similar to that of the unmatched AS patients (26.2 (23.8-28.1) vs. 26.7(25.1-30.3), p=0.625). BASDAI was comparable (6.2 ±1.8 vs. 6.2 ± 1.7, p=0.978) as well. Likewise the ESR and CRP levels in serum were also similar to that of the unmatched AS patients. The proportion of patients in both groups who had iritis, psoriasis and inflammatory bowel disease were similar. Thus, the sub group of AS patients did not differ from the total cohort of cases in terms of gender distribution, duration, BASDAI, mSASSS, BMI, comorbid illnesses, ESR or CRP.

Patients with AS had lower volumetric BMD at the radius compared to non-AS subjects. Data are shown in Table 25. Patients with AS had significantly lower total and cortical vBMD at the radius. The mean difference in trabecular vBMD at the radius between the two groups of subjects was 20.96± 59.9 but the difference did not reach statistical significance.

Trabecular, cortical and total vBMD at tibia were lower in AS patients than non-AS subjects. However, the difference reached statistical significance only for trabecular vBMD. Trabecular thickness at radius was lower in AS patients than non-AS subjects (Table 20). AS patients had thinner cortices than non-AS patients at the radius. Trabecular separation at tibia was greater in AS patients than non-AS subjects. Cortical porosity at radius and tibia was not different between AS patients and non-AS subjects. Bone stiffness and stress at the radius were significantly lower in AS patients than non-AS subjects.
Table 2: Comparison of HRpQCT parameters between age and gender matched AS patients and non-AS subjects

<table>
<thead>
<tr>
<th></th>
<th>AS patients (n=24)</th>
<th>Non-AS subjects (n=24)</th>
<th>Paired difference (Mean±SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>151.14±46.15</td>
<td>172.09±41.23</td>
<td>20.9±59.9</td>
<td>.100</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>282.05±54.93</td>
<td>337.54±50.49</td>
<td>-55.5±81.4</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>818.65±50.87</td>
<td>864.90±50.11</td>
<td>-46.3±59.4</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>1.95±.424</td>
<td>2.076±.315</td>
<td>-.131±.548</td>
<td>.254</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.060±.012</td>
<td>.069±.0102</td>
<td>-.006±.01</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.429 (.382-.481)</td>
<td>.396 (.364-.472)</td>
<td></td>
<td>.407</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.126±.038</td>
<td>.144±.033</td>
<td>-.018±.049</td>
<td>.076</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>.690±.135</td>
<td>.805±.127</td>
<td>-.114±.160</td>
<td>&lt; .005</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.021 (.016-.025)</td>
<td>.018 (.013-.023)</td>
<td></td>
<td>.602</td>
</tr>
<tr>
<td>Stress</td>
<td>1.17±.36</td>
<td>1.44±.34</td>
<td>-.276±.474</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular vBMD</td>
<td>149.89±38.78</td>
<td>170.97±37.06</td>
<td>-21.3±49.6</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Total vBMD</td>
<td>276.04±48.25</td>
<td>310.67±40.94</td>
<td>-21.7±93.8</td>
<td>.269</td>
</tr>
<tr>
<td>Cortical vBMD</td>
<td>830.51±78.09</td>
<td>858.34±45.35</td>
<td>-30.4±86.8</td>
<td>.107</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>1.85±.465</td>
<td>1.981 (.408)</td>
<td>-.127±.525</td>
<td>.257</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>.069±.0131</td>
<td>.073±.014</td>
<td>-.004±.019</td>
<td>.322</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>.45 (.39-.56)</td>
<td>.43 (.35-.51)</td>
<td></td>
<td>&lt; .005</td>
</tr>
<tr>
<td>BV/TV</td>
<td>.125±.032</td>
<td>.143 (.031)</td>
<td>-.018±.041</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>.675±.118</td>
<td>.732±.139</td>
<td>-.057±.188</td>
<td>.359</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>.065 (.041-.092)</td>
<td>.055 (.045-.065)</td>
<td></td>
<td>.627</td>
</tr>
<tr>
<td>Stress</td>
<td>1.41±.51</td>
<td>1.42±.32</td>
<td>-.137±.517</td>
<td>.206</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>28.6±12.9</td>
<td>28.8±8.1</td>
<td>3.71±12.05</td>
<td>.145</td>
</tr>
</tbody>
</table>

Data expressed as mean ±SD or median (IQ). Comparisons made by paired t tests or Wilcoxon signed rank tests.
4.7.2 Sub group analysis of areal BMD in age and gender matched AS patients and non-AS subjects

BMD at the total hip and femoral neck was lower in AS subjects than non-AS subjects (Figure 9). BMD measured by DXA at the lumbar spine and distal forearm were not different between the two groups (.989 ± .205 gm./cm² vs. 1.045± .169 gm./cm², p=.086 at the spine, )

Figure 11: Comparison of areal BMD between age and gender matched AS patients (n=24) and non-AS subjects (n=24).

![Comparison of areal BMD between age and gender matched AS patients (n=24) and non-AS subjects (n=24).](image)

X-axis represents areal bone mineral density expressed as gm./cm²
*p value <.001  AS patients vs. non AS subjects (Paired difference (Mean± SD): -.272± .384 and -.362± .162 for total hip and femoral neck respectively)
CHAPTER 5

DISCUSSION
5.1 Summary and relevance of the results

This study confirms the hypothesis that patients with AS have lower trabecular volumetric BMD than healthy controls. However, greater disease severity, as measured by BASDAI, was not predictive of compromised bone strength.

This is one of the first studies to have assessed both bone strength and microarchitecture in patients with AS. This study has not only identified information on the HRpQCT outcomes, but has also provided important insights, not explored previously, into the bone strength by finite element analysis of the HRpQCT images. Both trabecular and cortical compartments were affected suggesting that patients with AS have high risk for fractures in the axial and appendicular skeleton. The results suggest that patients with AS have lower volumetric BMD, abnormal bone microarchitecture and poor bone strength. These alterations were more distinct than those revealed by DXA based measurements of areal BMD. Patients with AS had lower volumetric BMD compared to non-AS subjects. For instance, total volumetric BMD at radius and tibia as well as cortical volumetric BMD at the radius were decreased. Cortical and trabecular volumetric BMD at tibia also showed a trend towards being significantly less in patients than in non-AS subjects. Another important finding of this study is that patients with AS had abnormal cortical porosity when compared to non-AS subjects. BV/TV and trabecular parameters such as trabecular separation were also affected negatively. FEA parameters such as bone stiffness and stress at radius were found be lower in patients than non-AS subjects. The results also suggest that BASDAI, an important indicator of disease-activity of AS, is not a significant indicator of bone loss. AS patients with elevated mSASSS had worse bone microarchitecture than those with normal mSASSS suggesting that radiological progression and osteoporosis occurs in parallel. More importantly, when AS patients were compared with age and gender matched non-AS subjects, areal BMD measured by DXA were similar at the lumbar spine and distal radius and was different only at the femoral neck and total hip regions. Thus HRpQCT has uncovered bone structural abnormalities that were not evident on DXA.

These results are important given that abnormalities of HRpQCT and FEA based measures of bone quality have been related to fracture risk independent of areal BMD (Sornay-Rendu 2007). Particularly, low volumetric BMD and architectural deterioration of trabecular and
cortical bone at the radius and tibia are related to vertebral fractures independent of areal BMD of the lumbar spine (Sornay-Rendu 2009, Rudäng 2013, Chevalley 2013). In addition, HRpQCT based imaging has been shown to have the ability to better discriminate between patients with or without fractures or document abnormal bone microarchitecture in conditions such as chronic renal failure, SLE, rheumatoid arthritis, hemodialysis, Klinefelter’s syndrome, Turner’s syndrome, primary hyperparathyroidism, obesity, thalassemia and type 2 diabetes (Cejka 2011, Patsch 2013, Kocijan 2014, Tang 2013, Shanbhogue 2014, Hansen 2012, Stein 2013). For instance, primary hyperparathyroidism is widely thought to cause cortical bone abnormalities. However, an HRpQCT based study conducted in postmenopausal women (51 patients and 120 controls), showed that primary hyperparathyroidism is also associated with trabecular abnormalities such as trabecular separation and trabecular stiffness (Stein 2013). Similarly, Tang and colleagues reported that cortical vBMD at the radius could be used to differentiate lupus patients on long-term steroid treatment with and without vertebral fractures (Tang 2013). It is widely considered that bone loss in SLE is mediated mostly by glucocorticoids despite their potential to negate the inflammatory bone loss. However, the study by Tang et al. demonstrated that even SLE patients who were steroid naïve had lower vBMD at the radius, decreased cortical thickness ad compromised bone stiffness and failure load (Tang 2013). HRpQCT based analysis has also helped to uncover bone structure abnormalities that might explain fracture risk in patients with type 2 diabetes (Patsch 2013). This is particularly important in patients with diabetes as they typically have normal or higher areal BMD (Vestergaard 2007). The cross sectional study by Patsch and colleagues demonstrated that fragility fractures in patients with type-2 diabetes were explained by significantly greater cortical porosity (Patsch 2013).

5.2 Findings in the context other studies on bone microarchitecture in AS

To date, only one study has analyzed bone microarchitecture in patients with AS. This was a cross sectional study conducted in Sweden by Klingberg and colleagues (Klingberg 2013). The results of the current study confirm the findings of this recent study including 61 patients with AS. The cortical vBMD at radius (850±55 vs. 875±41 mg/cm3 in non-AS subjects; p=0.004) and trabecular vBMD at tibia (187±35 vs. 201±41 mg/cm3 in non-AS subjects; p=0.033) were reduced in patients with AS. It is worth mentioning that the study by Klingberg and colleagues had some differences and limitations when compared to that of the current study. Firstly only
male subjects were included and hence the results of the study lack generalizability. Secondly the cases and controls were not matched for ethnicity or geographic location. The data on controls were obtained from USA, though the study cohort belonged to Sweden, thus introducing bias from various unmeasured confounding variables. The study cohort had different mSASSS scores (median (IQ): 8 (0-72) vs. 2 (0-14)) when compared to that of the present study. BASDAI was also lower (3.1+ 2.0 vs. 6.6+ 1.7 in the current study). Next, the authors used syndesmophytes and not mSASSS as a measure of radiological involvement even though mSASSS is a composite measure and a more reliable indicator of radiological severity.

AS patients had lower body weight (84+14 vs. 89+16 kg, p=0.052 in controls) when compared to controls and the difference in body weight might have influenced their results. Further, even though an attempt was made to describe the abnormalities of bone microstructure in patients who had vertebral fractures, the number of patients with fractures (n=8) was small limiting statistical power for that analysis and the reliability of their conclusions. Moreover, the type of vertebral fractures included for the analysis was of doubtful clinical significance. This is because the study had only analyzed morphometric vertebral fractures. Also most fractures were (12/14 vertebral fractures) of Genant grade 1 type and the diagnosis of these fractures can be quite subjective. Besides, grade 1 fractures are not always caused by osteoporosis and hence have limited ability to predict future vertebral fractures. Finally, a significant number of patients in this study were being treated with methotrexate, sulfasalazine or TNFi and the authors did not adjust for the use of these disease-modifying drugs. About, 22% of subjects were taking TNFi (n=15), 20% were on methotrexate (n=13) and 7% on sulfasalazine (n=5). Though the effect of methotrexate and sulfasalazine on areal BMD in AS are neutral, no clear data exists on their effect on bone microarchitecture in AS. Given the fact that TNFi likely improve BMD at the spine and maintain BMD at the hip it is possible that TNFi might have a potential beneficial effect on bone microarchitecture (Visvanathan 2009, Durnez 2013).

Conversely, in the present study, only two patients had ever received methotrexate but none of the patients had received TNFi, and hence the effect of these medications would not have affected the results. The use of steroids and bisphosphonates was also negligible in this cohort.

5.3 AS and HRpQCT parameters

5.3.1 Association between BASDAI and HRpQCT parameters
It is important to identify predictors of bone loss in order to distinguish those patients with higher fracture risk. This study is the first to assess if BASDAI is a predictor of bone microarchitecture and strength in AS. But it was found that BASDAI was not a predictor of bone microarchitecture and strength. This is in contrast to the findings observed in studies using areal BMD as their outcomes (Grazio 2012, Arends 2011). However, there are also reports of BASDAI not being different in AS patients with lower BMD (van der Weijden 2011, Taylan 2012). In a recent study by van der Weijden MA and colleagues, BASDAI scores were not significantly different between AS patients with normal (n=70, BASDAI: 4.4±2.4) or low BMD (n=60, BASDAI: 4.1±2.2) (van der Weijden 2011).

Several reasons exist as to why BASDAI did not predict bone loss. BASDAI is a measure of disease activity at any given moment and hence it may not truly reflect longstanding systemic inflammation. Second, it may be that bone microarchitecture, measured by HRpQCT, reflects the influence of the disease on bone turn over several years, while BASDAI reflects the current status of disease activity. Another limitation of BASDAI is that it a subjective measure and may not completely reflect the true disease state. BASDAI does not always reflect underlying state of inflammation as pain and fatigue can arise from other causes too. Furthermore, all patients in this study had high BASDAI and hence there was no variability in its distribution. This may have influenced the results of the regression analysis. It can be argued that use of ASDAS as a composite marker of disease activity may have yielded better predictive results (Kilic 2014). However, calculation of ASDAS needs use of ESR or CRP and in the current study, both ESR and CRP did not show significant correlations with HRpQCT parameters.

### 5.3.2 Role of mSASSS in predicting abnormal bone microarchitecture

Patients who had elevated mSASSS (defined as mSASSS greater than zero) had worse bone microarchitecture than those who had an mSASSS of zero. This suggests that bone loss occurs in parallel with radiological progression in AS. The differences in bone microarchitecture were mainly noted to affect the cortical bone. Specifically, patients with elevated mSASSS had lower cortical volumetric BMD at sites, abnormal cortical thickness and cortical porosity. Trabecular parameters did not differ between the two groups. Bone stiffness and stress also tended to be lower in patients with elevated mSASSS when compared to those who had an
mSASSS of zero. Another interesting finding was that BMD measured by DXA at the lumbar spine, total hip and femoral neck were not different between the two groups. Thus, HRpQCT has uncovered bone abnormalities that were not evident from DXA based measurements. These results also suggest that patients with AS that have elevated mSASSS are at higher risk of developing fractures. Klingberg and colleagues had observed that trabecular thickness, number, cortical thickness and BV/TV at the radius were abnormal in male AS patients with vertebral fractures compared to those without vertebral fractures (Klingberg 2013).

### 5.3.3 Association between disease duration and HRpQCT parameters

This study tried to analyze the effect of disease duration on bone microarchitecture in patients with AS. Patients with long standing disease were found to have a negative effect on cortical parameters such as cortical thickness and cortical porosity. Contrary to what was expected, there was a positive relationship between duration of disease and trabecular vBMD on the multivariable regression analysis. However, no significant association existed between disease duration and trabecular vBMD on correlation analysis. The positive relationship between disease duration and trabecular parameters are in contrast to results found in studies that have assessed the relationship between areal BMD and duration of disease. Conversely, AS patients with long standing disease, have been found to have high spinal BMD and this is likely because of the presence of syndesmophytes and abnormal new bone formation (Ulu 2013). Femoral neck and total hip BMD have been found to be low in those with early disease and hence it is likely that disease duration may not have a temporal relationship with bone microarchitecture or BMD. Even patients with disease duration of less than ten years were noted to have low BMD (van der Weijden 2012). Bone microarchitecture is very sensitive to changes in systemic health and it may be possible that patients with recent onset disease had higher levels of inflammatory markers in serum possibly causing abnormal bone microarchitecture and this effect may have partly masked the negative association between disease duration and trabecular vBMD in the whole cohort.

### 5.4 Cortical bone abnormalities in AS

Microstructural properties of cortical bone are important determinants of bone strength (Sornay-Rendu 2007, Burghardt 2010, Rudäng 2013, Ostertag 2013, Nicks 2012, Vilayphiou 2010). Results of a recent population based cross sectional study conducted in 833 young adult men between the age of 23-25 years, showed that prevalent fractures (n=292) in childhood and early adulthood were associated with lower cortical thickness in addition to abnormalities in
the trabecular bone (Rudäng 2013). Younger and older subjects (age <50 years vs. ≥ 50 years) matched for areal BMD were found to have comparable trabecular microarchitecture but different cortical microstructure at the radius suggesting that the major effect of age on bone loss that is independent of aBMD occurs at the cortical bone (Nicks 2012). In a study by Chevalley et al, though cortical thickness and cortical volumetric BMD at the distal radius were associated with fractures in young healthy women, cortical porosity did not differ between women with fracture and without fracture (El Maghraoui 1999). Older subjects had lower cortical volumetric BMD, significantly higher cortical porosity (by 91% and 56%, in women and men respectively), total cortical pore volume (77% and 61%), and mean cortical pore diameter (9% and 8%) compared with younger subjects (Nicks 2012). These results highlight the role of abnormal cortical structure and cortical porosity in determining fracture risk.

In this study, cortical thickness and cortical volumetric BMD were significantly decreased in AS patients when compared to non-AS subjects at both radius and tibia. The trabecular parameters adversely affected in patients with AS were trabecular vBMD, BV/TV and trabecular separation. Underscoring the notion that trabecular component is more vulnerable to the effect of inflammation, the trabecular microarchitecture was affected less than the cortical bone. The contribution of lower cortical volumetric BMD, cortical thinning and porosity to fracture risk in AS patients is currently unknown. It was observed that cortical porosity was higher in AS patients than controls and similar to our results, Klingberg et al. reported that cortical porosity measured by HRpQCT was deteriorated in patients with AS (Klingberg 2013). However, the authors commented that higher cortical porosity in AS was likely exaggerated by periosteal bone apposition that had resulted from secondary osteoarthritis. It is also worth mentioning that mechanical loading has not ameliorated the catabolic actions of inflammation on cortical bone, as cortical microarchitecture was negatively affected at the distal radius and tibia.

Another novel and significant finding is the fact that patients with AS had poor cortical bone structure despite their relatively young age. It is known that age related loss of trabecular bone starts early in life in both sexes but the loss of cortical bone do not begin until middle age in women (Riggs 2004, Riggs 2008). In men, although small deterioration in cortical bone begins in the young adulthood, the accelerated cortical bone loss happens only late in life (Riggs 2008). In 2008, the findings from a prospective longitudinal study demonstrated that before the
age of 50, men and women suffered lesser decline in their cortical bone (15 and 6% respectively) than trabecular bone (42 and 37% respectively). It is likely that AS exaggerates loss of cortical bone (Riggs 2008).

Several reasons likely explain loss of cortical bone in patients with AS. First and most important pathogenetic mechanism is TNF alpha induced inflammation. Second explanation is the trabecularization of cortical bone happening due to increased endocortical resorption and decreased periosteal formation (Kawalilak 2014). The role of systemic inflammation in causing cortical bone abnormalities is known, though it is widely thought that trabecular bone is more affected due to inflammation, as it is more metabolically active. The factors that regulate cortical porosity are still not well understood. A third likely cause for cortical bone-loss is abnormal vitamin D metabolism. Vitamin D deficiency is common in patients with AS and is aggravated in persons who have active disease, poor dietary intake, malabsorption, gut inflammation and inflammatory bowel disease. Low vitamin D status causes secondary hyperparathyroidism and this may have produced cortical bone abnormalities (Yajima 2007).

Forth, the causative role of abnormal vitamin K metabolism needs to be explored further. Patients with AS develop clinical and subclinical bowel inflammation and it is now well established that gut inflammation is a risk factor for causing low vitamin K status in the body. Serum undercarboxylated osteocalcin levels were significantly higher in patients with Crohn’s disease than healthy subjects (Nakajima 2011). The levels of undercarboxylated osteocalcin were correlated to high disease activity, but not with BMD (Nakajima 2011). Vitamin K is a coenzyme of γ-carboxylase for Gla proteins and hence plays a role in bone mineralization (Hamidi 2013). The undercarboxylation of osteocalcin limits its ability to bind to the bone mineral. Vitamin K may also be having an effect on carboxylation of matrix Gla protein. Still, no clear data exists on the effect of vitamin K deficiency on bone microarchitecture. Although, randomized controlled trials of vitamin K1 or K2 supplementation in Caucasians did not observe any increase in BMD at lumbar spine or hip (Hamidi 2013), a recent study from Oslo observed that vitamin K1 and vitamin D had independent and synergistic links with the risk of hip fracture and this further strengthens the hypothesis that vitamin K may have a role in mediating bone microarchitecture (Torbergsen 2014). Treatment with oral vitamin K (2) for 6 weeks of treatment was shown to improve cortical bone strength without altering BMD in rats with renal insufficiency (Iwamoto 2012). A fifth factor that is likely to be associated with
cortical bone abnormalities is hypogonadism. Testosterone is a very important hormone that regulates bone structure during puberty and attainment of peak bone mass. Positive correlations between BMD of the femoral neck BMD and free testosterone levels in serum have been noted to exist in men with AS (Franck 2004). Previously, high prolactin and low testosterone have been linked to low BMD in AS (Franck 2004, Aydin 2005, El Maghraoui 2005). Various other reasons that may have aggravated loss of cortical bone include abnormal mechanical loading due to irregularities in gait and posture, low BMI, malabsorption, low peak bone mass and low lean mass (El Maghraoui 1999).

Certain limitations of HRpQCT in assessing cortical porosity need mention here. First, the measures of cortical porosity obtained by HRpQCT can differ by the type of algorithm applied to calculate the pore diameter. Second, HRpQCT has limited resolution to detect smaller pores in cortex. In addition, motion artifacts and consequent precision errors occur more commonly with HRpQCT based measurement of cortical porosity.

5.5 Abnormal microarchitecture at tibia in AS

Patients with AS were found to have abnormal bone microarchitecture at both radius and tibia. Adverse changes in tibia are more likely to reflect abnormalities in hip since both hip and tibia are weight-bearing sites. The protective effects of skeletal loading are likely to benefit bone structure at the tibia. However, in this study, both tibia and radius were affected in AS patients. This further confirms the role of systemic inflammation as the main cause for bone loss in AS. AS predominantly affects the axial skeleton and as a result many patients develop abnormal kyphosis of the spine. Kyphotic posture causes displacement of body’s the center of mass forwards and downwards (Helliwell 1989). Additionally, some AS patients have difficulty in fully extending the hip joints while standing due to pathologic involvement of the hip joints. Consequently, the displaced trunk and the diseased hip joints may fail to contribute to balance control. Body tries to compensate partly and one such compensatory mechanism is flexion of the knees and ankles plantar flexion of the ankles (Bot 1999). This posture may potentially affect the weight bearing at the tibia. Whether changes in posture and gait in AS can blunt the protective effect of skeletal loading on tibia needs to be studied (Del Din 2011).
5.6 **Finite element analysis**

This is the first report on finite element analysis in patients with AS. The finite element analysis offers distinct insights about bone strength that cannot be derived from vBMD or DXA based measurements. Microarchitecture is a key determinant of the stiffness of bone; both trabecular and cortical compartments maintain bone strength (Cheung 2013). In this study bone stiffness and stress were 15 and 19% lower at radius in AS patients when compared to non-AS subjects. Patients with AS also had thinner cortices and hence, cortical thinning must have likely caused low bone stiffness. Abnormal bone stiffness is not solely likely to be related to increased cortical porosity since it was not different between AS patients and non-AS subjects. It was also observed that the estimates of bone strength were affected differently at radius and tibia. Though lower in AS patients than non-AS subjects at the radius, bone stiffness and stress did not differ between AS patients and non-AS subjects at the tibia. The likely explanation for this is that skeletal loading may negate the adverse effect of inflammation on bone stiffness at tibia. Two recent FEA based studies have suggested that young men and premenopausal women, who suffer fractures, have abnormal microarchitecture, reduced stiffness, and increased failure load compared with those who did not have fractures. Reduced bone stiffness might explain the high fracture risk in AS. But this needs to be studied further. The current study was not powered enough to assess whether individual FEA parameters can discriminate between AS patients with or without fractures.

5.7 **Gender differences in bone microarchitecture**

This study provided important information about certain gender differences in bone microarchitecture and strength that were previously not known. The results indicate that AS affects bone health in men and women differently. Men had higher areal BMD at all sites and this is likely to be due to differences in skeletal between men and women (Tuck 2005). Likewise, the volumetric BMD in the trabecular compartment was also significantly higher in men. The cortical bone was affected more in men whereas trabecular parameters were abnormal in women. Specifically, men had lower cortical vBMD, thinner cortices and more cortical porosity. It was found that cortical porosity was 30-52 % greater in men. In a study by Macdonald et al, cortical porosity was 31% to 44% lower in young women than in men.
Women had fewer and thinner trabeculae at the radius. BV/TV and trabecular separation at radius and tibia were lower in women. But trabecular separation was more in women. The differential effects may partly be explained by menopause, as 8/19 women were postmenopausal. Some of these results are not surprising given the gender differences observed by other authors in the general population. For instance, young men tend to have thicker trabeculae than women (Nicks 2012). As young adults, men have higher BV/TV (by 26%; p=0.001) and trabecular thickness (by 28%; p < 0.001) (Nicks 2012). Similarly, trabecular number and thickness were 7% to 20% higher in young men than in young women at radius and tibia in a study done by Macdonald et al (Macdonald 2011). However, the data on gender differences in trabecular number and separation is conflicting. Though, trabecular number was favorable for men in the study by Macdonald et al, trabecular thickness at radius declined more with age in men (-16%) than in women (-2%, p < .01) (Macdonald 2011). Also, trabecular number and separation were not different between young men and premenopausal women in the study by Khosla et al (Nicks 2011). Similar to that of this study, another study done by MacDonald et al reported that women had a greater degree of trabecular separation than men (Macdonald 2011). Trabecular number at radius and tibia was 11-12% less in young women with AS than men in this study as opposed to 15-20% in the study by Macdonald et al. Though it is reported that in young adults, bone stiffness is lower in females than in men, this study found that bone stiffness were similar in men and women with AS (Dalzell 2009). The presence of higher mSASSS and longer disease duration in men might have caused abnormal bone strength and stiffness in men and consequently masking gender differences in FEA parameters. In summary, these results suggest that gender differences in bone microarchitecture and FEA parameters are exaggerated by AS.

5.8 Relationship between serum inflammatory markers and HRpQCT parameters

In this study, an inverse correlation was noted between ESR and many of the parameters of bone microarchitecture and this may suggest that inflammation was the major cause for bone loss. However, serum CRP correlated significantly only with bone stiffness and stress at the tibia. The lack of correlation between CRP and HRpQCT parameters could be explained by the fact that these parameters reflect different time courses. CRP indicates the current status of inflammation, but parameters of bone microarchitecture are longitudinal variables. It may be
that systemic inflammation might be more relevant to longitudinal bone loss than the absolute values of HRpQCT parameters at a single time point (Ding 2008). In addition, 18% of study subjects had IBD and this condition is associated with elevated CRP. This may have affected the interaction between CRP and HRpQCT parameters (Vermeire 2004).

Data on the link between CRP and other inflammatory markers and low areal BMD have been inconsistent. Some longitudinal studies have found a link between high levels of inflammatory makers and bone loss in older women (Gertz 2010, Scheidt-Nave 2001). Higher serum levels of CRP have been associated with lower BMD (van der Weijden 2011, Taylan 2012, Lee 2011), vertebral fractures and hip fractures independent of BMD (Barbour 2014). Similarly, prospective data from the study of osteoporotic fractures in elderly Caucasian women showed that the hazard ratio of incident hip fracture was 1.64 (95% CI: 1.1-2.5) in those who had the highest level of inflammatory markers such as interleukin-6 as well as soluble receptors for IL-6 and TNF (Barbour 2014). However, some other studies have reported a lack of association between BMD and high levels of IL-6, TNF-α, and CRP. Sponholtz and colleagues studied this association in community-based individuals from the Framingham offspring cohort and reported that no association existed between inflammatory markers and BMD in middle aged and older men and postmenopausal women (Sponholtz 2013). Likewise, the association between high CRP and fracture risk is still not proven though a composite measure of high inflammation markers such as CRP, IL-6, and TNF alpha was noted to be able to discriminate those at high risk of fracture (Cauley 2007).

5.9 Potential mechanisms responsible for poor bone microarchitecture and strength in AS

The mechanisms responsible for reduced BMD and microarchitecture in patients with AS are still unclear. Initial studies had suggested that only bone resorption was adversely affected in AS. Bone formation is expected to increase in the setting of increased resorption. However, in the presence of an inflammatory microenvironment, there can be uncoupling of bone turnover due to inappropriately normal or reduced bone formation. TNFs and other proinflammatory cytokines have been discussed as the main mediators of bone loss. Prolonged high circulating TNF concentrations in AS increases bone resorption. Newer studies suggest a direct role of TNF alpha in the suppression of osteoblastic mediated bone formation. Osteoblasts are derived
from mesenchymal stem cells where as osteoclasts are produced from macrophages and monocytes. There are several mechanisms by which high levels of inflammatory cytokines such as tumor TNF-a, interleukin-1 and interferon gamma affect bone formation. One such mechanism is the inhibition of differentiation of the mesenchymal stem cells in to osteoblasts and osteocytes (Kotake 2014, Yang 2013). TNFα induces the phosphorylation of GSK3β, and p-GSK3β, which leads to β-catenin accumulation, and inhibition of Runx2-associated osteogenesis of mesenchymal stem cells (Kong 2013). In addition, TNF alpha inhibits the osteoblast transcription factors. TNF alpha has also been shown to promote the apoptosis of osteoblast and its progenitor cells. Others have established suppression of the Wnt signaling pathway. TNF alpha has the ability to up regulate DKK-1 and sclerostin molecules that act as Wnt inhibitors (Diarra 2007, Findlay 2011).

5.10 Limitations and strengths

5.10.1 Limitations of the study

Some limitations of the study should be acknowledged while interpreting its findings. First, the causal association between AS and abnormal bone microarchitecture cannot be confirmed due to the cross sectional design of the study. A longitudinal study is needed to further determine the effect of AS on bone microarchitecture and strength. Second, the study was originally designed as exploratory and hence no power calculation was done. The sample size was relatively small but given the lower prevalence of AS, the size of the sample may be justified (Dean 2014). The number of subjects for the sub group analyses was also smaller and hence some differences may not have been detected due a possible lack of power. Moreover, the study may not have had enough power to analyze the gender differences. But this is a reasonable sample size given that women are three times less affected by AS than men. Third, patients in this study were relatively young; therefore, the results cannot be extrapolated to older patients with AS. Whether AS can accelerate bone loss related to age, needs to be studied further. Fourth, the results cannot also be generalized to AS patients belonging to other ethnicities, as most subjects in this study were Caucasians. Fifth, the study cohort consisted of only patients with active disease and hence the findings may only reflect abnormalities in those patients with severe inflammation. Furthermore, increasing number of patients with AS are treated with TNFi now. This study however, included only patients who had never received TNFi. Bone microstructure might be affected differently in patients being treated with effective
control of inflammation. Next, serum levels of bone markers were not analyzed. As a result, the effect of high bone turnover on impaired bone microarchitecture could not be analyzed. Additional drawback of the study is the relatively less number of fractures observed. Consequently it was not possible to study the ability of HRpQCT to identify patients with high fracture risk. Finally 18% of patients in this study had associated IBD, and the HRpQCT parameters are likely to be much worse in such patients.

5.10.2 Limitations of HRpQCT assessment

The limitations of HRpQCT need mention here. HRpQCT assesses microstructure at peripheral sites; on the other hand the fragility fractures related with worst morbidity and mortality are the ones that occur at hip and the spine; therefore, abnormalities of bone microarchitecture detected by HRpQCT may not truly reflect the fracture risk. Although data are still emerging, the few studies that have examined the relationship between HRpQCT of the radius and tibia have shown a moderate correlation ($r=0.56–0.70$) to the axial skeleton. Measures such as cortical porosity are not actual measurements but only estimates of the true value. Also, the use of HRpQCT in routine clinical practice is not practical at the moment as the scanners are not widely available and very few centers can afford to purchase it. The lack of normative data available for comparisons is also a limiting factor. Further, HRpQCT provides information only about the mineralized portion of the bone. This is more important when subjects have vitamin D deficiency and secondary hyperparathyroidism. As mentioned above, HRpQCT does not provide much information about non-mineralized matrix of the bone. Matrix changes are considered to affect bone strength (Sroga 2012). Finally, the sensitivity of HRpQCT in detecting on-going bone loss in AS is unknown. In addition, the discriminative ability of HRpQCT to detect AS patients who are at high risk for fractures is yet to be proved. This is because AS is a complex disorder in which systemic bone loss and new bone formation occur in parallel and its pathogenesis is still uncertain. No data exists regarding the precision of HRpQCT imaging in AS patients. The FEA algorithm applied on the HR-pQCT data only represent axial loading that simulates a fall to the outstretched hand. It does not evaluate bending strength, which might be pertinent to assess the mechanism of peripheral fractures.
5.10.3 Strengths

This is only the second study to provide detailed information of bone microarchitecture in patients with AS. In addition to the standard morphologic outcomes of HRpQCT, this study is strengthened by the assessment of bone strength and stiffness estimated from FEA. It is now well known that structural and mechanical parameters of bone are even better predictors of vertebral fractures than areal BMD assessed by DXA. Consequently the availability of information on structural and mechanical parameters of bone might improve the prediction of vertebral fractures. Moreover, bone mechanical properties assessed by FEA have also been shown to enhance the prediction of wrist fractures (Vilayphiou 2010, Graeff 2012, Vilayphiou 2011). Another strength of this study is the assessment of bone composition at radius and tibia in both healthy and diseased subjects. Strikingly, the differences between the certain parameters in AS patients and in non-AS subjects, obtained at the distal radius and tibia were similar and characterized by increased cortical thinning, decreased total volumetric BMD and increased cortical porosity. Next, HRpQCT provides separate measurements of trabecular and cortical bone compartments and hence the study has generated novel findings that both trabecular and cortical parameters were affected in AS. Future studies can now be designed to assess changes in bone strength within differing compartments in response to disease or therapy. Also noteworthy are the facts that the participants were well characterized. Another important highlight of the results is that areal BMD was different only at the hip and femoral neck, between AS patients and non-AS subjects. Both patients and non-AS subjects had similar BMD at the lumbar spine and distal radius. Thus the study has generated important findings that would have been ignored if patients were assessed by DXA studies alone. Finally, the potential confounding effect of TNFi that influence bone structure were eliminated by excluding subjects who had been on treatment with TNFi, whereas the other studies was unable to do so (Klingberg 2013).
CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS
6.0 Conclusions

6.1 Conclusions
The results indicate that trabecular and cortical bone microstructure as assessed by HRpQCT were altered in AS patients when compared to non-AS subjects. There was decreased trabecular and cortical bone volumetric BMD. Moreover, cortical thinning and cortical porosity were aggravated by AS. These changes translate into reduced bone strength and stiffness. The role of BASDAI as a predictor of poor bone quality could not be demonstrated. AS patients with elevated mSASSS had worse bone microarchitecture when compared to those with normal mSASSS. Men with AS had more cortical bone abnormalities than women. However, trabecular bone was more affected in women compared to men. Thus, the current study data suggest that bone quantity and quality are significantly decreased in the appendicular skeleton of AS patients.

6.2 Future directions

1. Use of HRpQCT to discriminate those at high risk of fractures
Certain limitations do exist regarding the use of DXA in diagnosing or monitoring bone loss in AS. HRpQCT presents the potential to identify patients with high risk of fractures with possibly greater discrimination. Future research should focus try to explain if the abnormalities of bone strength and microarchitecture demonstrated at the peripheral sites such as radius and tibia are related to vertebral fractures in AS.

2. Gender differences in bone microarchitecture
Further studies are needed to further assess why bone microarchitecture is affected differently in men and women with AS.

3. Bone markers in AS
The role of various bone formation and resorption markers in predicting abnormalities of bone strength and microarchitecture should be explored.
4. Inflammatory markers and fracture risk
The role of various inflammation markers such as CRP, ESR, IL-6, and TNF alpha in identifying those at high risk of fracture should be explored further

5. Longitudinal assessment of bone microarchitecture in AS

Presently, there are no published studies on longitudinal data and normative values for HRpQCT in patients with AS. This is an area that requires further research.

6. Treatment of bone loss in AS
The results of the study should be kept in mind while deciding which treatment agent should be chosen to address bone loss in AS. This is because there exist some differences in the effect of bisphosphonates on bone microarchitecture. Both alendronate and zoledronic acid have been proven to maintain or increase areal BMD, cortical vBMD, trabecular BV/TV and cortical thickness at tibia. But the effects of alendronate have been consistently reported to only occur at the tibia and not radius. Though zoledronic acid provides significant improvement at both radius and tibia and also maintains bone strength on FEA, there is conflicting evidence of its effect on cortical porosity. Hence therapeutic agents that have a favorable profile on the various parameters of bone microarchitecture including cortical porosity should be preferred. Denosumab has been shown to improve morphological parameters, total and cortical vBMD in postmenopausal women better than bisphosphonates in postmenopausal women, so might be preferred for AS. In a recent randomized control trial, denosumab was shown to reduce bone remodeling and cortical porosity more rapidly and completely than alendronate. More research needs to be done in this regard by conducting head to head trials between oral bisphosphonates, zoledronic acid and denosumab. Researchers are also trying to study the effect of intravenous bisphosphonates such as zoledronic acid, pamidronate and neridronate in AS (Viapiana 2014, Clunie 2014, Kiltz 2012). Such an approach might be beneficial in reducing disease activity and improving BMD. Randomized controlled trials are warranted to study this further.

7. Role of TNF alpha inhibitors in managing bone loss in AS
TNFi are now being increasingly used to treat AS. These agents have been found to improve or
maintain BMD at the spine and hip (Haroon, 2014). Hence the effect of these agents on bone microarchitecture and strength needs to be explored as well. Various TNF inhibitors such infliximab, etanercept, adalimumab and golimumab differ in their therapeutic effects based on their affinity to the TNF alpha-receptor and duration of action (Tracey 2008). Hence it is likely that their effects on bone architecture may be different and this needs to be studied further. Infliximab has the maximum affinity and binds to TNF longer. The biologic effect of infliximab can last for up to two months. Conversely, etanercept has a shorter half-life of three days. Infliximab can bind to both transmembrane and soluble TNF but etanercept binds only to soluble TNF. Consequently, the binding between etanercept and TNF alpha is reversible whereas infliximab binds to TNF alpha in an irreversible fashion.

TNF-alpha and RANKL have some similarities in their structure and biological activity. Therefore, development of single molecules that can inhibit both RANKL- and TNF-alpha should be attempted. Such agents that possess the ability to control both inflammation and bone resorption will have an important role in the treatment for inflammatory diseases (Coste 2013, Qian 2014).

8. Continuation of the research project

A detailed analysis of radiographs of the thoracic and lumbar spine of all the patients will be done to document potential morphometric vertebral fractures. If enough vertebral fractures are identified, a secondary analysis will be done to identify how abnormalities of bone microarchitecture and strength predict fracture risk in this cohort. The study cohort will be expanded to 100 by recruiting more patients and this will improve the power of the study. Further, the patients who consent to continue in the study will be monitored annually to study longitudinal changes in HRpQCT measurements for two years. All of the study patients have started treatment with TNF alpha inhibitors. HRpQCT based assessment of bone microarchitecture and strength will be done to analyze how treatment with TNF alpha inhibitors can change bone microarchitecture and strength over a period of two years.
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