Chapter 5

Etanercept increases bone mineral density in Ankylosing spondylitis, but does not prevent vertebral fractures

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Abstract

Objective: Ankylosing spondylitis (AS) is characterized by chronic inflammation leading to ankylosis, but also to low bone mineral density (BMD) and vertebral fractures (VF). Treatment with TNF blockers decreases inflammation and has shown to be effective in increasing BMD. We studied the effects of etanercept on BMD and VF in AS patients after two years treatment. Furthermore we studied changes in bone-markers (CTXI, CTXII, RANKL, OPG, Osteocalcin) and radiological damage.

Methods: Patients with active AS, treated with etanercept for two years, were included. BMD lumbar spine and hips was measured at baseline and after two years, as well as the radiological damage (mSASSS, including thoracic spine), VF (Genant method) and change in bone-markers.

Results: Forty-nine AS patients were included**.** After two years of etanercept, BMD hip raised with 2.2% ($p=0.014$) and BMD lumbar spine with 7.0% ($p<0.001$). The BASDAI decreased significantly ($p<0.001$) as well as CRP and ESR ($p<0.001$). Despite etanercept therapy, the number of patients with VF more than doubled (from 6 to 15 patients, $p=0.004$). Also the radiological damage increased significantly over time (from 12.1 to 18.5, $p<0.001$), however no significant change in bone-markers was found.

Conclusion: This prospective longitudinal cohort study showed that after two years etanercept, BMD of hip and spine increased significantly, but the number of patients with VF and the severity of VF increased as well. Besides that, radiological progression, including the thoracic spine, increased significantly. Thus, the favourable bone preserving effect is accompanied by unfavourable outcomes on VF and radiological damage.

Introduction

Ankylosing spondylitis (AS) is characterized by chronic inflammation leading to ankylosis of the spine and sacroiliac joints. Bone loss is a well known complication of AS [1-3], which is highly prevalent after long disease duration, but starts already at an early stage [4-7]. Bone loss and inflammation are probably responsible for the occurrence of vertebral fractures (VF) in this patient group [8-10]. The pathogenesis of the decrease in bone mineral density (BMD) is complex. Persistent inflammation (by inflammatory cytokines like TNF-α) might be an important etiologic factor [11-13].

A way to decrease inflammation is treatment with TNF-α blockers. In Rheumatoid arthritis, several studies showed an increase of BMD, additionally to the positive effects on disease activity and radiographic progression [14-18]. In AS many studies showed positive effects of TNF-α blockers on inflammation and disease activity, although effects on decreasing radiographic progression are disappointing [19, 20]. Considering effects of TNF-α blockers on BMD, patients treated with infliximab showed significant increases in BMD scores over two years [21]. A small study (n=10) of Marzo-Ortega et al. showed that etanercept increased BMD in a short follow-up study [22]. Arends et al. also showed an increase in BMD and, in addition an effect in favour of bone formation by measuring bone-markers in patients treated with different TNF-α blockers [23]. However, clinically relevant outcome measures like vertebral fractures, radiographic progression and disease activity combined in one study were not performed.

Therefore, the aim of this study was to measure the effects of two years of etanercept at bone quality by measuring change of BMD and the incidence of VF. Further, we assessed changes in bone-markers and the effects on radiographic damage.

Patients and methods

Study population

AS patients who fulfilled the modified New York criteria for AS and were eligible for treatment with anti-TNF-α (etanercept) according to the ASAS guidelines were recruited from the Jan van Breemen Research Institute, a large outpatient rheumatology center in Amsterdam [24]. The data for this open prospective follow-up study were collected systematically, every three months during the first year and twice yearly thereafter.

AS patients were treated during two years with etanercept (25 mg twice a week or 50 mg once a week) as decided by their physician if they had previously failed on at least two non-steroidal anti-inflammatory drugs (NSAIDs) and if they had active disease (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥4).

Demographic data and data on known risk factors for osteoporosis, such as age, sex, race, smoking, disease duration, HLA-B27 status, body mass index, extra-articular manifestations, current use of NSAIDs, DMARDs, corticosteroids and anti-osteoporotic drugs were collected at baseline.

The protocol was approved by the local Medical Ethics Committee (of the Slotervaart hospital Amsteram) and all patients provided written informed consent.

Outcome measures

Bone mineral density

The primary outcome measure was the change in BMD of the lumbar spine (L2-L4) and left proximal femur after two years of etanercept. Each patient was measured by dual energy X-ray absorptiometry (DXA) using Lunar (Lunar expert DPX-IQ, Oldelft). Results were presented as BMD (g/cm2), T-scores and Z-scores. The T-score corresponds to the number of standard deviations (SD) from the normal mean obtained from young healthy adults and the Z-score is the T-score with a correction for age. Osteopenia and osteoporosis are defined according to the World Health Organization (WHO): 1) osteoporosis (T-score ≤-2.5 in spine and/or hip), 2) osteopenia (-2.5< T-score <-1.0 in spine and/or hip, without osteoporosis) [25].

Vertebral fractures

The secondary outcome measure was the occurrence of VF. Radiographs of thoracic and lumbar spine were made at baseline and after 24 months. The lateral radiographs were evaluated chronologically for VF by two experienced investigators (WL and BD) who were blinded for medication the patients received. Vertebral deformities were determined by grading each vertebral body (T4-L5) according to the Genant criteria for fractures [26]. In Genant's semi-quantitative assessment, the vertebrae receive a severity grade based on the visually apparent degree of vertebral height loss. The reduction in height is divided in grades on a scale of 0-3; grade 0 (normal) represents a reduction in anterior, middle and/or posterior vertebral heights of less than 20%, grade 1 (mild) represents a reduction of 20-

25%, grade 2 (moderate) reduction of 25-40% and grade 3 (severe) more than 40% reduction. VF were defined as a reduction of ≥20% of the vertebral body height [26].

Markers of bone turnover

The tertiary outcome measure were the bone-markers CTX-I, CTX-II, RANKL, OPG and Osteocalcin which were obtained at 0, 3, 6 and 12 months. Non-fasting serum and urine were collected and stored at -20°C until analyses. Bone resorption was measured by CTX-I and CTX-II. CTX-I was determined by β-isomerised carboxy terminal telopeptide of type 1 collagen (β-CTx) in serum using commercial assays according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany). CTX-II was determined using a urine Cartilaps ELISA (from Immunodiagnostic Systems; IDS) for the quantification of degradation products of C-terminal telopeptides of type II collagen in human urine. Levels of osteoclast-regulating proteins, including total RANKL and OPG, were determined in serum using an ELISA (from Immundiagnostik AG, Bensheim, Germany). Bone formation was measured by osteocalcin using commercial assays according to the instructions of the manufacturer (Roche Diagnostics, Mannheim, Germany). All assays on the analyzer had an intra-assay and inter-assay coefficient of variation of ≤ 5%. The ELISA's had an intra-assay and inter-assay coefficient of variation of ≤10%.

Radiographic progression

The final outcome measure was the degree of radiological damage of the spine after treatment with etanercept. The modified Stokes Ankylosing Spondylitis Spine Score (mSASSS) was used as the index of radiological damage of the cervical and lumbar spine [27]. The lateral radiographs of the spine were examined by two experienced investigators (CvD and IvdH) of each patient at baseline and after 24 months. Additionally, the radiographs of the thoracic spine were assessed as well (T9-T12), although they are not implemented in the official scoring method of the mSASSS. However, since the low thoracic spine might have an additive effect of the sensitivity to change [28] and as it is a predilection place for VF, the X-rays were included in the measurement.

Disease activity

Disease activity measures included the disease activity score BASDAI [29], whereby the functional capacity scores BASFI (Bath Ankylosing Spondylitis Functional Index) [30] and

BASMI (Bath Ankylosing Spondylitis Metrology Index) [31] measured the physical function. The ASAS Working Group criteria for response were applied to define response [32, 33] as a 50% improvement or as an absolute improvement of 2 points of the BASDAI (0-10 scale) and an expert opinion in favour of continuation of treatment after 3 months.

Statistical analysis

Categorical variables were calculated as frequencies and percentages. Continuous variables were reported as mean and SD, or when skewed, as median and IQR. To examine the longitudinal changes in BMD (and T- and Z-scores), BMD was first tested for normality (with the Shapiro-Wilks test) and subsequently the paired t-test or Wilcoxon's signed rank test was used. Differences in VF were tested with the McNemar test. Bone-markers were tested for a linear trend with regression analyses. To detect differences between different time moments the Friedman test was used. With univariate analyses (Pearson correlation coefficients) and multivariate analyses (by multivariate linear regression) the relation between BMD (changes) and bone-markers was investigated. Radiological damage was analysed first by testing for normality and subsequently the change over time was tested with the non-parametric Wilcoxon's signed rank test. Disease activity changes in BASDAI, CRP and ESR were also analysed with the non-parametric Wilcoxon's signed rank test because of the skewed distribution. P-values less than 0.05 were considered as significant. Statistical analyses were performed with SPSS statistical software, version 20.0 (SPSS, Chicago, IL).

Results

Patient characteristics

In total, 49 patients with AS were enrolled and monitored after starting with etanercept. The mean follow-up duration of these patients was 2.3 years. The baseline demographics and clinical features are shown in Table 1.

Most patients were male (82%), the mean age was 42 years and the mean disease duration was 12.2 years. Three patients had a total hip replacement at inclusion. There was a high disease activity before start of therapy and the majority responded well.

Table 1. Baseline characteristics AS-etanercept cohort

	N=49
Demographic variables	
Men[a]	40 (81.6)
Age[b], (years)	41.8 (9.2)
Caucasian race[a]	38 (77.6)
Disease related variables	
Disease duration[b], (years)	12.2 (9.1)
Symptom duration[c], (years)	15.8 (9.9-23.4)
Follow-up duration[b], (years)	2.3 (0.7)
HLA-B27[a], (positivity)	43 (87.8)
ESR[c], (mm/hr) [<20]	20.0 (6.0-39.0)
CRP[c], (mg/l) [<10]	14.0 (3.0-39.0)
BASDAI[b], (0-10)	5.7 (1.6)
BASFI[b], (0-10)	5.7 (2.1)
BASMI[b], (0-10)	4.4 (2.3)
Uveitis[a], (history of)	17 (34.7)
Psoriasis[a], (history of)	6 (12.2)
Inflammatory bowel disease[a], (history of)	3 (6.1)
Peripheral arthritis[a], (history of)	16 (32.7)
Radiographic damage	
Total mSASSS[c], (0-72)	10.0 (3.8-35.5)
Total mSASSS+ThSpine[c], (0-90)	12.1 (6.8-42.7)
BMD related variables	
BMI[b], (kg/m^2)	26.3 (3.4)
Menopausal status[a]	2 (4.1)
One or more prevalent vertebral fractures[a]	6 (12.2)
Smoking[a], (current)	24 (49.0)
BMD variables	
sBMD hip[b]	0.903 (0.152)
sBMD L2-L4[b]	1.141 (0.203)
T-score hip[b]	-0.92 (1.14)
T-score L2-L4[b]	-0.29 (1.77)
Z-score hip[b]	-0.81 (1.04)
Z-score L2-L4[b]	-0.31 (1.71)
Medication	
NSAIDs[a], (current)	49 (100)
DMARDs[a], (current)	12 (24.5)
Corticosteroids[a], (current)	1 (2.0)
Bisphosphonates[a], (current)	3 (6.1)

ESR= Erythrocyte Sedimentation Rate; CRP= C-Reactive Protein; BASDAI= Bath Ankylosing Spondylitis Disease Activity Index; BASFI= Bath Ankylosing Spondylitis Functional Index, BASMI= Bath Ankylosing Spondylitis Metrology Index; BMI= Body Mass Index; BMD= Bone Mineral Density; NSAIDs= Non-Steroidal Anti-Inflammatory Drugs; DMARDs= Disease-Modifying Anti-Rheumatic Drugs. [a]Number (%), [b]Mean (SD), [c]Median (IQR).

Bone mineral density

All 49 patients had two DXA scans of the spine and 46 patients had a DXA of the hip (three patients had a total hip replacement). At baseline 12% of the patients had osteoporosis, 45% osteopenia and 43% had a normal BMD. After two years of etanercept this changed to 4% osteoporosis, 41% osteopenia and 55% normal BMD (Table 2).

After two years of treatment, BMD hip raised significantly with 2.2% (5.7), p=0.014 and BMD lumbar spine raised significantly with 7.0% (9.5), p<0.001. The mean T- and Z-scores showed the same significant increase of the hip (p=0.037 vs. p=0.002) and lumbar spine (both p<0.001).

Table 2. Bone mineral density measurement at femur and spine

	t=0 year, before etanercept			t=2 years, after etanercept		
	Hip*	Spine	Total BMD	Hip*	Spine	Total BMD
Normal BMD[a]	23 (46.9)	29 (59.2)	21 (42.9)	27 (55.1)	36 (73.5)	27 (55.1)
Osteopenia[a]	19 (38.8)	16 (32.7)	22 (44.9)	17 (34.7)	12 (25.5)	20 (40.8)
Osteoporosis[a]	4 (8.2)	6 (12.2)	6 (12.2)	2 (4.1)	1 (2)	2 (4.1)

Normal BMD= T-score ≥-1.0, Osteopenia= -2.5< T-score <-1.0, Osteoporosis= T-score ≤-2.5. [a]Number (%), *calculated in 46 patients because 3 patients had a hip replacement.

Vertebral fractures

At baseline six patients (12.2%) already had at least one VF. After two years of etanercept, the number of patients with one or more VF was more than doubled to 15 patients (30.6%) (p=0.004). Most VF were localised in the (mid)thoracic spine. Not only the number of patients with VF increased significantly over two years, but also the severity (grade) of the VF, from 4 fractures (out of 8) graded 2 or more to 13 fractures (out of 21) graded at least 2 (Table 3). Analyses for risk factors for the development of VF did not show any parameter to be associated with these incident VF (such as age, BMD, disease activity, radiological damage; data not shown).

Markers of bone metabolism

Boxplots of the distribution of bone-markers over time as well as the change in disease activity (BASDAI) and inflammation (CRP) are shown in Figure 1. Bone-markers were tested for linear trend with regression analyses, but there was no significant trend over time for the bone 'resorption' markers, the 'osteoclast-regulation' markers, nor for the bone

'formation' marker. To detect differences between different time moments the Friedman test was used. No significant changes in bone-markers were detected over time, except for OPG (which showed a decreasing trend). However the RANKL/OPG ratio did not change significantly over time.

Table 3. Vertebral fractures before and after etanercept in AS (N=49)

	t=0 year, before etanercept		t=2 years, after etanercept	
Vertebral fractures	Patients with VF	Number of VF	Patients with VF	Number of VF
0 Vertebral fracture[a]	43 (87.8%)		34 (69.4%)	
1 Vertebral fracture[a]	4 (8.2%)		9 (18.4%)	
Grade I		2		5
Grade II		2		4
Grade III		0		0
2 Vertebral fractures[a]	2 (4.1%)		6 (12.2%)	
Grade I		2		3
Grade II		2		7
Grade III		0		2
Total number of VF[a]		8 (16.3%)		21 (42.9%)
Total patients with VF[a]	6 (12.2%)		15 (30.6%)	

VF= Vertebral Fracture, Grade I= reduction of vertebral height 20-25%, Grade II= reduction of vertebral height 25-40%, Grade III= reduction of vertebral height >40%. [a]Number (%).

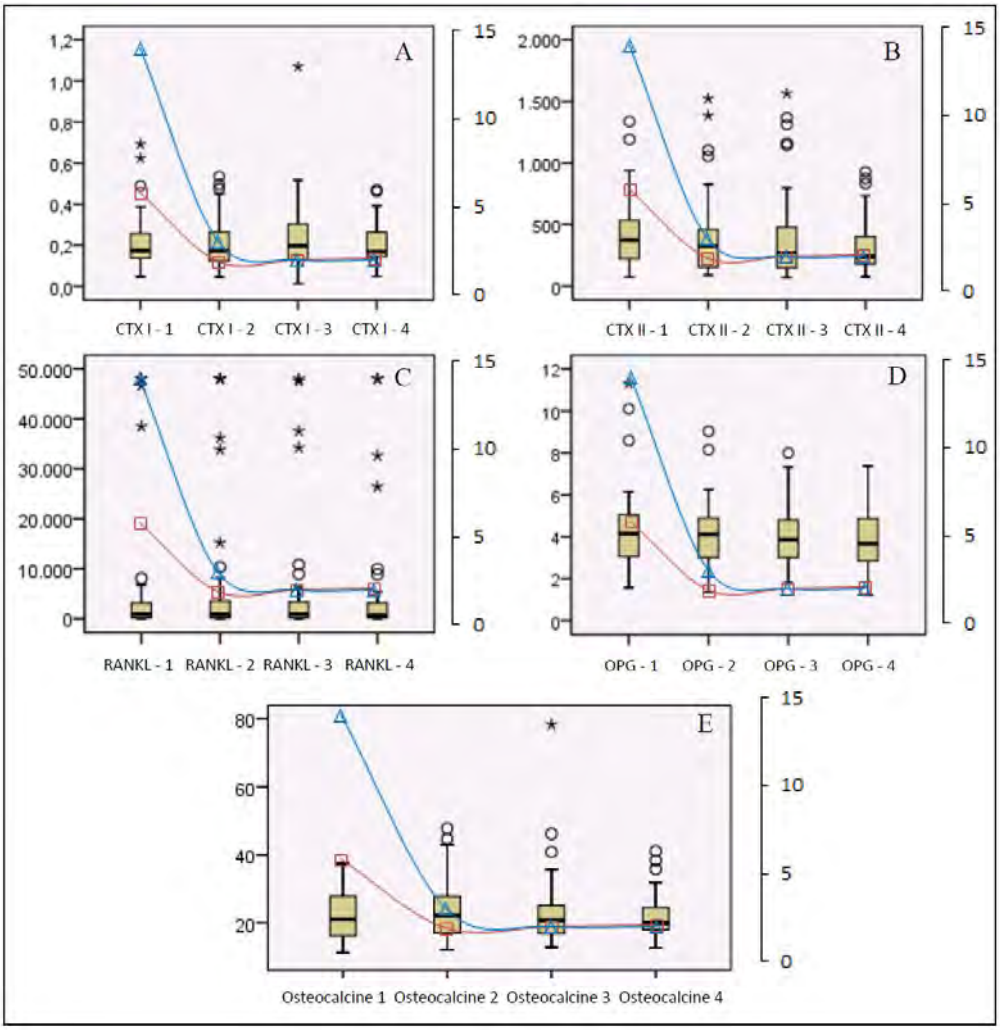
Radiological progression

The median radiological damage (total mSASS-score) at baseline was 10.0 (3.8-35.5) and increased after two years to 15.5 (5.5-42.5). The total mSASSS+ThSpine had a median at baseline of 12.1 (6.8-42.7) and progressed to 18.5 (8.7-52.0). There was a significant difference between the radiological damage at baseline and after two years ($p<0.001$) measured by both the mSASSS as well as the mSASSS+ThSpine. Correlation tests did not show a significant relation between BMD change over time and the radiological progression. Further, there was neither a correlation between bone-markers and radiological progression, nor between disease activity parameters and radiological progression.

Disease activity

Disease activity measured by BASDAI decreased from 5.8 (5.1-6.8) at baseline to 2.1 (1.0-4.1) after 12 months and to 2.8 (1.0-4.4) after 24 months ($p<0.001$ at all time points compared with baseline). According to the ASAS major clinical response criteria, 85% of the patients responded to etanercept at 3 months, 69% at 12 months and 63% at 24 months.

Figure 1. Distribution of bone turnover markers during treatment with etanercept



Vertical scale left: scale of different bone markers. Vertical scale right: scale of BASDAI and CRP. Horizontal: different time moments; 1= 0 month, 2= 3 months, 3= 6 months, 4= 12 months. Boxplots= different bone markers, Δ= CRP, □= BASDAI

Disease activity measured by inflammation markers like CRP and ESR also decreased significantly ($p<0.001$ at all time points compared with baseline). CRP decreased from 14.0 (3.0-39.0) at baseline to 2.0 (1.0-6.0) at 12 months. ESR decreased from 20.0 (6.0-39.5) at baseline to 5.0 (2.0-9.0) at 12 months. ESR and CRP changes were strongly correlated ($p<0.001$).

There was a significant relation between the change in BMD hip and spine and the change in inflammation markers (i.e. ESR and CRP) over 12 months. Both the decrease in ESR and CRP were significantly associated with an increase in BMD hip and spine (ΔBMD hip: $p=0.040$ resp. $p=0.005$ and ΔBMD spine: $p=0.012$ resp. $p<0.001$).

Discussion

This prospective observational cohort study in patients with active AS demonstrated that after two years of TNF-α blocking therapy with etanercept, BMD of the hip as well as BMD of the spine increased significantly, whereas the number and severity of VF and the radiographic damage increased. This observation suggests that despite the decrease in inflammation and increase in the amount of bone, the anticipated increase in bone quality stays behind. In addition, the ongoing bony proliferation is also unfavourable, which emphasizes that despite TNF-α blockers bone-(patho)physiology is still not optimal.

Since persistent inflammation might be an etiologic factor of bone loss in AS, anti-TNF-α therapy has been proposed as treatment that controls inflammation with subsequent prevention of osteoporosis and associated VF [34, 35]. This study shows that after two years of etanercept, the BMD increased significantly in the lumbar spine as well as in the hips. This finding is in concordance with other studies that also showed an increasing trend in BMD after treatment with TNF blockers [21, 23, 36, 37].

Strikingly, this is the first study that describes the rapid progression of the number and severity of VF over two years, despite lowering disease activity and inflammation through etanercept therapy and despite the increase of BMD. The interpretation of VF in AS is a challenge, since the method of Genant does not differentiate between AS-related deformities, degenerative changes or osteoporotic fractures. The presence of low BMD and the localization of the VF suggest that these fractures are 'real' osteoporotic fractures. We have documented earlier in a cohort of early Spondylarthropathy patients a high number of patients (15%) with VF in especially the thoracic spine [10]. Unfortunately, VF in AS are often missed in clinical routine procedures, however diagnosing these fractures is important because the knowledge of existing fractures is necessary for optimal assessment of risk for future fractures and treatment [9, 38, 39].

The increased prevalence of VF despite the increase of BMD suggests that it is more likely that despite the increase in 'quantity' of bone mass, the problem in AS is more due to a

decrease in 'bone quality'. A specific definition of the quality of bone is difficult to be given, because multiple factors contribute to the structural integrity of bone: not only the total bone mass, but also bone geometry and properties of constituent tissue [40]. As BMD has been shown to be a limited predictor of fracture risk [41], now more clinical interest is needed for complementary measures of bone quality that could improve fracture risk prediction [42]. One of these measures could have been bone-markers, but unfortunately we did not find a linear trend of bone 'resorption' markers (CTX-I, CTX-II), 'osteoclast-regulating' markers (RANKL, OPG) or of the bone 'formation' marker (Osteocalcin) over time during etanercept treatment, whereas the inflammatory parameters (CRP and ESR) and disease activity responded well. A decrease in bone-resorption markers and osteoclast-regulating markers was therefore expected alongside an increase in the bone-formation marker [16, 21, 23]. Still, our results were in line with the study of Allali et al. who also found in 29 AS patients an increase in BMD during treatment with TNF-α blockers and no change in biochemical markers (osteocalcin and total deoxypyridinoline) [36]. However, an early increase after 2-12 weeks in markers of bone formation (bone alkaline phosphatase) was found in other studies [21, 43], but no change in levels of CTX, OPG and RANKL [43]. Arends et al. however, showed an increase in BALP (bone formation marker) but also a decrease of serum collagen-telopeptide (bone resorption marker) [23]. It is not fully clear why we did not find changes in the investigated bone-markers, it could be related to methodological errors (samples were not taken in a fasting state), type of TNF-α blocker or type of the measured bone-markers.

Interestingly, despite etanercept the radiological damage increased significantly over time ($p<0.001$). This is confirmed by other studies with TNF blockers, including etanercept, which show no delay in radiological progression in AS [19, 44]. Kang et al. recently wrote about the 'paradoxical effects' of TNF inhibitors on BMD and radiographic progression in AS, since they also found an increase in BMD in combination with radiographic progression of the spine, like we did [45]. Although they didn't study VF in combination with radiological progression. Maksymowych et al. hypothesized that early inflammatory lesions resolve after treatment with TNF blockers before the induction of reparative changes whereas in more mature inflammatory lesions (visible on MRI as focal fat infiltration which reflects post inflammatory tissue) new bone will be formed. New bone will be formed once the signalling pathways have been activated (through down regulation of dickkopf-1 which upregulates the Wnt pathway). Our study population consisted of

patients with highly active disease and a long disease duration. It could be that the resolution of inflammation in these more mature lesions following etanercept treatment may have caused the ongoing process of new bone formation [45-47].

The fact that there are no studies at this moment that investigated the effects of etanercept on both BMD, the occurrence of VF and radiological progression in combination with bone-markers, makes this study unique. As such this is a very clinically relevant combination of outcome measures. However, there are some potential limitations to be mentioned, such as the limited size of the cohort (n=49). Nevertheless, this number is higher compared to other studies [22, 36, 45] and the results are clear enough to show the challenges we are facing on this topic. Further, the duration of the study is limited by two years and the measurements of the bone-markers are performed within a maximum treatment duration of one year. However, it was to be expected that changes in biomarkers would have occurred the first year of etanercept treatment since it is known to have a strong and early effect on disease activity and subsequently also on bone-markers as has been shown in Rheumatoid arthritis [16]. Finally three patients used bisphosphonates. In our opinion this has not influenced the outcomes of this study because they used it already for more than three years and the results including these patients were not significantly different from when excluding them (results not shown).

Conclusion

This prospective cohort study shows that after two years of TNF-α blocking therapy with etanercept, BMD of the hip and spine increases, but also both the number and severity of VF. Besides that, the radiological damage, including the thoracic spine, increased significantly. With this study we showed that with increasing the BMD, bone quality is not necessarily increased. The favourable bone preserving effect is accompanied by unfavourable outcomes on VF and radiological damage, suggesting both a lack of increase in bone strength and also a further ankylosis of the spine. More attention and research is needed to investigate the aspects of quality of bone in AS patients and the thoracic spine may not be overlooked as important place for VF!

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