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BIOMARKERS LABORATORISTICI RELATIVI AL COINVOLGIMENTO OSSEO NELLE ARTROPATIE INFIAMMATORIE

S.S.D. Area 06 - Scienze mediche, MED/16 REUMATOLOGIA

Coordinatore: Prof. Giovanni Targher

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LABORATORY BIOMARKERS OF BONE INVOLVEMENT IN INFLAMMATORY ARTHROPATHIES

S.S.D. Area 06 - Scienze mediche, MED/16 REUMATOLOGIA

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ABSTRACT

Objectives: The purposes of the study are multiple: first to investigate the determinants of Dickkopf-1 (Dkk1) in ankylosing spondylitis (AS), second to understand the role of anti-citrullinated protein antibodies (ACPA) on systemic bone health and third investigate the effect in the short term of tumor necrosis factor α inhibitors (TNFI) on bone density, turnover markers and Wnt modulators in rheumatoid arthritis (RA).

Methods. Three different groups were involved: biologic naïve AS, a large RA biologic naïve cohort for the ACPA part, and a subgroup of RA biologic naïve patients about to start TNFI due to disease activity. All patients must not have serious comorbidity and taking bone targeting drugs. According to the part patients underwent blood sample for serum variables, bone density and clinimetric evaluation. Treatment and demographic data were also collected.

Results: In the first part we found that one of the determinants of Dkk1 in AS in parathyroid hormone (PTH) and this is truer in patients with longer disease duration. In the second part ACPA turned out to negatively affect systemic cortical bone in a titer dependent manner. Third we reported an increase in bone turnover after TNFI, together with an increase also in PTH hormone, a decrease in Dkk1 and a slight decrease in femoral bone mineral density (BMD). Dkk1 also in the third part showed a correlation with PTH.

Conclusions: Despite differences among inflammatory arthropathies bone and immune system are tightly connected and influencing each other. Wnt system, PTH, ACPA seems to be some of the key actors of this connection and also therapies, like TNFI, can influence their balance. Those evidences underline once again the importance of integrating bone in the evaluation of patients and in the development of new treatment of new way of using the existing ones.

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CHAPTER 1: INTRODUCTION

Autoimmune inflammatory arthropathies (AIA) are a wide group of diseases characterized by a reaction of the immune system against musculoskeletal structure of the host. The main ones are rheumatoid arthritis (RA) and the seronegative spondyloarthritides; of the latter, the most common are psoriatic arthritis (PSA) and ankylosing spondylitis (AS).

The pathophysiology of the diseases inside this big group is quite different but there are some common features like the link between immune system and bone.

For many decades AIA has be regarded from the pathophysiologic point of view as dependent only on the immune response with different inflammatory cytokines and immune cells involved. Musculoskeletal structures and especially bone has been considered for long time just the immobile victim of the inflammatory response led by the immune system.

In the last decade many evidences from the literature revealed a much more complex picture were bone is metabolic active, producing mediators that deeply connect it to the immune response both at systemic level in term of risk of osteoporosis, and at local level were the diseases give their structural damage.

Cytokines, immune cells, receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL)-osteoprotegerin (OPG) axis, Wnt System, parathyroid hormone (PTH) and vitamin D are the key actors of this connections but are differently involved in different diseases (giving reason of the different structural features clinically observed) and could be differently affected by therapies.

AIA can impair the normal homeostatic bone remodeling process were bone resorption by osteoclasts and bone formation by osteoblasts are coupled in order to maintain a stable balance.

1.1 BONE INVOLVEMENT IN ANKYLOSING SPONDYLITIS

In AS the inflammation takes place initially at the sites where ligaments and tendon attach to the bone, called entheses. The final event of the process is osteoblast bone forming activity resulting in syndesmophytes and ankylosis. There are no certainties if this process is always subsequent an erosive phase or not. Moreover to further complicate the picture in AS there's also evidence of systemic bone loss with an increase incidence of low bone mineral density (BMD) and vertebral fractures (1-3).

The bone formation process follows the endochondral ossification and this has been demonstrated both at syndesmophytes and sacroiliac joint (4, 5). There are studies describing expression of mRNA for inflammatory cytokines at sites of bone formation; the pathway that seems mostly involved are the IL-23/IL-22/IL-17 axis, the bone morphogenetic proteins (BMP) and the Wnt-β catenin pathway.

The Wnt-β catenin pathway is constitutively activated and stimulates bone formation. As many activated systems, its regulation is dependent on inhibitors, the most important are Dickkopf-1 (Dkk1) and sclerostin.

Sclerostin serum levels has been described to be lower than healthy controls (HC) and coherently its tissue expression at joint site studied with histochemical staining is lower than HC. Moreover, a study reported lower levels of sclerostin in AS patients with

increased presence of syndesmophytes (6). Tsui et al demonstrated also the presence of autoantibodies against sclerostin in the sera of AS patients (7).

Those evidences suggested that sclerostin either is low or its signal disrupted and thus the Wnt pathway would be more active leading to the increased bone formation characteristic of the structural damage in AS.

Another Wnt inhibitor, Dkk1, has been investigated in last years and seems to play an important role in AS. In fact, the inhibition of Dkk1 signaling through neutralizing antibodies in mice leads to sacroiliac fusion (8), Dkk1 levels have been described to be low in osteoproliferative disease like diffuse idiopathic skeletal hyperostosis (9) and its reduced level in mice is associated to a higher bone mass phenotype (10).

In AS data on Dkk1 are heterogeneous, it has been described to be higher (11, 12), normal (13-15), normal but dysfunctional (16) or lower (17, 18). Our previous experience found lower levels of Dkk1 in our cohort; of note Dkk1 levels were negatively correlated with BMD levels and with vertebral fractures (19).

Heiland et al reported a negative correlation also between Dkk1 levels and syndesmophytes (20).

The evidences above underline the role of the Wnt system and its regulators in the pathophysiology of AS even if more need to be investigated, especially about the determinants of Dkk1 levels. On that topic there are data in other settings, like RA, primary hyperparathyroidism, mastocytosis and teriparatide treatment where Dkk1 levels were significantly correlated to PTH levels (21-24). Those elements took us to investigate the possible relation between PTH and Dkk1 also in AS in the first part of this project.

1.2 BONE INVOLVEMENT IN RHEUMATOID ARTHRITIS

Bone involvement in RA is extensive and comprehends different aspects: focal at erosion site, periarticular and systemic. The first is the site direct contact with inflamed synovium, the second is due to local mediators that influence the bone close to the primary inflammation site and the last is the known osteopenia-osteoporosis of RA patients where systemic mediators and also iatrogenic factors influence bone health.

The loss of bone observed in RA is due to an imbalance between resorption and formation.

Osteoclasts are the cells responsible for bone resorption and they are essential to the erosive process as well as for the systemic loss; one of the key molecule for their differentiation and activation is the RANKL. Mice knock out for RANKL can develop arthritis and cartilage damage but not bone erosions as they lack of osteoclasts (25). The inhibition of the RANKL-RANK axis with denosumab has shown beneficial effects at all three the cited levels of bone loss demonstrating that osteoclasts are important physiopathological actors (26-28).

Osteoclasts differentiation, recruitment and activation are dependent on many factors.

Anti citrullinated peptides antibodies (ACPA), a hallmark of RA known mostly for diagnostic purpose, are growing also as true pathogenetic factors with accumulating clinical and laboratories evidences.

Anti citrullinated vimentin antibodies in vitro can bind osteoclasts surfaces but also can led to a strong induction of osteoclastogenesis and bone resorptive activity (29).

Moreover, the adoptive transfer of these antibodies into mice caused and increased osteoclastogenesis and osteopenia (29).

Of interest, human osteoclasts express peptidylarginine deiminase (PADI), the enzyme required for citrullination of proteins and some author described during osteoclast differentiation a specific N-terminal citrullination of vimentin (29).

Kleyer at al published a very interesting paper involving healthy subjects found to be positive for ACPA but without any sign or symptom of arthritis. Those patients had alteration of the periarticular bone with thinning, fenestration and reduced density of the cortical bone (30). For the second part of the study we start from the data of Harre and Kleyer supposing a systemic effect of ACPA.

In the same period we performed and published the second part of the study Bugatti et al reported in a cohort of early RA a negative effect of ACPA on spine BMD and only at high titer and together with rheumatoid factors at femoral level (31).

Another important pathway is the above mentioned Wnt system with its inhibitors, Dkk1 and sclerostin, those molecules inhibit bone formation. Dkk1 has been found higher than HC in RA patients and associated with erosive disease phenotype (22, 32) and furthermore, inflamed synovial tissue has been demonstrated to be a source of Dkk1. Inhibition of Dkk1 alters the RANKL-RANK-OPG axis with an increase in OPG leading inhibition of RANKL activity and thus of erosion development (33).

Other important influencers of bone metabolism in RA are inflammatory mediators.

Many pro-inflammatory cytokines, elevated in RA, are known to have a stimulatory effect on osteoclasts and thus on bone resorption. Among them one of the better studied is TNF

that has a double negative effect on bone: it inhibit bone formation and it boosts bone resorption (33-35).

TNFα has the ability to stimulates synovial fibroblasts and osteoblasts to produce Dkk1 (33) and that gives reason of the fact that the two systems, important for bone loss in RA; are tightly linked and can mutually influence each other.

In the therapeutic armamentarium for RA the first biotechnological targeted drugs developed are the TNFα inhibitors (TNFI).

Their efficacy in controlling inflammation and articular bone damage accrual has been proved in several trials. Fewer studies (36-49) investigated their effect on bone turnover markers (BTM) and systemic BMD with a general agreement on an increased bone formation and decreased resorption even if with some discording experiences. An important problem is that most of these studies were small and inadequately controlled important factors that can deeply influence bone metabolism such other medication.

For the last part of the study we wanted to investigate BTM in a more controlled setting in the short term after TNFI exposure, but also to understand how TNFI affects Dkk1 and one of its determinants which is PTH and the relation among those variables.

CHAPTER 2: STUDY OBJECTIVES

The purpose of our study was to investigate the role of bone in inflammatory arthropaties. This has been pursued looking at different aspects and settings.

First we investigated, in a transversal design, bone biomarkers in AS in a biologic-naive population. Bone loss and fragility fracture has been underlined recently as one of the burden of disease with great impact on disability. In a previous work vertebral fractures have been described as associated to Wnt pathway alteration. In AS Wnt system works differently at syndesmophytes formation sites and at systemic level, particularly patients with fracture had lower Dkk1 levels. The first part of the study investigated the determinants of Dkk1 levels (with a particular interest for PTH) and relations also with other variables such as BMD and non-steroidal anti-inflammatory drugs (NSAIDs).

The second part of the study was a transversal observation and dealt with RA patients with focus on the effect of ACPA on systemic bone health. This aspect is of great importance knowing the role of ACPA on osteoclastogenesis and the relation between BMD and erosions.

A third part involved in a longitudinal design a group of RA patients about to start an anti-TNF biologic treatment with the objective of understanding the modification of bone biomarkers and Wnt modulators induced by such treatment. How the new target therapies affect bone metabolism is still a matter of debate and scientific research.

CHAPTER 3: METHODS

PATIENTS AND DISEASES

In the first part we recruited the same cohort of patients of a previous study performed between January 2012 and March 2014. Patients must have a diagnosis of AS according to the modified New York criteria (50). Exclusion criteria were clinical or laboratory evidence of hepatic, renal or bone metabolic diseases, concomitant diseases with relevant impact on bone metabolism like inflammatory bowel disease or treatment with drugs known to interfere with bone or mineral metabolism, including TNF-a blockers, glucocorticoids, and bisphosphonates. Anthropometric, demographic and therapeutic data were also collected. NSAIDs use was defined as chronic if greater than 3 times a week.

Disease duration was defined as time passed from onset of symptoms to recruitment. Disease activity and disability were assessed by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Metrology Index (BASMI), and Bath Ankylosing Spondylitis Functional Index (BASFI). Blood samples from 71 healthy sex- and age-matched volunteers (healthy controls, HC), mostly coming from hospital personnel or patients' relatives were collected to be used as controls.

For the second part were included patients recruited consecutively from our outpatient clinic between 2013 and 2015. They must satisfy the new American College of Rheumatology(ACR)/ European League Against Rheumatism (EULAR) classification criteria (51). Exclusion criteria were bone targeted drugs (excluding calcium and vitamin D) such as bisphosphonates, biological disease modifying antirheumatic drugs (bDMARDs), severe renal, kidney, heart or metabolic disease. Disease activity and

disability were measured through Disease Activity score 28 (DAS28 CRP) (52) and Health Assessment Questionnaire (HAQ).

The third part involved 54 patients with definite diagnosis of RA according to ACR/EULAR criteria (51) who needed to receive anti-TNF therapy due to failure of (or intolerance to) conventional synthetic DMARD (csDMARD) to control disease activity. Treatment administration and screening tests were done according to current guidelines (53).

Patients taking bone targeted therapies (other than calcium and vitamin D) or other that could influence bone metabolism (e.g. diuretics, thyroid hormone, etc.) were excluded as well as patients with severe comorbidities as mentioned before.

Before entering the study, each patient was informed about its aims and gave informed consent to participate. The study protocol was in line with the Declaration of Helsinki. The studies obtained the approval from the Ethical Committee of our institution.

BMD AND RADIOLOGIC EVALUATION

BMD was assessed in all patients using due energy x-rays absorptiometry (DXA) (QDR Hologic Delphi) at lumbar spine (L1–L4), total hip, and femoral neck. Measurements were expressed as T-score (standard deviation –SD- below the mean of young healthy adults) and Z-score (number of SD above or below the mean for the patient's age, sex and ethnicity). Osteoarthritic or fractured vertebrae were excluded from the analysis. The coefficient of variation was 1,0% for the spine and 1,2% for the hip BMD. Osteoporosis was defined as a lumbar spine or hip BMD T-score of -2,5 or less, according to the WHO criteria (54).

Furthermore, in AS patients the spine was evaluated in all of them with standard x-rays (anteroposterior and laterolateral projections) in order to detect the presence of vertebral fractures and also the occurrence of syndesmophytes.

BLOOD SAMPLES AND MARKERS.

Blood samples were obtained in a fasting state from 8.00 to 9.30 AM. Levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were analyzed immediately by using standard laboratory techniques. Aliquots of serum sample were stored at -80°C until needed for analysis. Serum Dkk1 and sclerostin were measured with an enzyme immunoassay (Biomedica Medizinprodukte GmbH & Co. KG, Wien, Austria) with sensitivities of 0.89 and 8.9 pmol/L and an intra-assay coefficients of variation of 7.8 and 5.6%, respectively.

25OH-Vitamin D and PTH were measured by ELISA (IDS Ltd. Boldon, UK) with intra-assay variabilities of 3 and 6 % and inter-assay variabilities of 6 and 7 %, respectively. All samples were measured in a single batch in order to limit inter-assay variability.

Bone turnover markers (intact N-propeptide of type I collagen, P1NP, bone alkaline phosphatase, bAP, and C-terminal telopeptide of type I collagen, CTX) were measured by the IDS-ISYS Multi-Discipline automated analyzer (Immunodiagnostic System, Boldon, UK) based on chemiluminescence technology. The coefficients of variation intra-assay measured in our laboratory were 3.0% for P1NP, 2% for CTX and 4% for bAP.

For the second part were obtained serum sample for the measurement of rheumatoid factor (RF) and ACPA levels and analyzed at Verona hospital central lab. For ACPA has

been used a EIA kit by Inova diagnostic and were considered positive if above 20 U/ml, RF was measured by nefelometry.

STATISTICAL ANALYSIS

Continuous variables were expressed as mean with standard deviation or median with interquartile range (25^{th} -75^{th}), as appropriate. Non-normal variables were transformed in their logarithmic, when needed (second part). Categorical variables were expressed as absolute numbers and percentages. In the third part the patients were divided in two subgroups based on trend of corticosteroid consumption. Differences between groups were analyzed by t-test (independent samples, one sample, paired samples) ANOVA with Bonferroni correction for multiple comparisons, Mann-Whitney, Chi-square or Fisher's exact test, as appropriate. Correlation between continuous variables were analyzed with Pearson or Spearman correlation, as appropriate. In the second part for multivariate analysis a general linear model was used in order to account for possible confounders and ACPA titers were analyzed either as positive/negative or divided in four levels, i.e. negative, first, second and third tertile of positive values.

A p value<0.05 was considered statistically significant. Statistical analysis was performed by SPSS 17.0 (SPSS Inc., Chicago, IL).

CHAPTER 4: RESULTS

4.1 ANKYLOSING SPONDYLITIS

In the first part we analyzed the AS population; it was composed by 71 adults with a mean age of 44 (±12.1) years, predominantly males (83.1%) with a mean disease duration of 138.5 (±108.6) months and a peripheral disease in 19.7%. In table 1 and 2 are displayed the main clinical characteristics and biochemical panel.

Table 1. Clinical characteristics of the study population.

Characteristics (n=71)	**Values**
Disease duration (months)	138.5 ± 108.6
Peripheral involvement present	19.7% (14/71)
HLA-B27 positives	74.6% (53/71)
Current smokers	36.6% (26/71)
NSAIDs users	56.3% (40/71)
VAS pain (0-10)	2.76 ± 2.35
VAS morning stiffness (0-100)	27.1 ± 25.5
Patient Global Assessment (0-10)	2.85 ±2.42
BASMI	2.25 ± 1.98
BASDAI	2.68 ± 2.18
BASFI	2.25 ± 1.92
Vertebral fractures (prevalence)	25.3% (18/71)
Syndesmophytes (prevalence)	46.5% (33/71)
Lumbar Spine Z-score	-0.32 ± 1.50
Neck Z-score	-0.16 ± 0.93
Total Z-score	-0.31 ± 0.88

Values have been reported as percentages (proportions) or mean ± standard deviation as appropriate. NSAIDs = chronic users of Non-Steroidal Anti Inflammatory Drugs, VAS = visual analogic scale, BASMI = Bath Ankylosing Spondylitis Metrology Index, BASDAI = Bath Ankylosing Spondylitis Disease Activity Index, BASFI = Bath Ankylosing Spondylitis Functional Index, vertebral fractures: patients with vertebral fractures, syndesmophytes: patients with syndesmophytes.

Table 2. Serum measurements in the ankylosing spondylitis population (n=71).

Markers	Values
ESR (mm/h) (nr < 20)	14.7 ± 12.3
CRP (mg/l) (nr < 5)	5.1 ± 6.5
25OH-Vitamin D (ng/ml) (nr 20-100)	29.7 ± 14.2
P1NP (ng/ml) (nr 27.7-127.6)	49.5 ± 26.2
CTX (ng/ml) (nr 0.14-0.70)	0.27 ± 0.21
PTH (pg/ml)(nr 10-65)	33.8 ± 14.1
Dkk1 (pmol/L)	23.3 ± 13.1

Values have been reported as median ± interquartile range. ESR = erythrocytes sedimentation rate, CRP = C reactive protein, P1NP = pro-collagen type I N-terminal, CTX = C-terminal telopeptide of collagen type I, PTH = parathyroid hormone, Dkk1 = Dickkopf1, nr = normal range.

Our group previously described that Dkk1 serum level is lower in AS patients compared to HC (23.3±13.1 vs 29.8±15.9 pmol/l, respectively; p=0.009, table 3) and among AS patients those with vertebral fractures were the ones with higher Dkk1 serum levels (19).

Table 3. Serum measurements in the ankylosing spondylitis and healthy controls.

Markers	AS n=71	HC n=70	p value
Sclerostin (ng/ml) (nr 0.14-0.70)	0.27 ± 0.21	0.38±0.17	<0.001
PTH (pg/ml)(nr 10-65)	33.8 ± 14.1	24.8±13	0.002
Dkk1 (pmol/L)	23.3 ± 13.1	29.8±15.9	0.009

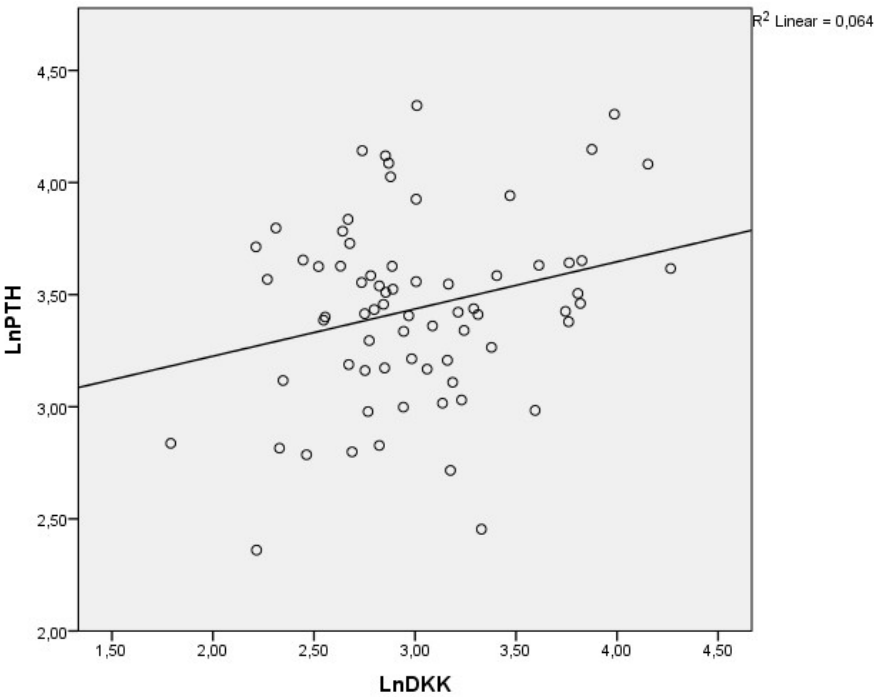
Values have been reported as median ± interquartile range. PTH = parathyroid hormone, Dkk1 = Dickkopf1, nr = normal range. AS: ankylosing spondylitis, HC = healthy controls

This part of the study had as purpose to understand the role of PTH in AS and its relation with bone turnover modulators, especially Dkk1 and also with disease

characteristics. We found PTH serum levels, compared to HC, to be significantly higher in AS patients (33.8±14.11 vs 24.8 ±13 pg/ml, p=0.002, table 3).

The other laboratory variables did not reach any significant difference between patients and controls. We observed at bivariate correlation analysis a strong negative correlation between PTH and 25OH-vitamin D (PTH as Ln, Pearson's coefficient -0.39 p=0.001) and a positive correlation between Dkk1 and PTH serum levels (both Ln, correlation coefficient +0.25, p=0.03, Fig. 1).

Figure 1. Correlation between PTH and Dkk1 (Ln scales).



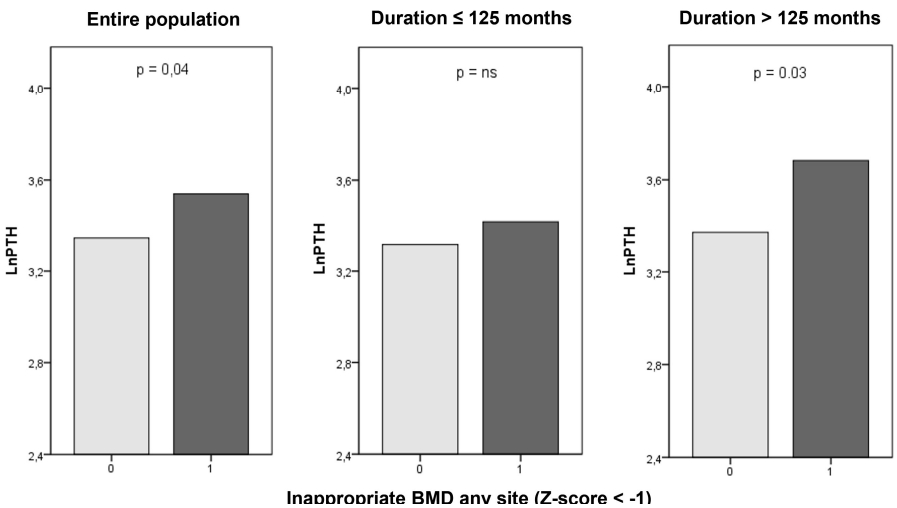
Dkk1 in pmol/l as Ln scale. PTH in pg/ml as Ln scale. Dkk1 = Dickkopf 1, PTH = parathyroid hormone.

We tested this last correlation also after correction for 25OH vitamin D levels without losing in significance (Ln, correlation coefficient=0.267, p=0.02). Moreover, we performed a multiple regression analysis testing on the predictors of Dkk1 and we

observed that it was not influenced by age, BMI, disease duration and CRP level whereas PTH remained significant (Ln, β= 0.284, p=0.02).

The study population was divided in two equal groups for size according to disease duration (above or below the median duration, 125 months). The two groups were tested separately for Dkk1-PTH correlation and this remained only in the group with longer disease duration and Dkk1 in this group was also positively correlated also with CTX (Ln, coefficient 0.42, p=0.01). Those two groups were not statistically different for Dkk1, CRP or PTH serum levels. Looking at bone mineral content, PTH (Ln) was found to be higher in those patients with observed inappropriate low BMD (Z-score ≤-1) at any site (p=0.04), and in the subanalysis by the same grouping above mentioned this relation remained again only in the group with longer disease duration (Fig. 2).

Figure 2. LnPTH according to the presence of inappropriate low BMD.



LnPTH = parathyroid hormone on logarithmic scale, Inappropriate bone mineral density at any site defined as Z-score <-1: 0 =absent, 1= present.

Moreover, patients with syndesmophytes showed a longer disease duration (179.6±122.1 vs 102.8±81.3, p=0.003) and a tendency to a higher serum PTH (Ln, 3.35±0.43 vs 3.54±0.37, p=0.06).

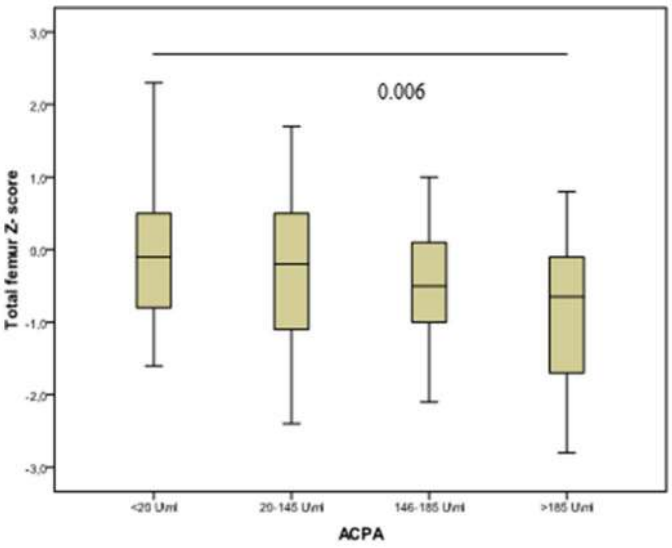
Dkk1(Ln) was also found to be higher in NSAIDs users (p=0.05).

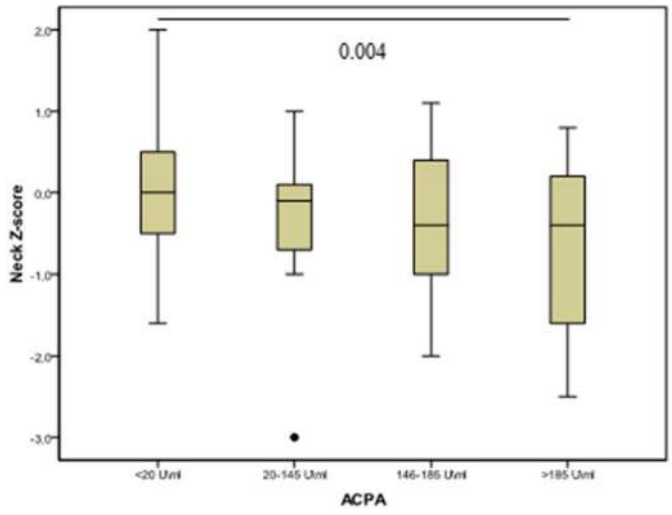
4.2 ANTI CITRULLINATED PROTEINS ANTIBODIES IN RHEUMATOID ARTHRITIS.

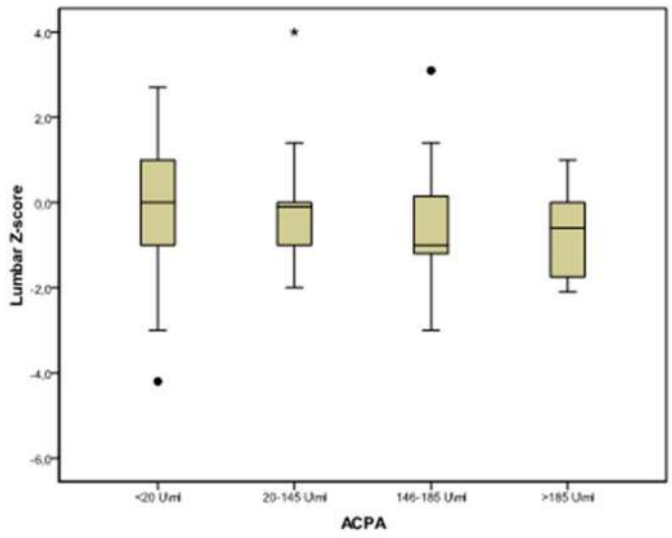
We enrolled 127 adult patients with RA according to EULAR/ACR 2010 criteria (51), among them the 78.7% were females and 59.8% were seropositive for ACPA. At observation they have in mean a high disease activity (DAS 28 CRP 4.86 ± 1.46) and median HAQ was 0.750 ± 0.875. Most of them were taking conventional synthetic disease modifying anti rheumatic drugs (csDMARDS), being methotrexate in 65% and leflunomide in 22% of them; half of the population (54%) were taking glucocorticoids. Normal BMD T-score at all sites was present only in 21.3% of the patients. Univariate model analysis (table 4) showed lower BMI (p=0.003), shorter disease duration (p=0.014) and a higher cumulative steroid dose (p=0.055) in ACPA-positive patients; no difference in terms of disease activity as measured at the moment of DXA assessment. ACPA-positive patients had also lower femoral Z-scores (p=0.006 for total femur and p=0.001 for femoral neck), and a higher osteoporosis prevalence compared to ACPA negatives ones (37.5 % vs 15.9%, p=0.02), lumbar Z-score showed an almost significant trend to be lower in ACPA-positives (p=0.059).

ACPA titer showed an inverse correlation with femoral neck, total femur and lumbar Z-score (r=-0.297 and p=0.001, r=-0.256 and p=0.004, r=-0.175 and p=0.055, respectively). Considering Z-scores at femur and spine accordingly to ACPA levels, defined as negative, within the first, second or third tertile of positive values, there was a statistically significant difference between groups for the femoral sites (p=0.008 for total and p=0.004 for neck) (Fig.3).

Figure 3. Hip and spine Z-scores according to anti citrullinated protein antibodies (ACPA) titers.







Z-scores were also negative correlated with steroid cumulative dose (r=-0.217 and p=0.022 for total femur; r=-0.254 and p=0.007 for femoral neck) whereas they seem to be unrelated to smoking habit, disease duration or DAS28-CRP.

Two distinct multivariate analysis model were applied: the first included ACPA seropositivity as dichotomous variable with a cutoff of 20 U/ml, the second ACPA divided into negative, within the first, second or third tertile of positivity; the results of these analysis are displayed in Table 5.

Model 1 shows that Z-scores were not different between ACPA positive and negative patients, while in model 2 the higher was ACPA titer the lower hip Z-scores, both at total femur (p=0.002) and femoral neck (p=0.03). In particular, estimated marginal mean was 0.1 (CI95%: -0.2 to 0.5) in negative patients and -0.8 (CI95%: -1.6 to 0.1) in patients with ACPA>185 U/ml for total femur Z-score, while 0.0 (CI95%: -0.3 to 0.3) in negative patients and -0.6 (CI95%: -1.4 to 0.2) in patients with ACPA>185 U/ml for neck Z-score.

Table 4. Population characteristics according to ACPA status.

		ACPA		p value
		No (n=63)	Yes (n=64)	
Age (years)#		58.3 (13.2)	57.3 (12.8)	0.658
Sex§	Male	13 (20.6)	14 (21.9)	0.864
	Female	50 (79.4)	50 (78.1)	
BMI (kg/m^2)#		27.7 (4.5)	25.5 (3.7)	0.003
Smoking habit (past or current)§		10 (15.9)	14 (21.9)	0.498
Disease duration (months)*		48 (71)	76 (174)	0.014
Cumulative steroid dose (g)*		4.5 (10.4)	18.5 (17.5)	0.055
DAS28 CRP#		4.48 (1.51)	4.86 (1.40)	0.152
ESR (mm/h)*		29 (33)	34 (25)	0.435
CRP (mg/l)*		3.2 (4.7)	4.5 (6.5)	0.202
Bone health§	Normal	15 (23.8)	12 (18.8)	0.022
	Osteopenia	38 (60.3)	28 (43.8)	
	Osteoporosis	10 (15.9)	24 (37.5)	
Total femur Z- score#		0.0 (0.9)	-0.5 (1.0)	0.006
Femoral Neck Z-score#		0.1 (0.8)	-0.4 (0.9)	0.001
Lumbar Z-score#		-0.1 (1.4)	-0.5 (1.2)	0.059

*Abbreviations: ACPA=anti-citrullinated protein antibodies, BMI=body mass index, DAS28 CRP=disease activity score on 28 joints, ESR=erythrocyte sedimentation rate, CRP= C-reactive protein.# values expressed as mean (standard deviation), § values expressed as absolute number (percentage), * values expressed as median (interquartile range).*

Table 5. Multivariate analysis for Z-scores BMD.

			Total femur Z-score		Neck Z-score		Lumbar Z-score	
			p value	B (CI 95%)	p value	B (CI 95%)	p value	B (CI 95%)
Model 1	BMI		<0.001	0.091 (0.030 to 0.151)	<0.001	0.100 (0.063 to 0.137)	0.004	0.091 (0.030 to 0.151)
	Cumulative steroid dose		0.022	-0.012 (-0.212 to 0.189)	0.014	-0.153 (-0.274 to -0.032)	0.908	-0.012 (-0.212 to 0.189)
	Disease duration		0.525	0.037 (-0.245 to 0.319)	0.940	0.006 (-0.161 to 0.174)	0.795	0.037 (-0.245 to 0.319)
	Smoking habit	No vs. Yes	0.200	0.123 (-0.683 to 0.929)	0.764	0.197 (-0.299 to 0.692)	0.826	0.123 (-0.683 to 0.929)
	ACPA*	Negative vs. positive	0.130	0.494 (-0.740 to 1.728)	0.149	0.440 (-0.320 to 1.201)	0.196	0.494 (-0.740 to 1.728)
Model 2	BMI		<0.001	0.129 (0.089 to 0.168)	<0.001	0.104 (0.067 to 0.140)	0.003	0.094 (0.032 to 0.157)
	Cumulative steroid dose		0.121	-0.102 (-0.233 vs 0.028)	0.066	-0.114 (-0.235 to 0.007)	0.753	0.033 (-0.174 to 0.239)
	Disease duration		0.819	0.020 (-0.156 to 0.196)	0.087	-0.24 (-0.189 to 0.140)	0.938	0.011 (-0.275 to 0.297)
	Smoking habit	No vs. Yes	0.819	0.359 (-1.300 to 2.018)	0.354	0.435 (-1.114 to 1.983)	0.433	1.186 (-1.426 to 3.798)
		<20 vs. >185		1.303 (-0.471 to 3.077)		0.887 (-0.769 to 2.543)		1.672 (-1.104 to 4.448)
	ACPA§	20-145 vs >185	0.002	1.897 (0.117 to 3.678)	0.030	1.136 (-0.526 to 2.798)	0.145	1.889 (-0.900 to 4.678)
		146-185 vs >185		-0.147 (-1.926-1.632)		-0.163 (-1.824 to 1,497)		0.673 (-2.110 to 3.457)

*In model 1 ACPA * are expressed as positive (i.e. ≥20 U\ml) or negative (i.e. <20 U\ml). In model 2 ACPA § are expressed as negative (<20 U\ml), positives at 20-145 U\ml, 146-185 U\ml, >185 U\ml. Abbreviations: ACPA=anti-citrullinated protein antibodies, BMI=body mass index, BMD=bone mineral density*

4.3 TNFα INHIBITORS IN RHEUMATOID ARTHRITIS.

Table 6: Main clinical characteristics of the RA-TNFI population.

Gender	Male= 9 (17%) Female= 45 (83%)
Age (years)	51.6 ± 13.3
Disease duration (months)	63 ± 18
BMI (kg/m2)	25.7 ± 4.6
CRP mg/L	13.5 ± 19.4 mg/L
DAS28 CRP	4.6±1.3
TNFI Infliximab Etanercept Adalimumab Golimumab Certolizumab	100% 5 (9.3%) 6 (11.1%) 22 (40.7%) 4 (7.4%) 17 (31.5%)
DMARDs Taking Hydroxychloroquine Methotrexate Leflunomide Cyclosporine None	42 (77.8%) 1 (1.9%) 37 (68.5%) 2 (3.7%) 2 (3.7%) 12 (22.2%)
Glucocorticoids Taking Not Taking	 29 (53.7%) 25 (46.3%)
Mean daily dose (mg)* (among users)	6.7± 2.5
P1NP (ng/ml)	43.5 ± 21.2
bAP (µg/l)	11.5 ± 4.6
CTX (ng/ml)	0.340 ± 0.208
BMD lumbar spine (mg/cm^2)	993 ± 194
BMD femoral neck (mg/cm^2)	787 ± 145

** = prednisone equivalents; DMARD= disease modifying anti-rheumatic drugs; CRP= C reactive protein; P1NP= intact N-propeptide of type I collagen; bAP = bone alkaline phosphatase; CTX= C-terminal telopeptide of type I collagen; BMD = bone mineral density. TNFI = TNFα inhibitor, RA = rheumatoid arthritis. Variables are reported as mean ± standard deviation or absolute number (percentages) as appropriate.*

In table 6 are summarized the main characteristics of the RA population enrolled for this part of the study. Most of the patients were females (83%) mostly in postmenopausal state (89%) for an average of 12.1 years. Only the 16.7% had osteoporosis defined as a

T-score below -2.5 and the 11% had an history of fracture being fragility ones only in half of the cases. The 78% were taking a csDMARD, the remaining did not due to intolerance or contraindication. Corticosteroids were used by the 54% with a dose between 5 and 15 mg/day (mean 6.7mg/day). They had a high disease activity according to the mean DAS28-CRP (mean 4.6±1.3) and a mean HAQ of 0.97 ± 0.72.

As per protocol they all received TNF inhibition therapy being adalimumab in 40.7%, certolizumab in 31.5%, etanercept in 11.1%, infliximab in 9.3% and golimumab in 7,4% of the patients.

CRP values at baseline ranged from 0.5 to 93 mg/L (mean 13.3±19.2mg/L), bone turnover markers (BTM) were within normal range in all patients.

Table 7: Six month changes (absolute and % means ± standard deviation) in clinical measured parameters after TNFI.

	Absolute changes	P value	% changes	P value
DAS28	-1.48±1.40	<0.001	-29.4±26.6	<0.001
CRP (mg/l)	-7.8±20.9	<0.001	-39.1±58.6	<0.001
P1NP (ng(ml)	8.5±18	0.001	30.6±64.3	0.001
bAP (µg/l)	1±2.38	0.003	8.6±20.7	0.003
CTX (ng/ml)	0.047±0.188	0.07	44.9±130.6	0.01
PTH (pg/mL)	6.7±11.7	0.002	32±55	0.002
Dkk1 (pmol/L)	-2.9±12.1	0.05	-4±34	Ns
Sclerostin (pmol/L)	0.2±7.3	Ns	4±24	Ns
25OHVitD (ng/ml)	-3.3±10.8	Ns	-5±49	Ns
Spine BMD	4.1±50.7	Not significant	0.2±5.2	Not significant
Neck BMD	-14.1±34.6	0.004	-1.8±4.6	0.005

DAS28 = Disease activity score, CRP= C reactive protein; P1NP= intact N-propeptide of type I collagen; bAP = bone alkaline phosphatase; CTX= C-terminal telopeptide of type I collagen; PTH = parathyroid hormone, Dkk1 = Dickkopf-1, 25OHVitD = 25OH vitamin D, BMD = bone mineral density. TNFI = TNFα inhibitor. Variables are reported as mean ± standard deviation.

At 6 months after TNFI therapy start we registered the expected clinical improvements with 42% patients achieving remission and 58.3% at least a low disease activity; 56.3% experienced a good EULAR response (55) and 75% at least a moderate one. DAS28 CRP decreased meanly of -1.5 ± 1.4 ($p<0.001$) and CRP of 7.8 ± 20.9 mg/L ($p=0.026$)

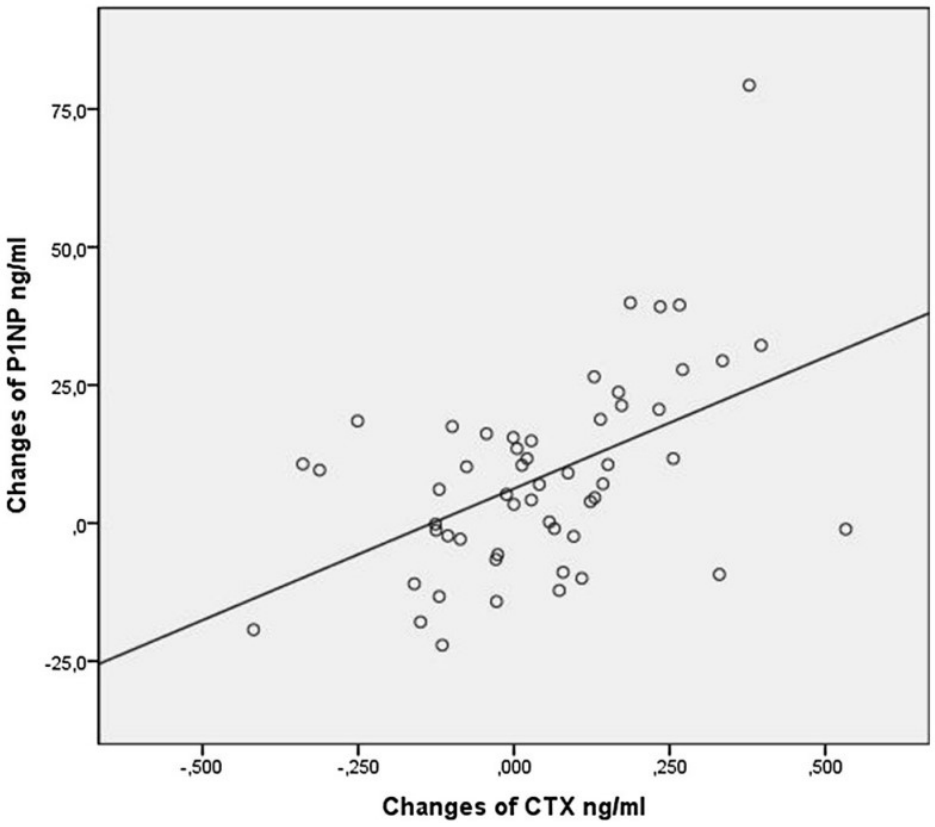
We assisted to a raise of all serum BTM measured: P1NP +30.6% ± 64.3% ($p=0.001$), bAP +8.6% ± 20.7% ($p=0.003$), CTX +44.9 ± 130.6% ($p=0.01$) (Table 7).

The study population has been split in two groups on the basis of whether the daily steroids dose at follow up was decreased (n=14) or remained unchanged (n=40) including in the latter group also the patients without glucocorticoid therapy since the beginning of the study (n=29). The mean daily dose of prednisone was 5.3 mg in the patients (n=11) with stable dose and from 7.7 mg to 2.2 mg in the reduced dose group.

The same analysis on BTM changes was performed by group with similar results, the only difference was that CTX increase did not reach significance in the reduced dose group. No significant difference was observed between group even if the changes showed a trend to be larger in the reduced dose group (data not shown).

In the whole population the 6-months changes of CTX and P1NP showed to be positively correlated in a linear regression model ($p<0.001$, $R^2=0.25$) (Figure 4).

Figure 4. Correlation between absolute changes of P1NP and those of CTX after 6 months of TNFα inhibitor therapy.



P1NP= intact N-propeptide of type I collagen; CTX= C-terminal telopeptide of type I collagen; TNFI = Tumor necrosis factor inhibitors.

Serum PTH increased significantly after 6 months by a mean of 22.8 % (p=0.002) Fig 5). that was not influenced by the trend in daily steroid dose (data not shown). PTH variation showed a positive correlation with the CTX one (R^2 =0.303, p=0.027).

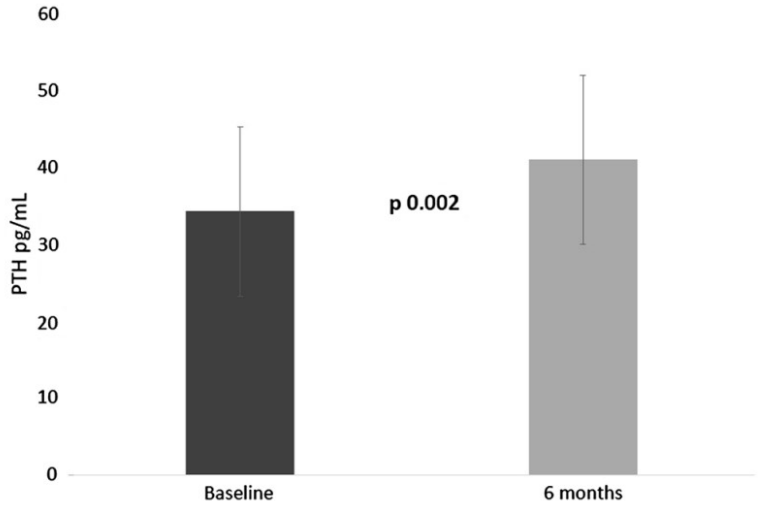
No significant changes in serum levels of 25OHVitD were observed during the 6 months of TNFI therapy (all patients were taking oral supplementation: 800 IU/day or weekly equivalent).

Dkk1 serum level decreased by a mean of 2.9 pmol/L (p=0.05); in particular, in the group with stable dose of steroids the decrease was greater and significant (-4.4 ± 12.1

pmol/l, p=0.026). Moreover, Dkk1 showed a positive correlation with PTH levels (R^2 =0.231, p=0.001). (Fig 6)

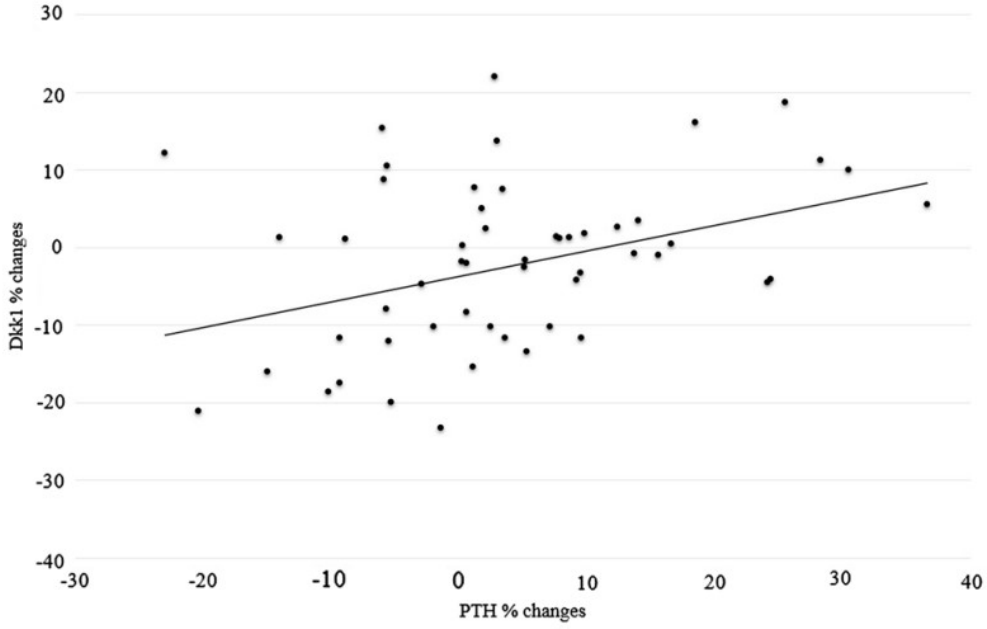
Sclerostin did not show any significant change with TNF administration or by different group of steroid trend.

Figure 5. Mean PTH serum levels (±SD) at baseline and after 6 months of TNF inhibitor therapy.



PTH = parathyroid hormone, TNF = tumor necrosis factor, SD = standard deviation

Figure 6. Correlation between percentage changes of PTH and those of Dkk1 after 6 months of TNF inhibitor therapy.



Dkk1 = Dickkopf-1, PTH = parathyroid hormone, TNF = tumor necrosis factor

No variation in lumbar BMD was reported whereas femoral neck BMD decreased (-1.8 ± 4.6%, $p<0.01$). We also tested if there were significant differences in BMD or bone markers changes regarding the achieving of remission or the presence of a DMARD through an independent samples T-test without finding any. No interactions (ANCOVA) were observed between the observed changes in bone turnover markers, gender or menopausal status and disease activity scores.

CHAPTER 5: DISCUSSION

. This project investigates multiple aspects of inflammatory arthropathies: different specific diseases (RA and AS), different settings (homeostasis and effect of treatment) and different biomarkers.

Besides the different aspects there is a unifying concept emerging from recent literature and our presented data: systemic bone health, disease pathophysiology and radiographic progression are entwined. In this tight relationship many factors play a role: immune system with ACPA and cytokines, calcium metabolism with vitamin D and PTH, Wnt pathway with its modulatory molecules (Dkk1, sclerostin). All these factors affects directly and indirectly each other resulting in final bone health and radiologic damage.

In the following paragraphs every specific part of the study will be discussed.

5.1 ANKYLOSING SPONDYLITIS.

In a previous work we demonstrated not only that AS patients, consistently with a bone apposing disease, had lower levels of Wnt inhibitors such sclerostin and Dkk1 but also that the latter correlated negatively with BMD and vertebral fractures (19).

In this paper we further analyzed our samples and data in order to investigate the determinants of Dkk1 finding that this was correlated with PTH serum levels.

This relation has been reported also in other condition like RA (22, 56), primary hyperparathyroidism (24) and following teriparatide administration (21).

Moreover, there are other evidences in literature suggesting that those systems are linked. In fact, either a constant PTH stimulation and Dkk1 could lead to an increased activation of the RANK/RANKL axis and osteoclastogenesis (57-59).

In the setting of RA we previously reported a positive correlation also between Dkk1 and CTX(22); in the AS population studied the same association was not present but when we divided the population in two groups according to disease duration, the correlation Dkk1-PTH and Dkk1-CTX were present and significant only in the longer duration of disease group.

A recent study by Boussoualim et al (60) supports our data, they reported that PTH serum levels are negatively correlated to trabecular bone score and BMD values and positively with disease duration even after multiple variables adjustments.

These data could also represent one possible explanation to the fact that epidemiologic studies indicates disease duration as a major risk factor for vertebral fractures and low BMD (61-66).

The relation pointed out between the metabolic factor (PTH) and the Wnt system seem to be the determinant of the bone content loss and fragility clinically observed in the later stage of the disease.

The role of the Wnt system in AS is still a matter of debate with probably different effects at local and systemic level or depending on the stage. There are authors reporting Dkk1 levels in AS to be lower than normal (17, 19) or higher but dysfunctional (67). In another osteoproliferative inflammatory arthropathy, PSA, we recently observed Dkk1 serum levels lower than RA patient and HC (68).

Intriguing is also the data of higher Dkk1 levels among NSAIDs users reported also by another author (18) that could offer a rationale for the slight positive effect on radiographic progression (69). This data could be also explained by the dose dependent effect of prostaglandin on Wnt/β catenin pathway even if this interplay is not completely understood (70).

5.2 ANTI CITRULLINATED PROTEINS ANTIBODIES IN RHEUMATOID ARTHRITIS.

We demonstrated that in established RA ACPA could influence negatively systemic BMD in a titer dependent manner with results similar to the findings of Bugatti et al in early RA (31).

This relation observed in clinical setting has a strong pathophysiologic background. Harre et al reported that ACPA can promotes osteoclastogenesis from hematopoietic precursors as seen in animal specimen treated with ACPA. Moreover ACPA correlated with markers of bone resorptive activity such as CTX, tartrate resistant acid phosphatase and cathepsin K (29). Of interest is the report that osteoclasts and monocytes express peptidylarginine deaminase (PADI), the enzyme responsible for protein citrullination, similarly to cells of the inflamed synovium of RA patients (71-73). Recently Krishnamurthy et al described that ACPA exert their effect on osteoclast through a PAD-dependent IL-8 mediated autocrine loop.

Another important and well known stimulator of osteoclasts activity and differentiation is RANKL; its level could be increased upon cytokines stimulation such TNFα, IL-1 and IL-6 and it has been proposed as biomarker of bone damage in RA (74-78). In fact, RANKL has been described to be elevated and associated with erosions in ACPA positive treatment-naïve early RA patients (79). Besides the effects on osteoclasts ACPA seems also to impair osteoblasts function (80). All these data support the detrimental role of ACPA on bone health.

The negative impact of ACPA seropositivity on periarticular bone is a more consolidated data.

Boyesen at al showed in a longitudinal cohort of RA patients that ACPA were predictors of a higher hand bone loss measured through radiogrammametry (DXR) (81). A very interesting study of Kleyer et al showed through micro-CT evaluation at metacarpal site a decrease in bone cortical thickness and an increase in porosity in ACPA-positive healthy subjects (30).

Our results suggest that the same detrimental effect exerted by ACPA at metacarpal site are present at systemic level especially in those site, like the femoral one, with a prevalence of cortical bone as in metacarpal bone.

The other study present on systemic bone health and ACPA, by Bugatti et al (31), reported a negative effect of ACPA on systemic bone present at either low and high titers at lumbar site whereas for the femoral site this was true only at high titer. They also found an addictive negative effect of rheumatoid factor in ACPA highly positive patients.

Both studies showed a negative effect of ACPA on systemic bone, the differences could be explained by the different RA population involved. Their population was composed by early RA patients opposed to ours of established RA were other confounding factors may have masked an effect at the lumbar site. Our stronger evidence at the cortical site may be more pathogenetically appealing considering that RA erosions involve cortical sites.

Considering previous data on highly erosive patients showing higher values of serum PTH (56), we can hypothesize a synergic effect of ACPA and PTH towards systemic bone resorption, especially cortical one.

On that basis systemic bone loss might be relevant also on erosion risk and not only for osteoporosis and fragility fracture risk.

In the literature there are studies indicating a tight relation between hand periarticular bone content and loss and development of erosions (82-84).

We also reported that femoral, but not spine, BMD was significantly lower in those RA patients with erosion compared to the ones without erosion suggesting a connection between systemic and focal bone loss (56).

More evidence comes from the use of denosumab in RA patients which proved to improve cortical BMD, cortical porosity, prevent metacarpal bone loss and progression of bone erosions (27, 85-88).

Among our population we found a correlation between the cumulative dose of corticosteroids and BMD at hip site but not at the spine giving probably reason of a higher reliability of BMD measurement at the hip.

Finally it is important to underline that our population were taking with good adherence vitamin D supplements minimizing the possible negative role of vitamin D deficiency elsewhere described (31). This supplementation was very important as vitamin D has been described to be more frequently deficient in ACPA seropositive patients (89).

5.3 TNFα INHIBITORS IN RHEUMATOID ARTHRITIS

The objective of this part of the study was to investigate the short term effect of TNFI on BTM.

TNF α inhibitors (TNFI) administration brought, as expected, an amelioration in disease activity with a decrease of CRP and DAS28 CRP.

In our population we registered an increase of both bone formation (bAP) and bone resorption (CTX) markers together with a small, statistically significant, reduction in femoral neck BMD.

A large part of the literature is discordant with such data, with different results among the few studies available on the topic and almost none reported an increased bone turnover (36-49).

A hypothesis of the different results could be the coadministration of other drugs targeted to the bone such as calcium, vitamin D and bisphosphonates or drugs with influence on bone and those medications were often not registered. Another hypothesis is that very often the study analyzes the co-therapy at the beginning but not their variation during the study. Changes in corticosteroid daily dose, a common practice when TNFI control the disease (or during flares), can greatly influence bone metabolism.

We decided to limit the observation to the first 6 months of treatment considering that the return to physical activity due to disease control could influence bone metabolism (90) and also that steroids and other medication could have multiple changes in a longer period adding more confounders. Moreover, it was also unethical to prohibit further the introduction of bone protecting agent in those patients unable to reduce corticosteroid under 5 mg/day or found to be osteoporotic at BMD measurement.

Bone formation markers (P1NP and bAP) increment should be seen as unrelated to the tapering of corticosteroids as the same changes were observed both in the subgroup reducing steroid and in the constant dose one.

We hypothesize that TNFI have an effect similar to teriparatide administration (91) where at the beginning there's a transient increase in bone turnover with a slight decrease in BMD before it starts to increase, It may be possible that with TNFI happens the same and if we could have observed patients longer without confounder they would have shown a gain in BMD.

This theory is also supported by the strong correlation found between CTX and P1NP that indicate the rapid coupling of formation and resorption. It is possible also that bone formation need a little more time to recover as also subtle inflammation could inhibit osteoblasts (92).

A step further of this part has been to investigate also the role of PTH and Wnt pathway in this setting of RA taking into account that they are two key factors of bone turnover regulation and that they may influence each other.

Those are the first presented data on PTH and its relation with bone turnover markers after TNFI administration, moreover studying Wnt modulators such as sclerostin and Dkk1 at the same time.

Surprisingly, we observed an increase in PTH serum level after TNFI. One of the main physiological drive for PTH increase is a decrease in vitamin D levels (93) and in our population those were unchanged with also unchanged supplementation. The same observation is true also for renal function, creatinine levels did not raise during the study (data not shown).

It could be hypothesized that TNFα could inhibit PTH and with TNFI we remove this blockade allowing PTH levels to raise. There are studies in the literature both in Crohn disease and in vitro involving the use of TNFI supporting this hypothesis, they observed low basal level of PTH to raise after drug exposure too (94-96).

Of interest is the correlation between changes in Dkk1 and PTH serum levels similar to what we observed in RA, hyperparathyroidism or teriparatide treatment (21, 22, 24).

PTH changes were also correlated to CTX ones reflecting the relation between PTH and the resorption process.

TNFI effect on bone is complex: it increases the turnover, it reduces Dkk1 favoring bone formation but conversely it also increases PTH with deleterious effect on bone.

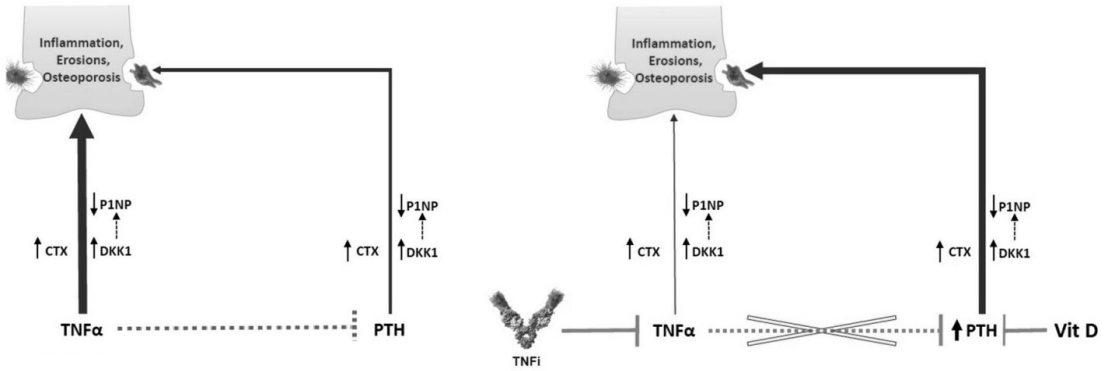
This behavior of PTH, similar to effect cited during teriparatide treatment (91), give further explanation to the effect observed in femoral BMD.

The negative variation in femoral BMD is probably an epiphenomenon of the increased bone turnover more than a true negative effect and moreover the entity, although statistically significant, is tiny and of doubtful clinical meaning.

Sclerostin levels did not shown any significant change, probably its levels are unrelated to Dkk1 levels and there is no feedback mechanism.

CTX and Dkk1 are important factors related to systemic bone loss and erosive damage in RA and they showed a strong relationship with PTH levels (22, 32, 97). The data presented might imply that PTH is involved in RA manifestation and also in TNFI failure (Fig.7).

Figure 7. Hypothesized role of TNFα, PTH, TNFα inhibitors and vitamin D in bone involvement in rheumatoid arthritis.



P1NP= intact N-propeptide of type I collagen; CTX= C-terminal telopeptide of type I collagen; PTH = parathyroid hormone, Dkk1 = Dickkopf-1, Vit D = vitamin D, TNFI = TNFα inhibitor, TNFα = tumor necrosis factor α.

The underlined role of PTH could have strong clinical implications, suggesting that a generous vitamin D supplementation, in order to keep PTH level normal/low could be important in RA treatment together with immunosuppressive drugs. Adequate vitamin D level, lowering PTH, might blunt the bone losing effect of RA at both systemic and articular levels.

5.4 LIMITATIONS

There are some limitations in the paper presented, the first and second part involved patient in a cross sectional design. The AS and the RA-TNFI groups were quite small in sample size.

The population of the second part was heterogeneous by DMARD treatment even if we could not find any difference between DMARD users and not-users, moreover the study wasn't powered enough to obtain information on different treatment regimen. A great limitation was also that in this part we excluded the probably most interesting population: the patients at higher risk of fracture and that because they were taking bisphosphonate that would have been a too high confounder to our purpose.

Another consideration to make on ACPA patients is that ACPA may characterize a subset of patients with other factors, such different levels and types of cytokines, that could influence bone independently from ACPA.

About the third part of the study a possible source of bias is again the heterogeneity of the population and the presence of patients taking glucocorticoids, these problems have been minimized keeping all fixed except the TNFI administration and the glucocorticoid dose tracked.

Giving the limitations above, although significant, some of these data need further confirmation especially through longitudinal and larger studies that can better investigate the associations of factors and also permit subanalysis with adjunctive variables.

CHAPTER 6: CONCLUSIONS

In conclusion, those data, even if coming from different settings of disease and therapy, underlined the tight relationship between the immune system and bone cells.

This interplay takes place through different mechanism but the Wnt pathway with his inhibitors (Dkk1 and sclerostin) and the parathyroid hormone seems to play a major role in both systemic bone health and radiographic progression of disease at local level. Moreover, these two systems seem to affect each other acting together to give the resulting effect on bone.

Together with those metabolic axes the fact that ACPA antibodies can have a detrimental effect on systemic bone is of utmost importance in the complexity of bone involvement in RA.

All those data, even if they need further investigation, give an important message, that bone should be taken into high consideration when treating patients for two main reasons.

First, all these factors lead to a higher risk of low BMD and fracture in both arthritis and spondylitis patients. Second treating the patients with bone targeted drugs and may be also with higher dose of vitamin D would probably lead to beneficial effects on disease control and damage as some initial evidence suggests (88, 98).

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