INFLUENCE OF ANTI-TNF α TREATMENT IN BONE METABOLISM MARKERS

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Abstract

Chronic inflammatory joint diseases are a heterogeneous group of disorders associated with local and systemic bone loss. Tumor necrosis factor alpha (TNFα), a pivotal pro-inflammatory cytokine common in the pathogenesis of these diseases, is known to induce bone loss by increasing osteoclast recruitment and activity. Anti-TNFα antibodies used in the treatment of inflammatory arthropaties may influence the risk of osteoporosis, by preventing bone loss. The exact mechanism of preventing bone loss has not yet clearly assessed.

The main purpose of this work was to examine the fluctuation of biochemical markers of bone metabolism induced by three different anti-TNF agents (Infliximab, Etanercept and Adalimumab), taking into account their different pharmacokinetic profiles.

We evaluate patients with inflammatory rheumatic diseases treated with biologics. Data on demographic characteristics, pharmacological treatment history, anti-TNF treatment duration and disease activity (Bath Ankylosing Spondylitis Disease Activity Index, BASDAI, Disease activity score for 28 joints, DAS28) were collected. Blood and urine samples were collected in the day of drug administration (day A), immediately before administration and in the estimated day of maximum plasmatic level of each drug (day I). We determined, by ELISA, serum bone-specific alkaline phosphatase (sBAP), serum osteocalcin (sOC) and urine deoxipyridinoline (uDPD) using creatinin (Cr) as a normalization factor for urine samples. Changes between days were analyzed by Paired sample T test. Statistical significance was assumed for p values <0.05.

These study enrolled 58 patients (67,2% females); 35 with rheumatoid arthritis (RA) (DAS28=3.8±1.4), 17 with ankylosing spondylitis (AS) (BASDAI=3.4±2.3) and 6 with psoriatic arthritis (PsA) (DAS28=3.1±1.1), with a mean age 48,9±14 years. The average biological treatment duration was 25±16 months. Changes in biological parameters from

baseline (day A) to day I were calculate and also presented graphically. We also performed ratios between formation and resorption markers (sOC/uDPDcr and sOC/uDPDcr).

Our study showed an oscillation towards a decrease of DPDCr, a bone resorption marker, between the day of minimum and maximum anti-TNFα antibodies plasmatic levels mainly for infliximab and etenercept groups. Contrariwise bone formation markers (OC and BAP) showed no statistical significant changes with an exception for sBAP for the Adalimumab group. The positive change on ratio bone formation/resorption markers lead to a net bone formation, which suggest that anti-TNFα antibodies prevent osteoporosis. Further research is warranted to clarify whether fluctuations and differences are reflected in the actual osteoporosis risk in these patients.

Key words:

Tumor necrosis factor alpha (TNFα)

Bone metabolism

Infliximab

Adalimumab

Etanercept

Introduction

Immune mediated inflammatory diseases are a particular group of chronic illness, which interfere substantially with patient's life quality and incur significant costs to patients and society. Chronic inflammatory joint diseases comprise an heterogeneous group of disorders characterized by chronic inflammation of synovial tissues and osteitis,[1] leading to destruction of joint cartilage and a significant bone loss.[2] These features lead to impaired function and disability, and to an increased risk of fracture. Rheumatoid arthritis (RA), spondylarthritis (SpA) such as ankylosing spondylitis (AS) and psoriatic arthritis (PsA) are all examples of diseases in which joint inflammation is linked with skeletal and bone pathology. Each disease has a unique impact on skeletal tissues but, as they share some of the mechanisms of bone remodeling, all of them are useful models for studying the influence of chronic inflammation in bone.[2]

Rheumatoid arthritis is the archetype for an inflammatory arthritis and is characterized by poliarticular synovitis with a symmetrical and additive involvement patern.[2] The chronic inflammatory process results in extensive local bone loss evidenced by justa-articular osteopenia and erosions,[3] a radiological features of "early" RA, and generalized osteoporosis, one of the most common extra-articular manifestations of the disease.[4,2,5]

Infiltration of inflammatory cells such lymphocytes, activated macrophages and plasma cells results in the marked expansion of synovial tissue with cell proliferation and villi formation, which occupy the joint space and is known as "pannus".[6,7,8] The pannus invades the articular cartilage and adjacent bone tissue and the inflammatory mediators released by these cells contribute to cartilage and bone destruction. Tumor necrosis factor alpha (TNFα) is considered to be the predominant pro-inflammatory cytokine in RA and plays a pivotal role in

the synovitis and destructive process of this disease.[5]

It is believed that disturbance of bone homeostasis is driven by the cellular action of osteoclasts. The enhance of formation of these cells in RA is due to local and systemic production of inflammatory cytokines such as TNFα, Interleukin (IL)-1, IL-6, and IL-17 as a results of an intensive crosstalk between those cells and osteoblasts, osteocytes, synovial fibroblast-like cells, and activated T and B cells which express the receptor activator of nuclear–kB ligand (RANKL), an essential mediator of osteoclastogenesis.[5] Numerous TNF family members including RANKL, TNFα, Fas ligand (FasL) and TNF–related apoptosis induced ligand (TRAIL) play pivotal roles in the differentiation, function, survival and/or apoptosis of osteoclats.[9] Binding of RANKL to the RANK receptor on the OC precursors and mature osteoclasts, leads to stimulation of several signalling pathways of osteoclast differentiation and activation.[5] RANKL synthesis is under influence of pro-inflammatory cytokines, especially TNFα [10] which are abundantly present in the inflamed synovium and the systemic circulation.[5] Besides that this high level of RANKL expression is not balanced by the production of its physiologic inhibitors, mainly osteoprotegerin (OPG)—an osteoblast-derived soluble decoy receptor of RANKL which blocks RANK-RANKL interaction, thereby inhibiting osteoclast formation.[5,10] The RANKL/OPG ratio determines the degree of proliferation and activity of osteoclasts, this system imbalance may be the final common pathway and mediator of osteoclastic bone resorption yielding a negative net effect on bone mass in RA.[5,10]

The knowledge of molecular basis of inflammatory process of RA has led to research and development of biological agents, which inactivate these cytokines. In addition to corticosteroids, non-biologic DMARD's, the most recent approved therapies (Biological

DMARD's) focus on TNFα blockade and more recently the altering IL-6 signaling and anti-lymphocyte biologic therapeutic strategies provided potent therapeutic effects in protecting bone demineralization in RA.[5] There are three monoclonal antibodies (mAb) (Infliximab, Adalimumab, Golimumab), a pegylated monoclonal antibody (Certolizumab) and a soluble receptor, which binds to soluble and membrane forms of TNFα that can counterbalance the pathological effects of TNFα in clinical use.[11,12]

Etanercept is a soluble TNFα receptor,[3] a dimeric protein that inhibits signal tranduction pathway, thus the proinflamatory activity.[11,13] Infliximab is a chimeric mAb that binds to both soluble and transmembrane TNFα and mediate programmed cell death.[11,13] Adalimumab is a recombinant human mAb that also binds to both soluble and transmembrane TNFα, thereby preventing TNFα binding to its receptor, demonstrating its effect on cell lysis and apopotosis.[11,13]

The clinical efficacy of these drugs, control of radiological progression and reduction of acute phase proteins in patients with RA, is well documented in numerous studies.[2,14,15,16] At the same time it is also known that the inhibitory potential of anti-TNF varies individually, and has been revealed to inhibit the formation of bone erosions in many patients in whom signs and symptoms remains present[17] Considering that the same cytokines are involved both on local and generalized bone loss, it is rational to speculate that biologic agents could directly perform a protective action on bone remodeling even if probably the different biologic drugs exert variable effects on local and systemic bone resorption typical of RA.[3] TNFα blockade by these biologic drugs can act directly by inhibiting the stimulatory effect of TNFα on osteoclastogenesis, but also trough the reduction of RANKL as its expression is increase in RA patients.[3] Currently, there are few data regarding the effects of treatments directed against TNFα, IL-1 and IL-6 on systemic bone loss in RA patients.[3]

Moreover, markers of bone metabolism comprise important tools in the evaluation of bone remodeling activity, as they change faster than bone mineral density (BMD) measurements used currently in clinical practice. Thus, the aim of this study as to evaluate the influence of biologic therapeutics in bone metabolism markers in patients submitted to anti-TNFα drugs.

As a specific aim this study will examine the fluctuation of biochemical markers of bone metabolism (formation and reabsorption) induced by three different anti-TNF agents (Infliximab, Etanercept and Adalimumab), taking into account their different pharmacokinetic profiles. The main reason for this study is the lack of knowledge about short-term effect of anti-TNF agents on bone markers, thus reflecting what is happening at a systemic level.

Methods

Patients and Study Design

This study included patients (n=58) with inflammatory rheumatic diseases such as rheumatoid arthritis (AR), ankylosing spondylitis (AS) and psoriatic arthritis (PsA), which were currently under biologic treatment with one of the following anti-TNF agents (Adalimumab, n=6; Etanercept, n=24 and Infliximab, n=28) for at least 8 weeks. They were randomly selected from the pool of patients under biologic treatment in the Rheumatology Department of Centro Hospitalar Universitário de Coimbra. The treatment regimes for these biologics agents were as follows: Etanercept 25 mg twice/week, subcutaneously, Adalimumab 40 mg each 2 weeks, subcutaneously and Infliximab 3mg/Kg every 8 weeks for RA patients and 5 mg/Kg every 6 weeks for PsA and AS patients. Stable non-biological DMARDs, oral glucocorticoids (maximum 10 mg/day prednisone or equivalent) or nonsteroidal anti-inflammatory drugs were permitted throughout the study. Data on demographics, pharmacological treatment history, anti-TNF treatment duration, disease activity (Bath Ankylosing Spondylitis Disease Activity Index, BASDAI; Disease activity score for 28 joints, DAS28), functional status (Bath Ankylosing Spondylitis Functional Index, BASFI; Health Assessment Questionnaire, HAQ) were collected from patients' files, after obtaining informed consent. Blood and urine samples (1^{st} or 2^{nd} morning urine after 12 hour's fasting) were collected in the day of drug administration immediately before administration (day A / baseline), and in the estimated day of maximum plasmatic level of each drug (day I- the 7^{th} and the 3^{th} day after administration of Adalimumab and Etanercept respectively). For Infliximab day I was assigned to the 28^{th} or 21^{st} day after administration, for 8/8 and 6/6 week regimes, respectively.[13,18,19] Only patients with a baseline sample and the post dosing sample were included.

Biochemical Marker Assays

Blood and urine samples, from both days (baseline and day of maximum concentration) were centrifuged at 3000 rpm during 15 minutes at room temperature. Several serum and urine supernatant aliquots were conserved at -80ºC until they were used for analysis of C-reactive protein level (CRP), erythrocyte sedimentation rate (ESR) and bone metabolism. In order to evaluate bone metabolism we choose 2 bone formation markers: serum bone-specific alkaline phosphatase (sBAP) and osteocalcin (sOC), and urine deoxipyridinoline (uDPD) as bone resorption marker all quantified by ELISA (Quidel Corporation, San Diego, USA). Urine creatinin (Cr) was measured in accordance with the modified Jaffe colorimetric reaction and used as a normalization factor for the urine DPD marker. The sensitivity of the abovementioned ELISA assays were 0,7 U/L, 0,45 ng/ml, 0,020 ng/ml, 1,1 nmol/L, respectively. The change in biological parameters from baseline (day A) to day I were calculate as (biological parameter day I - biological parameter day A)/ biological parameter day A). Given that the literature is not consensual in this respect, we also performed several other approaches such as (Δ)= (biological parameter Day I - biological parameter Day A) and ratio between formation and resorption (sOC/uDPDcr and sOC/uDPDcr) after converting to the same units.

Statistics

All the results from bone metabolism markers are presented as the mean ± standard deviation (SD) unless otherwise statement. Parametric and non-parametric tests were employed to assess differences with normally and non-normally distributed variables as estimated by the Kolmogorov-Smirnov Test. To compare each marker concentrations' on day A and I within treatment groups we used Paired sample T test. Statistical analysis and data processing were

performed using IBM SPSS Statistics software, version 22. Statistical evaluations were conducted at the 5% level of significance (2-tailed) (*p* values <0.05).

Results

Patient Demographics

Fifty-eight patients, with rheumatoid arthritis (RA, n=35), ankylosing spondylitis (AS, n=17) or psoriatic arthritis (PsA, n=6) were included. These patients comprise three subgroups according to type of therapy instituted (Infliximab, Etanercept and Adalimumab), and these where the bases of our comparisons. Baseline main clinical characteristics are listed in Table 1, categorized by type of treatment. The whole group comprised 39 women and 19 men, with a mean ± SD age of 48,9±14,0 years, mean ± SD disease duration of 15 years (range from 2 years to 42 years). The disease activity for each group were as follows: in patients with RA (DAS28=3.8±1.4), in AS (BASDAI=3.4±2.3) and in PsA (DAS28=3.1±1.1). Taking all together these patients were under biological treatment for 24.9±16.1 months (Table 1). During study forty two (72,4%) were under metotrexate treatment (median 12 mg/day), thirty four (58,6%) were taking corticosteroids (median 5 mg/day), twenty-three (39,7%) were taking calcium and twenty-four (41,4%) cholecalciferol, eighteen (31%) were taking biphosphonates, twelve (20,7%) were taking AINES and just one patient was doing hormonal replacement therapy.

As described in Table 1 there where no significant differences in demographic characteristics between groups (type of treatment) and the adalimumab group was the one that shown smallest disease duration, biological treatment period.

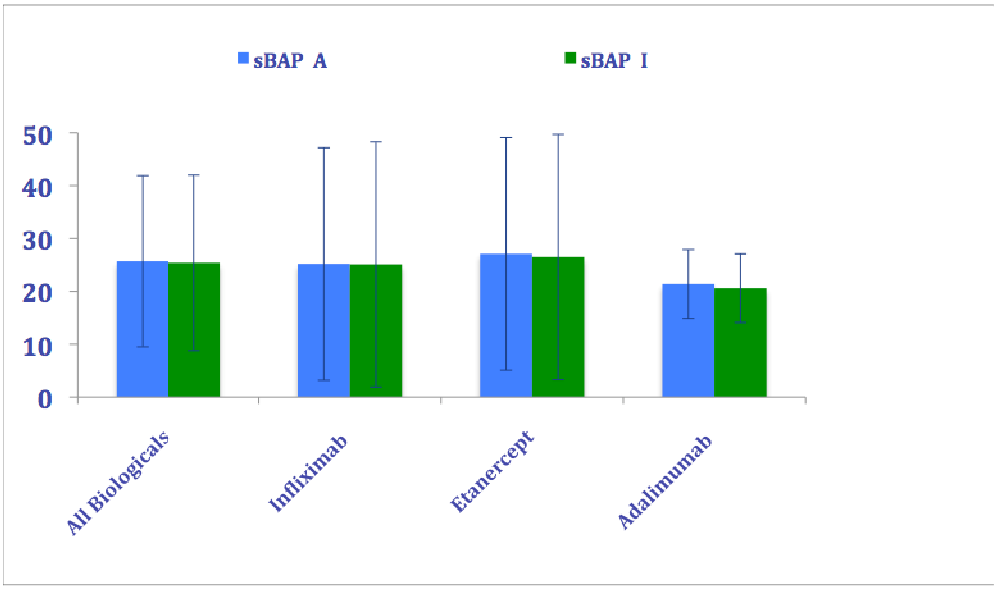
Table 1. Baseline demographic characteristics of patients, disease activity and treatment duration by type of treatment

	All Biologicals (n=58)/100%	Infliximab (n=28)/48,3%	Etanercept (n=24)/41,4%	Adalimumab (n=6)/10,3%
Age, years				
Mean± SD	48,9±14,0	48,6±14,5	49,8±14,4	46,2±11,6
Median	51	50,5	51,5	47
(Min, Max)	(14,75)	(17,75)	(14,72)	(27,56)
Sex, n/%				
Female	39/67,2	16/57,1	18/75	5/83,3
Male	19/32,8	12/42,9	6/25	1/16,7
Biological Treatment duration, months				
Mean± SD	24,9±16,1	28,6±18,9	21,3±10,8	17,4±13,8
Median	20,5	26	20	15,3
(Min, Max)	(3, 73)	(5,73)	(3,44)	(3,36)
Disease duration, years				
Mean± SD	14,6±9,0	14,1±8,2	15,5±10,4	11,7±614
Median	15	15	15,5	13
(Min, Max)	(2, 42)	(2,30)	(2,42)	(5,17)
Weight, kg				
Mean± SD	67.3 ±12.0	67.4±12.7	67.3±12.2	65.7±6
(Min, Max)	(42, 92)	(42, 87)	(46, 92)	(60, 72)

Temporal Changes in Biomarker Levels Following Treatment

When we observed the mean± standard deviation (SD) for each bone metabolism marker for day A and day I within each drug, as shown in Figure 1, it is not manifest differences between days. For the sBAP and sOC markers the mean group values for each day were similar with tiny changes (Figure 1A and B). For bone resorption we found, on day I uDPDCr mean± SD lower than day A for each group (Figure 1 C)

A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sBAP I
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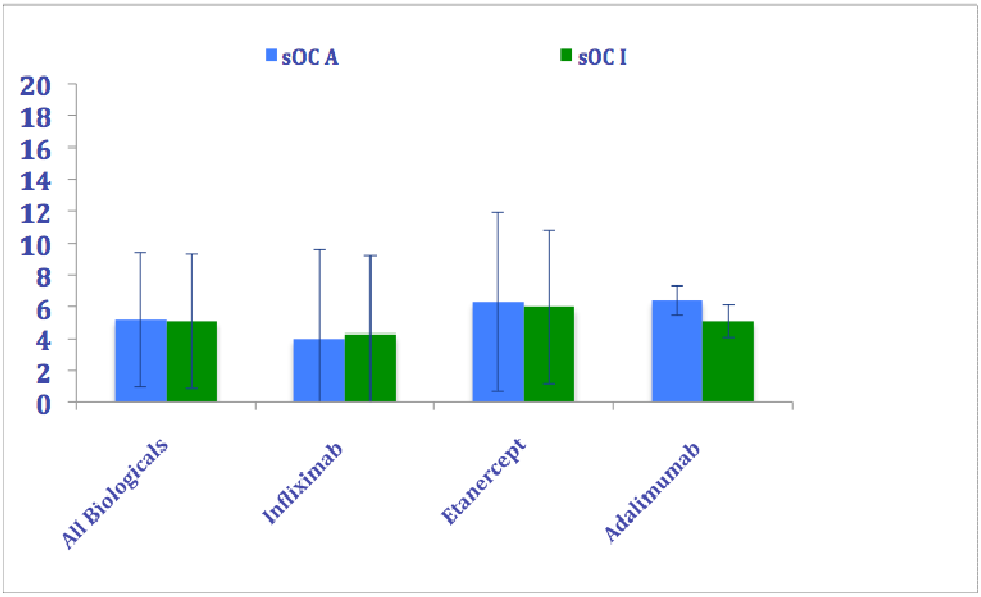
All Biologicals
Infliximab
Etanercept
Adalimumab

B


sOC A
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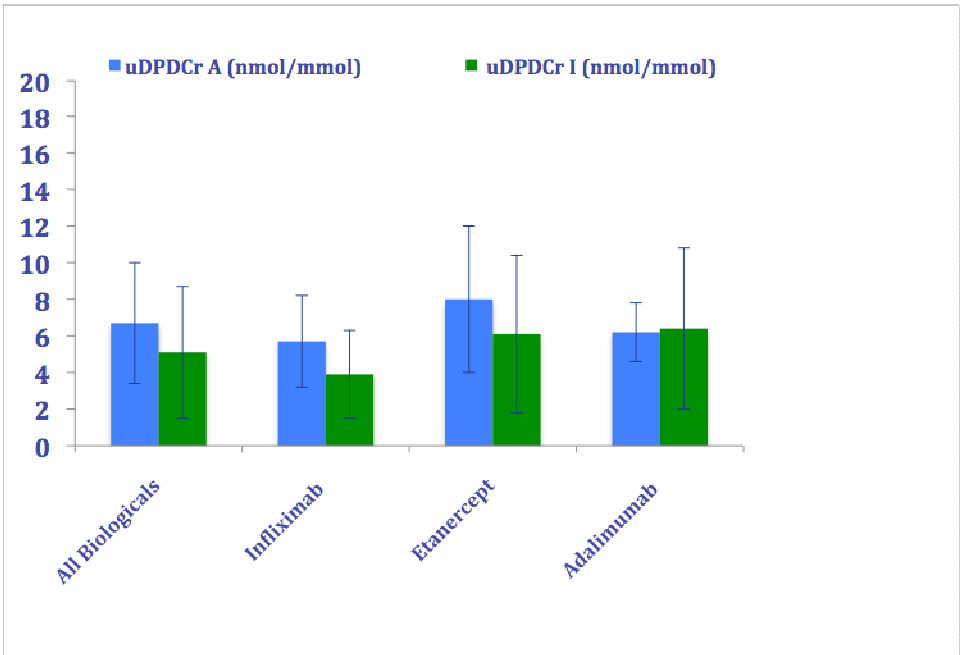
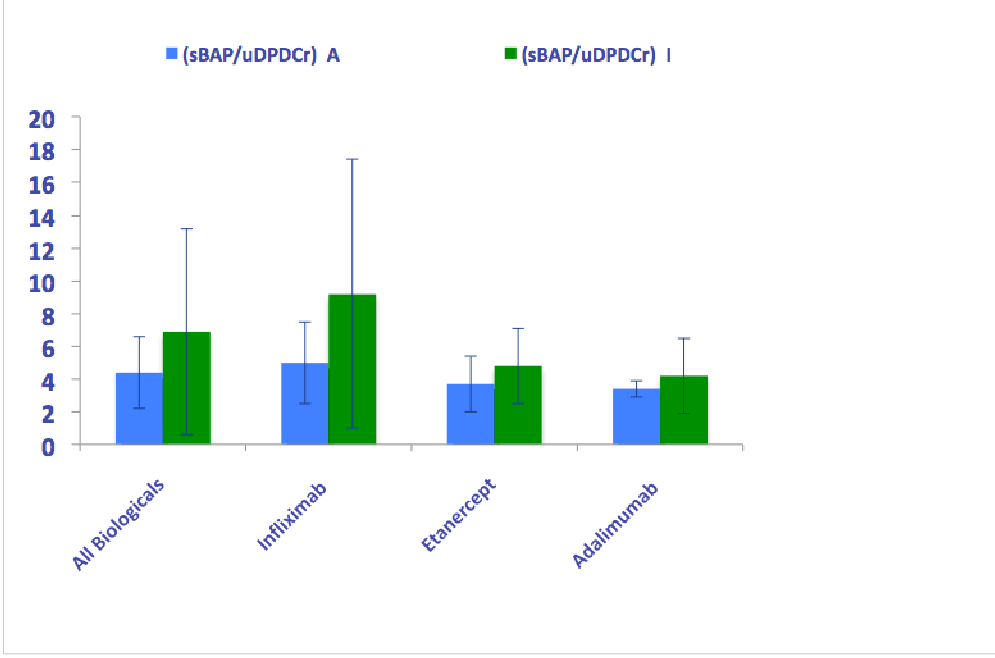


Figure 1– Mean and standard deviation values for Day A and Day I in patients treated with anti-TNFα by type of treatment for **A. Serum bone alkaline phosphatase**; **B. Serum osteocalcin**; **C. Urine DPDcr**. (***Abreviations:*** DPD- Deoxipyridinoline, BAP- Bone Alkaline Phosphatase, OC- Osteocalcin).

At the same time in bone balance assessment through the BAP:DPDcr (Figure 2 A) or OC:DPDcr (Figure 2 B) ratios showed an overall increase toward day I for infliximab and etanercept group.

A



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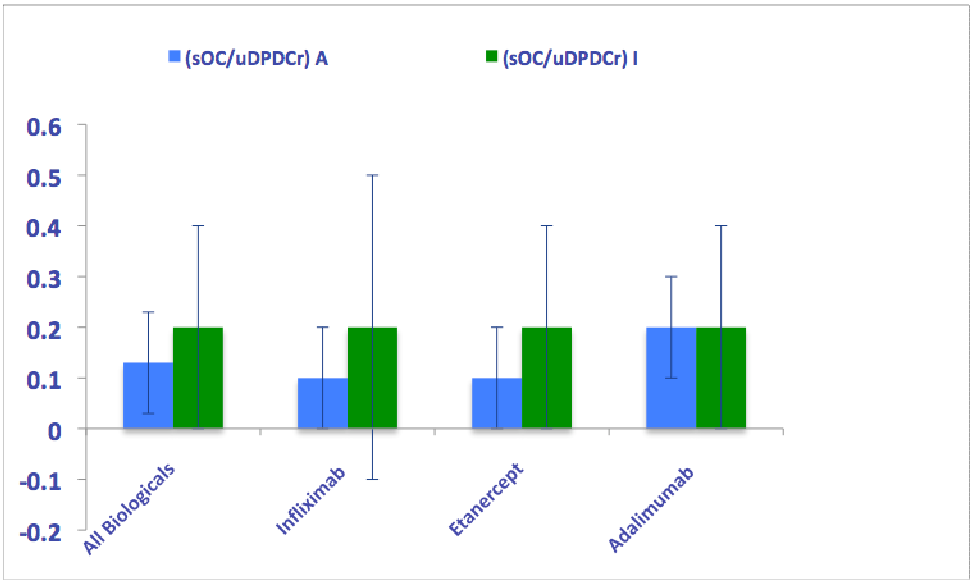


Figure 2– Mean and standard deviation values for Day A and Day I in patients treated with anti-TNFα by type of treatment for **A. Serum BAP/urine DPDCr Ratio**; **B. Serum OC/Urine DPDCr ratio** (***Abreviations:*** DPD-Deoxipyridinoline, BAP- Bone Specific Phosphatase, OC- Osteocalcin).

As the main goal of this study was to estimate the influence of biological therapeutic and examine the fluctuation induced by the three different anti-TNF agents (Infliximab, Etanercept and Adalimumab) on bone metabolism markers we performed paired sample T test that compares the means of two variables for a single group. Thus the differences between

values of the two variables were computed for each case and it was tested whether the average differs from zero. In those comparisons, as we can see in Table 2 we found paired sample statistics significance for uDPDcr, sBAP/uDPDcr and sOC/uDPDcr ratios for all biologicals, for infliximab and for etanercept groups. The adalimumab group only had statistical paired differences for sBAP (Table 2).

Table 2. Paired sample T Test (paired differences)

Delta (Δ)	All Biologicals	Infliximab	Etanercept	Adalimumab
Δ sBAP (U/L)				
Mean± SD	-0,3±3,9	-0,04±3,5	-0,6±4,6	**-0,88±0,3**
p	0,508	0,950	0,528	**0,09***
Δ sOC (ng/mL)				
Mean± SD	-0,1±2,6	+0,2±3,2	-0,3±1,8	-1,3±1,7
p	0,722	0,722	0,394	0,217
Δ uDPDCr (nmol/mmol)				
Mean± SD	**-1,7±2,8**	**-1,8±2,4**	**-1,7±3,3**	-1,3±1,4
p	**0,000***	**0,001***	**0,022***	0,416
Δ (sBAP/uDPDCr)				
Mean± SD	**2,7±5,8**	**4,2±7,6**	**1,2±2,1**	1,5±2
p	**0,001***	**0,009***	**0,019***	0,492
Δ (sOC/uDPDCr)				
Mean± SD	**0,1 ±0.2**	**0,1±0,2**	**0,052±0,1**	0,09±0,2
p	**0,002***	**0,021***	**0,027***	0,567

(***Abreviations:*** DPD- Deoxipyridinoline, BAP- Bone Alkaline Phosphatase, OC- Osteocalcin, u- urine, s- serum); **Δ=** biological parameter day I – biological parameter day A; * Statistical significance using paired sample T test analysis.

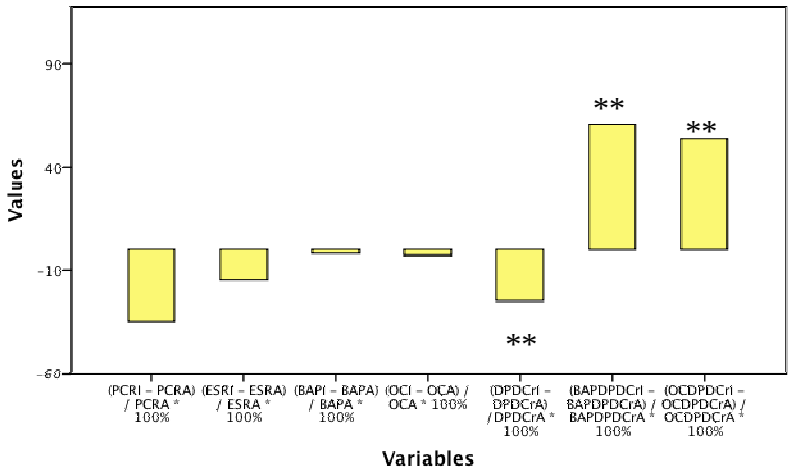
In order to better represent these differences we performed and represented graphically the change as a percent relative to baseline [(day I- day A)/day A *100)], as shown in figure 3.

Here we can observe that there was an overall decrease (expressed by the negative percentage changes), without statistical differences in CRP and ESR measurements in day I, except for ESR in the Etanercept group which had a non-significant percentage change (Figure 3 C).

Besides that, in these graphic representations of bone metabolism markers and bone ratios we saw a statistical significant decreases (uDPDcr) and increases (bone ratios) relative to baseline for all anti-TNF togheter (Figure 3A) and for infliximab and etanercept groups (Figure 3 B

and C). These changes for bone ratios rounded more than 50% and these means that, day I ratio presented, broadly, higher values which reflect a positive effect on bone balance with a decrease in resorption. The infliximab showed higher percentage changes for these bone ratios than the others biological drugs (Figure 3 B).

A



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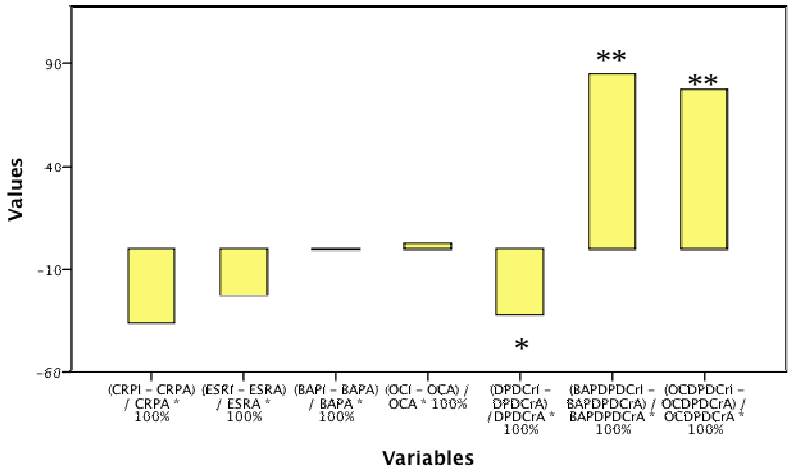

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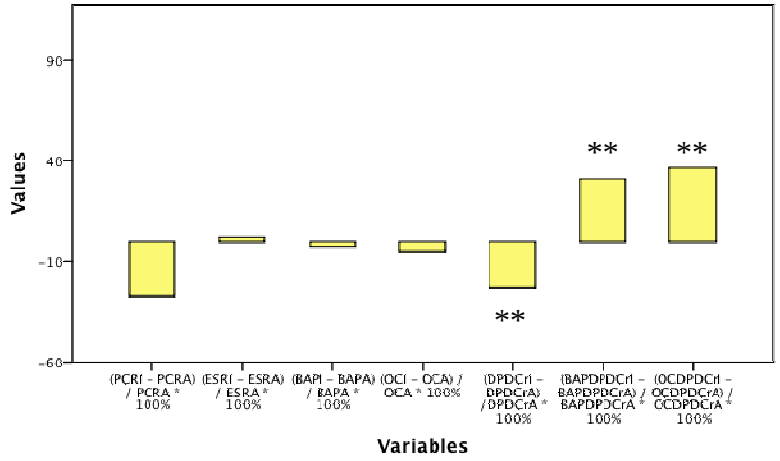

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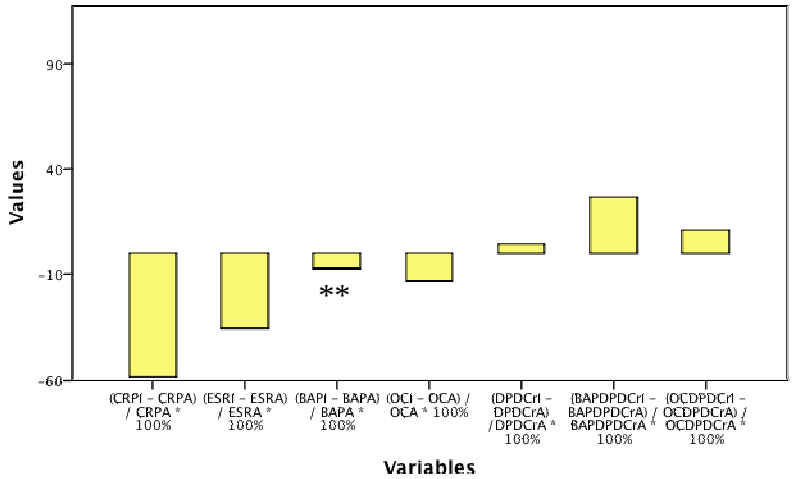

Figure 3- Mean percentage changes for biochemical (CRP, ESR), bone markers (sBAP, sOC and uDPDcr) and bone formation/bone resorption ratio, at day I relative to baseline (day A), **for all biologicals (A)**, **infliximab group (B)**, **etanercept group (C)** and **adalimumab group (D)**. ***(Abbreviations:*** C-reactive protein level, CRP; erythrocyte sedimentation rate, ESR; serum bone-specific alkaline phosphatase, sBAP; serum osteocalcin, sOC; urine deoxipyridinoline, uDPD; Creatinine, Cr). Statistical significance for paired sample T test comparisons between day A and day I *p<0.05 and **p<0.01

Discussion

In our first analysis of baseline demographic data, we deduce that this sample cohort behavior as a homogeneous subset with similar demographic characteristics (age, weight), as well disease and treatment duration. There were no significant differences between the 3 groups of biological treatment (Table 1). We also ensure that all the concomitant therapeutics didn't change during the study period. These observations allow us to perform comparisons between different biological treatment groups even with different pathologies included in each group.

The main goal of our study was to evaluate the influence/impact of each biological drug on bone metabolism, namely on maximum serum drug concentration, expressed by changes on several bone metabolism markers. In generality studies described on literature comprise long-term changes in several markers. Here, as we measure our parameters in a short period of time we've tried to provide evidence that changes might be related to the biologic itself rather than several cumulative factors arising in a long study period, such as changes in concomitant medication, disease progression, reducing active inflammation and the associated pro-inflammatory cytokines.

Recent evidences indicate that TNFα, IL-6 and IL1β[1] being important mediators of inflammation, plays a pathological role in joint destruction and osteoporosis.[20,21] These cytokines have been shown to stimulate osteoclast differentiation, increase osteoclast activation, inhibit osteoclast apoptosis and inhibit osteoblast differentiation. *In vitro* studies showed that they also reduce bone formation in cultured osteoblasts and can induce bone resorption,[20] supporting the knowledge that inflammation is one of the strong risk factor of osteoporosis. Being one of the most important cytokine in these pathologies, the anti-TNFα blockade inhibits the acute-phase response, proliferation of fibroblasts, and recruitment and activation of leukocytes,[20] thus providing significant protection against joint destruction and

osteoporosis. It is known that these inflammatory mediators in RA modify the relationship between bone formation and resorption by stimulating osteoclasts and inhibiting osteoblasts.[2] Our findings are in accordance to other studies that also reported more expressive results in bone resorption thus suggesting that TNFα antagonists may prevent the inflammatory bone demineralization in TNFα driven systemic arthritis.[5]

Biochemical markers of bone metabolism comprise a group of proteins that can be used to real-time assessment of resorption, formation and overall bone turnover.[22] Taking into account bone metabolism markers our results clearly showed (Figure 1 and Table 2) that bone resorption marker (uDPDCr) (Figure 1 C and Table 2) could predict a positive effect of infliximab and etanercept on bone turnover, as we found a statistical significant decrease thoward day I for these resorption marker. This marker showed nearly more than -25% percentage change relative to baseline with statistical significance (Figure 3 B). Our results are in accordance to others studies published.[20]

The molecules study in our work for estimate bone formation (sBAP and OC), as described also in literature, do not reveal difference/changes between days. Nevertheless, sBAP oscilation was statistically different in the Adalimumab group, with a lower value on day I. Studies in bone formation markers are not consensual. Some showed increase on OC and N-terminal propetide of type I collagen after 6 weeks of treatment with infliximab,[5,20] others showed no changes on OC (3 studies) and sBAP levels (1 study) and in another study, levels were actually decreased.[20]

An important aspect to consider in the interpretation of bone formation serum markers is the significant difference in biological half-life between sBAP (around 1.6 days) and sOC (under an hour).[22] As so, levels of osteocalcin best represent acute phenomena, while bone alkaline

phosphatase levels are more stable and reproducible.[22] Taking this into account this might be an explanation for the absence of differences between day A and I for sOC (Figure 1 B, table 2 and Figure 3).

It is important also to considerer that the changes within a certain marker will depend on intrinsic intra-individual variations[23] and on the source of those marker. Thus, urinary resorption markers require variations above 30% to be considered significant, whereas changes in serum formation and resorption markers could be slighter, in the range of 15 to 20%. This is in accordance with our results as we have lower than 15% percentage changes for the bone formation markers (Figure 3) and variations above 30 % for uDPDCr in the infliximab group (Figure 3 B).

Concerning adalimumab results, data in the literature are scarce and one study described that during adalimumab/methotrexate combination therapy, no overall erosive progression or repair occurred.[24] Our results showed a slight increase without statistical significance in uDPDcr, thus the positive percentage change for the bone ratios is also tiny, when comparing to the others biologic drugs (Figure 3). At the same time the number of patients in this group is small (n=6) so it is imprudent take any conclusions about this result.

The bone balance could be assessed in a more appropriate manner by changes in the delicate balance between bone formation and bone resorption. This measure might be crucial rather than assess the markers individually.[1] Normal bone turnover is a tight equilibrium between these parameters. Consequently the expression of bone balance through BAP:DPDCr or OC:DPDCr ratios describes a dynamic equilibrium of bone turnover, in which a higher ratio value indicates net bone gain, and a change from a lower to higher ratio level (positive *delta*) suggests a positive impact on bone.

Our results emphasis these postulate as we saw significant increase on day I mainly for the

OC:DPDCr (Figure 2 and Table 2) ratio (for all biologics) and for the BAP:DPDCr ratio for the infliximab and etanercept groups. Consequently infliximab and etanercept might have a significant positive effect favoring bone formation, expressed by more than 30% increase relative to baseline (Figure 3 B and C).

The bone balance between bone resorption and bone formation can be considered a tool for investigation of these balance, rather than an absolute measure that eventually will lead to a change in bone mass density (BMD), as the biomarkers have predicted in other studies.[25,26,28] Importantly, rather than the individual markers, the bone balance does not change, in case that bone formation and bone resorption change to the same extent. Thus the bone balance poses as a sensitive measurement of the net bone turnover. We must consider also a limitation on this study the lack of measurements of BMD in the beginning and end of the study in order to confirm conclusions of the true effect of anti-TNF therapy on bone density and the history of fractures would be the most important outcome to assess.

Conclusions

Our study showed an overall oscillation towards a decrease of uDPDCr, a bone resorption marker, on day of maximum anti-TNFα antibodies plasmatic levels. Bone formation markers (sOC and sBAP) showed no statistically significant changes. These variations suggest that anti-TNF shift the balance towards bone protection during peak plasma levels. Our results suggest that anti-TNF agents may differ on their impact upon some of the studied markers. These fluctuations are present despite long mean duration of treatment 25±16 months in our patient cohort. Further research is warranted to clarify whether these fluctuations and differences influence the prevention of osteoporosis associated with these agents. The positive effect seems to be heterogeneous in etiology. It's possible that several different mechanisms play a role in this positive effect of biologics on bone metabolism. First, reduction of TNFα, which has been shown to have a beneficial effect on bone metabolism in vitro, might also play a role in vivo. The literature support that this positive balance is also derived by reduction of other pro-inflammatory cytokines known to cause bone loss, such as IL-6 and IL-1β where biologics, is likely to play a pivotal role.[10] Finally, a third possible mechanism, also focused on literature is that improvement in general well-being and physical activity might also improve net bone formation.[10]

It is clear with this study that the improvement in the formation/resorption marker ratio suggest beneficial systemic and probably local bone effects (to be confirmed with BMD) in patients undergoing infliximab and etanercept treatment, and that these measurements may provide an important tool to include in clinical-laboratorial monitoring of these patients.

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