Bone-sparing effects of rituximab and body composition analysis in a cohort of postmenopausal women affected by rheumatoid arthritis – retrospective study

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Abstract

Objective: Osteoporosis is the most common bone tissue disease and it is characterized by a reduced bone mineral density (BMD). The main physiopathological mechanisms converge on the uncoupling between bone formation and resorption, thus leading to an enhanced risk of fractures. Several papers have documented the inverse relationships linking high inflammatory cytokines, anti-citrullinated protein antibodies, rheumatoid factor, and BMD in rheumatoid arthritis (RA). Rituximab (RTX) is a chimeric monoclonal antibody directed against the CD20 receptor of B cells. Since the Food and Drug Administration approved it for RA in 2006, there have been many clinical experiences regarding its use. Nevertheless, few studies evaluate the effect of rituximab on BMD. RA is a disease characterized by immune dysfunction with high levels of inflammatory cytokines, autoantibodies, and it is reasonable that a B cell depleting therapy could restore a physiological cytokine balance, thus exerting an osteoprotective effect on the bone tissue. The purpose of this paper is to highlight any difference in BMD and to assess differences in body composition over a retrospective 18-month follow-up period after RTX treatment with a B cell depleting therapy.

Material and methods: We analyzed by dual energy X-ray absorptiometry BMD expressed as g/cm² and body composition modifications over 18 months with RTX treatment of 20 postmenopausal RA patients.

Results: After eighteen months of therapy with RTX, a statistically significant increase in vertebral (L1–L4) BMD and the stability of femoral BMD were documented.

Conclusions: Rituximab is associated with an improvement of vertebral and preservation of femoral BMD, suggesting a bone-sparing effect due to B cell depletion. Furthermore, patients displayed a redistribution of fat masses toward the hip region.

Key words: bone, rituximab, osteoporosis, rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is defined by the latest American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria [1] as a chronic and systemic disease in which immunologically mediated inflammation of synovial joints may lead to destruction of articular structures. Osteoporosis is the most common bone tissue disease where pathophysiological mechanisms involve an uncoupling between bone formation and resorption, thus leading to a reduction of bone mineral density (BMD) and to an increased risk of fractures [2]. Bone homoeostasis is
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lyzed with DXA. Subsequently, we compared BMD of the lumbar spine, femoral neck, and whole femur with the $t$-test for paired observations if data followed a normal distribution, or using Wilcoxon’s test for paired statistics when data deviated from a Gaussian distribution. The same tests were run to compare each variable derived from body composition analysis. Results were considered statistically significant when $p < 0.05$.

**Results**

The results showed a significant increase in lumbar spine BMD ($1.031 \pm 0.11$ vs. $1.110 \pm 0.10$, $p < 0.005$), while no significant difference was found for femoral BMD. The data are shown in Figure 1 and Table II.

Erythrocyte sedimentation rate (ESR) expressed in millimeters/hour (available for only 13 matched comparisons) displayed a significant reduction from baseline ($32 \pm 16$ vs. $25 \pm 14$, $p < 0.0205$).

Lean mass and fat mass values increased from baseline, although not significantly (ALM $16.21 \pm 3.60$ vs. $17.84 \pm 4.03$; SMI $6.03 \pm 0.99$ vs. $6.42 \pm 1.85$ ns; body fat % $37.45 \pm 10.82$ vs. $40.19 \pm 8.44$ ns). The results are shown in Table III.

**Discussion**

The investigation highlighted a clear and significant increase of vertebral BMD and documented the stability of femoral mineralization in RA patients after 18 months of treatment with B cell depleting therapy. Obtained results may confirm the pathogenic role of activated B cells in bone loss in RA, accordingly with what is reported in the literature [6].

Several papers demonstrated the active role of B lymphocytes in modulating key osteoclastogenic cytokine RANKL and RANKL/osteoprotegerin (OPG) ratio, therefore actively intervening in bone homeostasis and being the bridge between immune and skeletal systems [6, 8, 9].

Osteoprotegerin belongs to the superfamily of tumor necrosis factor receptors (TNFR), exists only in soluble form and can prevent bone resorption acting as a decoy receptor binding RANKL, thus being a bone savior.

Receptor activator for NF-$\kappa$B ligand is also a part of the TNFR superfamily, but unlike OPG, which prevents bone loss, is the main actor of bone resorption by promoting osteoclast formation.

**Table II.** Differences in bone mineral density (BMD) at baseline and after 18 months of rituximab (RTX) therapy

| BMD [g/cm$^2$]         | Baseline ($n = 20$) | After 18 months ($n = 20$) | $p$-value |
|------------------------|--------------------|-----------------------------|-----------|
| Lumbar spine (L1–L4)   | $1.031 \pm 0.11$   | $1.110 \pm 0.10$           | 0.0029**  |
| Whole femur            | $0.848 \pm 0.15$   | $0.853 \pm 0.14$           | 0.622 (ns)|
| Femur neck             | $0.793 \pm 0.14$   | $0.808 \pm 0.12$           | 0.140 (ns)|

Mean values of BMD (g/cm$^2$) at baseline and after 18 months therapy with rituximab, **$p < 0.005$, ns – not statistically significant.
B lymphocytes represent an important source of OPG, nevertheless during inflammatory circumstances OPG production decreases, shifting toward RANKL produced by T lymphocytes activated by pro-inflammatory cytokines. The chronic inflammatory stimulus in arthritic patients plays a pivotal role in bone loss by promoting the shift toward RANKL production in B activated lymphocytes, thus altering the RANKL/OPG ratio and facilitating BMD reduction. A significant loss of bone mineralization attributable to an immunological dysfunction of activated B cells is also described in multiple myeloma, human immunodeficiency virus (HIV) [8] and periodontal pathology [9]. Diseases very different from each other but united by RANKL/OPG ratio imbalance and by a high inflammatory load which may lead to the development of bone loss and osteoporosis.

Our results differ from those reported in other papers, where RTX therapy did not produce effects on vertebral BMD and indeed displayed bone loss at the femoral level [10]. Although these investigations were conducted longitudinally, the follow-up of patients did not exceed 12 months, a time probably insufficient to observe significant changes in BMD. None of our patients were osteoporotic at the beginning of the study and perhaps, starting from a lower degree of mineralization and a larger sample, an improvement in BMD could be observed at the femoral level too. Furthermore, increases of lumbar spine BMD and of muscle masses occurred in patients despite low dose GC therapy in 7 patients and 800 mg of methylprednisolone used as premedication during 18 months of therapy. Although the deleterious effects of GCs on bone [11] and muscle tissues [12] are known, a paper [13] demonstrated that a high dose GC pulse therapy did not lower BMD of RA patients during 18 months of follow-up.

Whether these observations are due to a direct effect of therapy on cytokine resources, or to an improvement in quality of life and in an increase of physical activity among RA individuals, remains to be investigated.

An altered body composition, such as an increase in percentage of fat mass, a reduction of muscle mass and a prevalent android fat distribution, in concert with an increased incidence of metabolic syndrome, is widely described in RA [14]. Several studies have demonstrated that blocking IL-6 [15] or TNF-α [16] produced an increase in the BMD in RA patients and improved their body composition [17]. Contrary to what we expected, RTX therapy did not cause any significant change in patients’ muscle mass or fat mass representation. The anti-CD20 receptor can be expressed by other cell lines [18] and in RA there is reported an increase in circulating TNF-α as well as IL-6 [19, 20]. Many studies highlight the correlation between metabolic syndrome, increased inflammatory state, altered body composition and cardiovascular diseases [21]. An interesting paper reported that RTX may even lower IL-6 levels [12].

Based on these assumptions, it would have been expected that in our patients, alongside the preservation of BMD, the B cell depletion therapy produced a change in patients’ body composition too.

Lack of this effect could be due to normal body fat (BF) percentage and muscle mass values displayed by most of our cases, allowing us to speculate that perhaps, in patients with an altered body composition at baseline, an improvement could also be observed in this regard as a consequence of a reduction in pro-inflammatory cytokine serum levels. There was an increase in gynoid masses, although not statistically significant.

Furthermore, an altered body composition [14, 22] and low bone mineral density are proven to share the same pathological mechanism of cardiovascular diseases [23], and this paper may prompt further research on the topic by highlighting the bone sparing effect in RA patients treated with RTX: an impairment of the RANKL/OPG ratio is reported in correlation with arterial atherosclerosis [24] and in an increase of cardiovascular risk in RA [25], and thus RTX may play a cardioprotective effect.

### Table III. Changes in body analysis composition at baseline and after 18 months of rituximab (RTX) therapy

| Body composition analysis | Baseline | After 18 months | p-value |
|--------------------------|----------|----------------|--------|
| Total lean mass [kg]     | 39.94 ± 8.74 | 38.64 ± 8.19 | 0.278 (ns) |
| BF [%]                   | 37.45 ± 10.82 | 40.19 ± 8.44 | 0.248 (ns) |
| ALM [kg]                 | 16.21 ± 6.60 | 17.84 ± 4.03 | 0.476 (ns) |
| SMI [kg/m²]              | 6.03 ± 0.99 | 6.42 ± 1.85 | 0.445 (ns) |
| Total fat mass [kg]      | 25 ± 10.45 | 26.58 ± 9.43 | 0.382 (ns) |
| Android fat [%]          | 43.07 ± 13.33 | 45.89 ± 10.54 | 0.320 (ns) |
| Gynoid fat [%]           | 40.28 ± 10.08 | 43.02 ± 10.65 | 0.099 (ns) |
| Android/gynoid fat ratio | 1.07 ± 0.28 | 1.10 ± 0.31 | 0.999 (ns) |

Body composition features of RA patients before and after treatment with rituximab, BF – body fat, ALM – appendicular lean mass, SMI – skeletal muscle index.
in RA patients, although further investigations are needed to ascertain the validity of this hypothesis.

This study had several limitations due to its retrospective nature: it was not possible to further stratify patients according to their serum vitamin D levels, smoking habits, previous therapies and immunological status. Therefore, it will be necessary to assess prospectively and for an adequate time the effects of RTX on bone homeostasis and body composition.

Conclusions

Our study demonstrated that 20 non-osteoporotic RA patients had a significant increase in vertebral BMD concentration of this biological drug. Spite a bolus of GCs for premedication prior to administration of this biological drug.

The authors declare no conflict of interest.

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