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REVIEW

Biologic therapies and bone loss in rheumatoid arthritis

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C. R. Uña[4] · J. D. Adachi[5] · W. F. Lems[6] · C. Cooper[7,8,9] · N. E. Lane[10,11] · on behalf of
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Abstract

Introduction Rheumatoid arthritis (RA) is a common systemic autoimmune disease of unknown cause, characterized by a chronic, symmetric, and progressive inflammatory polyarthritis. One of the most deleterious effects induced by the chronic inflammation of RA is bone loss. During the last 15 years, the better knowledge of the cytokine network involved in RA allowed the development of potent inhibitors of the inflammatory process classified as biological DMARDs. These new drugs are very effective in the inhibition of inflammation, but there are only few studies regarding their role in bone protection. The principal aim of this review was to show the evidence of the principal biologic therapies and bone loss in RA, focusing on their effects on bone mineral density, bone turnover markers, and fragility fractures.
Methods Using the PICOST methodology, two coauthors (PC, LM-S) conducted the search using the following MESH terms: rheumatoid arthritis, osteoporosis, clinical trials, TNF- antagonists, infliximab, adalimumab, etanercept, certolizumab, golimumab, IL-6 antagonists, IL-1 antagonists, abatacept, tocilizumab, rituximab, bone mineral density, bone markers, and fractures. The search was conducted electronically and manually from the following databases: Medline and Science Direct. The search period included articles from 2003 to 2015. The selection included only original adult human research written in English. Titles were retrieved and the same two authors independently selected the relevant studies for a full text. The retrieved selected studies were also reviewed completing the search for relevant articles. The first search included 904 titles from which 253 titles were selected. The agreement on the selection among researchers resulted in a Kappa statistic of 0.95 (p < 0.000). Only 248 abstracts evaluated were included in the acronym PICOST. The final selection included only 28 studies, derived from the systematic search. Additionally, a manual search in the bibliography of the selected articles was made and included into the text and into the section of "small molecules of new agents."
Conclusion Treatment with biologic drugs is associated with the decrease in bone loss. Studies with anti-TNF blocking agents show preservation or increase in spine and hip BMD and also a better profile of bone markers. Most of these studies were performed with infliximab. Only three epidemiological

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studies analyzed the effect on fractures after anti-TNF blocking agent's treatment. IL-6 blocking agents also showed improvement in localized bone loss not seen with anti-TNF agents. There are a few studies with rituximab and abatacept. Although several studies reported favorable actions of biologic therapies on bone protection, there are still unmet needs for studies regarding their actions on the risk of bone fractures.

Introduction

Rheumatoid arthritis (RA) is a common systemic autoimmune disease of unknown cause, characterized by a chronic, symmetric, and progressive inflammatory polyarthritis [1]. Affected patients often experience inflammatory signs in the joints of the hands, wrists, and feet, but many other joints may be involved including the temporo-mandibular joints, elbows, shoulders, hips, knees, and ankles. A mono-articular involvement may occur initially, but the articular signs of inflammation usually become symmetrical. Many patients complain of joint stiffness early in the morning that can last for more than 1 h. The duration of this sensation is in direct proportion with the degree of the articular inflammation. Although considered primarily a disease of the joints, many extra-articular manifestations can develop during RA clinical course.

One of the most deleterious effects induced by the chronic inflammation of RA is bone loss [2, 3].

Bone loss in rheumatoid arthritis

Bone loss often occurs in chronic inflammatory diseases and can be diagnosed in rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel diseases, systemic lupus erythematosus, psoriasis, and many others [4]. During the development of chronic inflammation, a large amount of body energy is diverted to the activated immune system, and this leads to signs and symptoms that may enhance bone loss (Fig. 1). Anorexia, malnutrition, muscle wasting, cachexia, and depression are directly or indirectly related to this persistent allocation of energy to the cytokine network activation. Decreased functional capacity and lack of exercises associated with joint pain and deformities impair the development of a healthy life and also contribute to progressive bone loss. An excellent and comprehensive review of the evolutionary and adaptive aspects of bone loss in chronic diseases and the concept of sick behavior can be found in a recent publication by Straub et al. [6].

The use of corticosteroids during RA treatment, even as a small dose of prednisone 5 mg/day or equivalent for more than 3 months, is associated with a fast and persistent loss of bone [7]. After the initiation of oral glucocorticoid therapy, bone loss progresses quickly in the initial 3–6 months and fractures may occur in the first 6 months of treatment. Bone loss occurs mainly at the trabecular bone leading to increased risk of vertebral fractures, but cortical bones may also be affected. One study showed that continuous treatment with prednisone 10 mg/day during 90 days or more increased by 17-fold the risk of vertebral fractures and by 7-fold the risk of hip fractures [8].

Three different forms of skeletal involvement can be seen in patients with RA, and they are associated with a common pathophysiologic mechanism: alteration in bone remodeling.

The first is a peri-articular bone loss or "juxta-articular osteoporosis" related to a modification in the bone remodeling favoring bone resorption. There is a loss of peri-articular cortical and trabecular bone, which usually appears at the beginning of the disease and can be easily seen in hand radiographs.

A second form of bone loss in RA is characterized by marginal bone erosion. The immediate peri-articular cortical bone is lost as a consequence of synovial membrane inflammation.

A third pattern is a generalized osteoporosis involving the skeleton as a whole, even at distant sites of joint inflammation.

The prevalence of osteoporosis in RA is high compared to aged similar controls and can become a severe co-morbidity [9]. The risk of fracture is increased at vertebral and appendicular sites of the skeleton [10, 11]. Patients affected by RA, mainly those with high disease activity, have a twofold risk of developing osteoporosis compared to the general population and almost the double risk for hip and vertebral fractures independent of the adverse effects of corticosteroids therapy on bone mass [12–15]. Risk factors for vertebral fractures in RA include high inflammatory disease activity (high CRP), the presence of bone erosions, and long disease duration [6, 16, 17]. In addition, risk factors for generalized osteoporosis in RA subjects include long disease duration [18] and/or increased levels of biochemical markers of bone and cartilage degradation [19].

The fracture risk assessment tool FRAX, the most frequently used tool to determine fracture risk worldwide, has RA as one of the seven most important risk factors for fragility fractures [20].

The persistence of chronic inflammation in postmenopausal women, the main population affected by RA, adds a risk factor for the loss of bone mass and fractures in an already susceptible individual. RA patients have more loss of bone mass in peripheral bones than in the axial skeleton, which is in contrast with the characteristic vertebral bone loss seen in postmenopausal women [21]. Accelerated loss of bone mineral density in the hands has been associated with progressive joint disease in the hands and feet at the beginning of the disease [22].
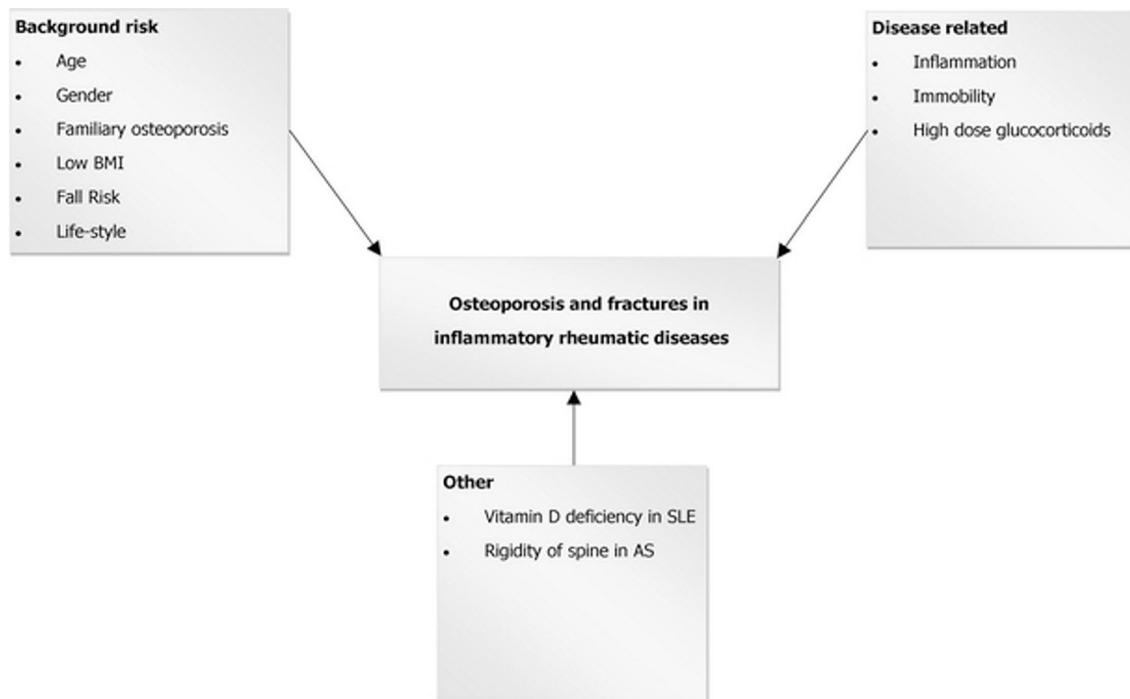
Fig. 1 Risk factors for osteoporosis and fractures in inflammatory rheumatic diseases. *AS* ankylosing spondylitis, *BMI* body mass index, *SLE* systemic lupus erythematosus [5]; with permission of W.F. Lems

Although disease duration and activity are the most important variables regarding the risk of systemic osteoporosis, bone loss may also occur in a preclinical phase, before the appearance of the first clinical symptoms [23, 24], which can only be disclosed by laboratory markers of inflammation such as C-reactive protein [25]. More recently, a link was described between the development of anti-citrullinated antibodies and bone loss leading to the speculation that early events of autoimmunity in RA may already be associated with adverse effects on bone, even before a clinical diagnosis is made [26] (see below).

Mechanisms of bone destruction in RA

The concomitant occurrence of inflammation and bone loss seen in RA is also present in other systemic inflammatory diseases with involvement of the immune system such as spondyloarthrites (ankylosing spondylitis, psoriatic arthritis) and inflammatory bowel diseases (Crohn's disease and ulcerative colitis). In these conditions, the close tie between inflammation and bone loss is directly linked to the interactions between cells of the immune system and those of bone. The study of these interactions has led to the development of the new field of research named Osteoimmunology [27, 28]. In the last 20 years, the advances in this field have provided to a better understanding of the molecular and cellular pathways linking the immune system and bone, allowing the development of new and better therapeutic approaches.

The health and maintenance of bones depend on the remodeling process characterized by coupled and balanced activities of bone resorption and bone formation. All forms of osteoporosis in inflammatory diseases are mediated by an imbalance in bone remodeling in favor of reabsorption. Osteoclasts, the bone-resorbing cells, are stimulated by inflammatory cytokines in different phases of their lifespan promoting bone loss in different parts of the skeleton.

Osteoclasts are large multinucleated cells, members of the monocyte/macrophage family, with the particular property of degrading the organic and inorganic parts of bone tissue. The presence, in the inflamed synovium, of a large amount of mononuclear cells favors the local development of osteoclasts from which they are derived. The production of local and systemic cytokines, mainly M-CSF, IL-17, TNF-α, IL-1, and IL-6, stimulates the recruitment of osteoclast precursors and regulates osteoclast formation and function. Pro-inflammatory cytokines regulate osteoclastogenesis by mediating some initial steps in its development. Early and non-specific differentiation of the monocytic cell to an early osteoclast precursor depends, in part, of macrophage proliferation and the survival cytokine M-CSF [29]. TNF-α may induce the expression of special receptors in the surface of monocytic cells promoting their differentiation in osteoclasts [30]. The activation of the receptor activator of nuclear factor-kB (RANK) on the early osteoclast precursor membrane by RANK ligand (RANKL) allows the commitment of the cell to the mature osteoclast. RANKL is the key molecule involved in the control of the osteoclast differentiation. This

molecule is mainly produced by osteoblasts and osteocytes but may be also expressed by other cells including activated T and B cells, chondrocytes, and synovial fibroblast-like cells. RANKL binds to the RANK in osteoclast precursors and mature cells leading to its differentiation and resorbing action. The inflammatory cytokines are important stimulators of RANKL synthesis, and its overwhelming production during the inflammatory process exceeds the production of its physiologic inhibitor and decoy receptor osteoprotegerin (OPG). The imbalance of RANKL/OPG ratio is directly responsible for bone loss in rheumatoid arthritis and other inflammatory diseases. Inflammatory cytokines can also influence osteoblastic function.

Osteoblasts are bone-forming cells that synthesize bone matrix, mainly type 1 collagen, but also other types of collagens in small amounts and other proteins like osteocalcin, osteopontin, thombospondin, sialoprotein, and osteoprotegerin. The differentiation and function of osteoblasts are stimulated by the Wingless (Wnt) proteins, which can induce the OPG production, thereby reducing the stimulus for reabsorption promoted by RANKL. TNF-α is a potent inducer of the protein dickkopf-1 (Dkk1), an inhibitor of the Wnt signal found in high serum levels of RA patients [31, 32]. The elevated production of Dkk-1 induced by TNF-α reduces the Wnt-induced production of OPG, which results in an increase in the RANKL/OPG ratio and an acceleration of osteoclast resorption leading to bone loss.

The TH17 subset of T cells is also implicated in the osteoclast-mediated bone resorption associated with RA synovial inflammation. These cells have the capacity to produce RANKL, TNF-α, and IL-17, a cytokine with the capacity to induce RANKL in mesenchymal cells and thus enhance osteoclast development [33, 34].

The progressive bone loss at sites of synovial inflammation is the result of an orchestrated production and action of different cytokines. IL-1 and IL-6 produced by T cells and activated macrophages have the capacity to up-regulate RANKL, increasing the survival and the resorbing activity of the osteoclasts [35–37]. A study in postmenopausal women with RA showed that IL-6 trans-signaling was predictive of the RANKL/OPG ratio [38]. IL-6 secreted by osteocytes undergoing apoptosis may regulate the adhesion of osteoclast precursors by enhancing the expression of the vascular endothelial adhesion molecule ICAM-1 [39].

Osteocytes comprise 90 to 95 % of all bone cells in adult skeleton and can live for decades in the mineralized bone tissue [40]. Osteocytes are descendants of osteoprogenitor mesenchymal cells through differentiation of osteoblasts. Osteocytogenesis, which is the transformation of osteoblasts in osteocytes, is an active process that includes the action of metalloproteinases on the cleavage of collagen and other bone matrix proteins as fibrin and fibronectin [41]. Osteocytes have their cell bodies encased in lacunae throughout the mineralized matrix and are connected to each other and other bone cells through a large network of dendritic processes traveling inside canaliculi (very small channels). Osteocytes have many functions in the bone metabolism: (1) they can act as a sensor of mechanical loading through their large channels network; (2) they behave as an endocrine cell, secreting many soluble factors with paracrine and endocrine actions, as the regulation of phosphate homeostasis through the production of FGF23; and (3) they play an important role as a regulator of bone remodeling through modulation of both osteoblast and osteoclast activity. They may stimulate bone formation and mineralization through the phosphate-regulating neutral endopeptidase on the chromosome X (Phex) and dentin matrix protein (DMP1) or promote their inhibition through the production of sclerostin and MEPE/OF45. Osteocytes induce osteoclast formation and activation through their death by apoptosis. Dying or apoptotic osteocytes, appearing in unloaded bone or at sites of microdamage, release apoptotic bodies expressing RANKL that can recruit and activate osteoclasts [42]. Bakker et al. made an interesting in vitro experiment to determine the relation between IL-6 and osteocyte mechano-sensitivity. MLO-Y4 osteocytes were incubated with/without IL-6 for 24 h. After this period, osteocytes were subjected to mechanical loading by pulsating fluid flow for 1 h. The results suggested that IL-6 is produced by shear-loaded osteocytes and that IL-6 may modulate osteocyte communication with osteoblasts [43].

Interleukins may interfere with functions of osteocytes. Estrogen deficiency increases serum levels of TNF-α and IL-1, which is reported to induce osteocyte apoptosis [40, 42]. Dkk-1 and sclerostin, which are potent inhibitors of osteoblast and bone formation, are highly expressed in osteocytes. As mentioned before, TNF-α is a potent stimulator of the production of Dkk-1, an inhibitor of the Wnt signal on osteoblasts, and is found in high serum levels of RA patients. Bakker et al. investigated the role of TNF-α and IL1-β in the modulation of the osteocyte response to mechanical loading. Osteocytes were maintained in culture and treated with TNF-α or IL1-β and exposed to mechanical loading by a pulsatile fluid flow technique. The cell response was measured by nitric oxide (NO) production. Both TNF-α and IL1-β inhibited the osteocyte mechanical loading–induced NO production and IL1-β also stimulated osteocyte apoptosis. The investigators suggested that this was a potential mechanism to explain how inflammatory cytokines could induce bone loss in RA [44]. Heiland et al. treated TNF transgenic mice with neutralizing antibodies against TNF, Dkk-1, or both, and analyzed bone architecture, gene expression of β-catenin, osteoprotegerin, and osteocalcin. They made also measurements of Dkk-1 and sclerostin in osteoblast cultures stimulated with TNF-α. Blockade of Dkk-1 completely protected the transgenic mice from inflammatory bone loss. This blockade was also associated with enhanced expression

of β-catenin, osteocalcin, and osteoprotegerin, and it neutralized TNF-induced sclerostin expression [45].

Biologic agents used in the treatment of RA may have a beneficial action on the osteocytes, and these actions may prevent inflammatory bone loss. These observations will need to be tested in clinical studies.

RA serum markers and bone loss

Most RA patients produce the immunoglobulin rheumatoid factor (RF). Testing for IgM-RF is associated with a high specificity and sensibility for RA (80 and 70 %, respectively). High serum levels of RF are associated with joint damage, radiographic progression, and systemic extra-articular features [46, 47]. RF may be negative in early RA, becoming positive as the disease progresses. Recently, diagnosis of RA has been changed to include the serologic detection of anti-citrullinated protein antibodies (ACPAs) [48]. Citrullination is the post-translational conversion of peptidyl-arginine to peptidyl-citrulline. This conversion is mediated by the calcium-dependent enzyme peptidylarginine deiminase (PAD). This enzyme is up-regulated by calcium.

ACPA-positive RA has a different profile from ACPA-negative RA. ACPA-positive RA has more aggressive clinical and radiological courses and appears to have distinct genetic associations. Indeed, it is possible that the distinction between two forms of RA will be made clearer by the positivity versus negativity for ACPAs than the same dichotomy for RF.

ACPAs and bone

Patients positive for ACPAs develop more bone erosions and more severe osteopenia than ACPAs− [49–53]. During the course of the disease, the presence of ACPAs is independently associated with severe trabecular bone loss, and this is specially seen in the hands and distal radius [54, 55].

The presence of ACPAs without signs of articular inflammation may be a clue for later appearance of RA. The set of healthy ACPAs+ individuals are considered at risk for the future development of the disease. Two questions may arise:

1. During this preclinical phase, is it possible that bone can be affected, implicating a direct effect of ACPAs on bone, independently of inflammatory mediators?

Individuals, without signs of inflammation but ACPAs+, were analyzed for the presence of bone damage. One study analyzing metacarpal bones using HRpQCT (high-resolution peripheral quantitative computed tomography) of such healthy ACPAs+ individuals showed reduced thickness and increased porosity of the cortical bone compared with controls [26].

2. How then would ACPAs interact directly with the bone inducing damage and loss?

A proposed explanation links ACPAs to the differentiation of osteoclasts and activation of bone resorption. Citrullinated vimentin, an autoantigen targeted by the ACPAs, is expressed in cells of the monocyte/macrophage lineage and also in osteoclast precursors. The binding of ACPAs to citrullinated vimentin in the surface of these cells induces differentiation of monocytes into osteoclastic lineage and also differentiation of osteoclasts. The differentiation of monocytes to osteoclasts is further enhanced by the release of TNF, after the binding of ACPAs to the cells of the osteoclast lineage. These findings may explain the bone loss seen in the RA preclinical phase and the role of ACPAs in its induction [56].

Recently, a study described a new way to explain the effects of ACPAs on bone loss independently of the inflammatory process. The study focused the role of ACPAs in osteoclast differentiation, activation, and the subsequent bone loss and destruction [57]. In vitro assays showed that protein citrullination on osteoclasts by peptidylarginine deiminases (PADs, see above) is essential for osteoclast differentiation from peripheral blood macrophage precursors and for its activation. When stimulated by ACPAs, osteoclasts produced high levels of IL-8 that had an autocrine effect on osteoclastogenesis. The neutralization of IL-8 by anti-IL-8 antibodies blocked osteoclast differentiation induced by M-CSF and RANKL. The in vivo section of the study showed that intravenous injection of ACPAs to mice was associated to a significant decrease of trabecular bone density, trabecular number, and the bone volume fraction (bone volume/tissue volume). These bone changes were reversed by subcutaneous injection of reparixin. Describing this new way of osteoclast activation by ACPAs, before the start of the RA inflammatory process, the authors stated that (1) during the differentiation and activation of the osteoclast precursors, a progressive and gradual citrullination occurred due to an increased PAD activity; and (2) circulating ACPAs binding to osteoclast precursors enhanced the osteoclasts' reabsorbing activity through an IL-8-dependent autocrine loop.

Kocijan et al. showed that patients with seropositive RA have greater alterations of trabecular bone than those with seronegative RA [54]. When compared with seronegative RA patients, those who are serum positive for RF and/or ACPAs had significant decreases in total trabecular density ($p = 0.007$) and inner trabecular density ($p = 0.007$) as evaluated by HRpQCT.

Effects of biologic DMARDs on bone

The introduction of biologic DMARDS (bDMARDs) for the treatment of RA allowed not only for the reduction of cartilage damage but also for the decrease of both localized and generalized bone loss. Several studies in RA reported beneficial effects on bone mass after treatment with bDMARDs

(Fig. 2). Most of them showed only results on bone markers and a few on BMD and fractures risk. In order to systematically for the articles needed for this review, we conducted a literature search as follows:

Studies search strategy

The aim of this review was to show the evidence of the principal biologic therapies on bone loss in rheumatoid arthritis, focusing on the effects of TNF-α inhibitors, interleukin-6 blockade, B-lymphocyte blockade, co-stimulation blockade, and biologic anti-osteoclast treatment.

An international group that included experienced authors and methodologists developed the PRISMA methodology (Preferred Reporting Items for Systematic reviews and Meta-Analyses) for the report of systematic reviews fully and transparently (1). This methodology includes a structured approach of five components to help the researcher formulate relevant and precise questions about studies during the making of the review. This approach is known by the acronym "PICOS," where each letter refers to a component: the patient population or the disease being addressed (P), the interventions or exposure (I), the comparator group (C), the outcome or endpoint (O), and the study design chosen (S). The additional component "T" used by some authors means time, and it refers to the date of the publication used in the review [58].

Using the PICOST methodology, two coauthors (PC-LMS) conducted the search using the following MESH terms: – rheumatoid arthritis, osteoporosis, clinical trials, TNF-α antagonists, infliximab, adalimumab, etanercept, certolizumab, golimumab, IL-6 antagonists, IL-1 antagonists, abatacept, tocilizumab, rituximab, bone mineral density, bone markers, and fractures.

The search was conducted electronically and manually from the following database: Medline and Science Direct. The search period included articles from 2003 to 2015. The first selection included titles of original adult human research written in English.

Titles were retrieved and the same two authors independently selected the relevant studies for a full text. The retrieved selected studies were also reviewed completing the search for relevant articles.

The first search included 904 titles, of which 253 titles were selected. The agreement on the selection among researchers resulted in a Kappa statistic of 0.95 ($p < 0.000$). Only 248 abstracts evaluated were included in the acronym PICOST. The final selection included only 28 studies, derived from the systematic search. Additionally, a manual search in the bibliography of the selected articles was made and included into the text and into the section of "small molecules of new agents."

A summary of the 28 selected studies is presented in Table 1.

Fig. 2 Cytokine network and biological treatment blockade

RF - Reumathoid Factor; ACPAs - Anti-Citrullinated Protein Antibodies; APC - Antigen Presenting Cell; RANKL - Receptor Activator of Nuclear Factor Kappa-B Ligand; BLyS - Lynphocyte Stimulator; OB - Osteoblast; OC - Osteoclast; OCY - Osteocyte — Stimulation — Inhibition
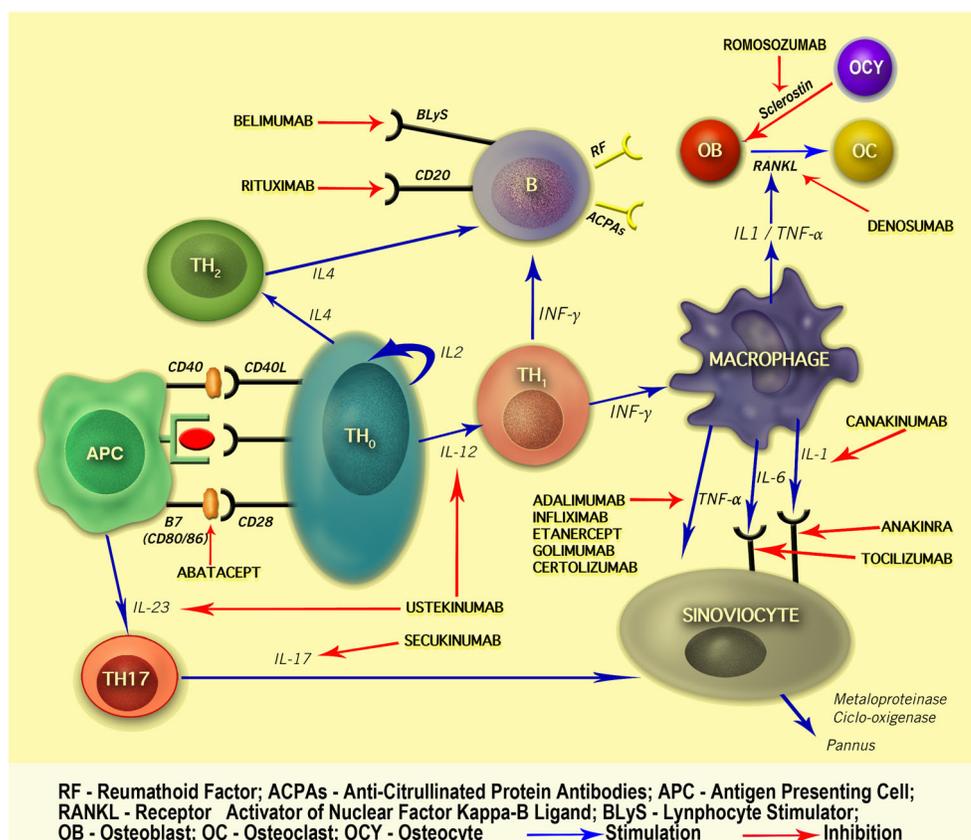
Table 1 Rheumatoid arthritis studies reporting the effects of biologic therapy on bone mass and on biological markers of bone turnover

Biological agents	Type study	Sample size	Follow-up	BMD	Hands bone mass	Bone formation markers	Bone reabsorption markers	Reference
Adalamumab	Prospective	184	4 years	Spine stabilizedHip ↓ (after 4 years)	↓MCP	–	–	[71]
Adalamumab	Prospective	50	1 year	Spine/hip stabilized	–	–	–	[67]
Etanercept	Prospective	30	6 months	–	–	↑BALPOPG stable	↓DPD↑ RANKLNTX-I stable	[87]
Infliximab	Prospective	102	1 year	Spine/hip stabilized	↓MCP	–	↓CTX-I↓ RANKL	[68]
Infliximab	Prospective	52	2 years	↑Spine/hip stabilized	↓MCP	–	–	[69]
Infliximab	Prospective open cohort	36	1 year	Non-significant↑ Spine↓ Hip	–	–	–	[73]
Infliximab	Prospective	90	1 year	Spine/hip stabilized	–	OC no change	CTX-I no change	[74]
Infliximab	Prospective randomized	342 (4 groups)Infliximab (1 group)	1 year	Non-significant↓ Spine/hipNo difference among groups	–	–	–	[76]
Infliximab	Prospective	36	1 year	–	–	↓OC	↓NTX↓ DPD	[81]
Infliximab	Prospective	48	1 year	Spine/hip stabilized	–	↑PINP/CTX-1↑ PINP/ICTP	–	[83]
Infliximab	Prospective	68	6 weeks	–	–	↑OC	–	[84]
Infliximab	Prospective open label	26	1 year	↑Spine↑ Hip	–	↑OC	↓Crosslaps	[85]
Infliximab	Prospective	17	6 months	–	–	Stable BALP	↓NTX-I↓ DPD	[86]
Infliximab	Prospective	43 Infliximab30 Controls	6 months	–	–	↓OPG	↓RANKL	[88]
Tocilizumab	Prospective	416	6 months	–	–	↑PINP (4 weeks)	↓CTX-I↓ ICTP	[98]
Tocilizumab	Cohort transversal	20	–	–	↓Large erosions	–	–	[96]
Tocilizumab	Prospective	302	1 year	–	↓Erosions (high-risk patients)	–	–	[97]
Tocilizumab	Prospective randomized	299	6 months	–	–	–	↓CTX-I↓ CTX-I/OC	[99]
Tocilizumab	Transversal	10	–	–	–	↑OPG (bone marrow histology)	–	[100]
Tocilizumab	Prospective	22	2 months	–	–	–	↓DkkI	[103]
Adalimumab + MTXMTX	Randomized	214 (ADA + MTX)188 (MTX)	52 weeks	–	MCP stabilized (for ADA Group)	–	–	[70]
Anti-TNF/ rituximab/ tocilizumab	Prospective observational	92	Anti-TNF 0–2 yearsAnti-TNF/rituximab 2–10 years	↓Bone lossSpine/hip for men and premenopausal women	–	–	–	[75]
EtanerceptInfliximabMTX	Prospective	11 Etanercept10 Infliximab10 MTX	6 months	Non-significant↑ Spine↑ Hip (TNF group)	–	↑OC	↓DPD/Cr	[72]
Denosumab			6 months	–	↑MCP	–	–	[115]

Table 1 (continued)

Biological agents	Type study	Sample size	Follow-up	BMD	Hands bone mass	Bone formation markers	Bone reabsorption markers	Reference
	Prospective randomized	43 Denosumab13 Controls	1 year	–	↓MCPBone loss	–	–	[116]
Denosumab	Prospective randomized	143 Denosumab75 Controls	1 year	↑Spine/↑hip	–	↓PINP	↓CTX-I	[117]
Denosumab	Prospective randomized	143 Denosumab75 Controls	15 months	–	–	–	↓DPD	[105]
Rituximab	Prospective	13	16 weeks	–	–	↑OPG/RANKL	↓RANKL	[106]
Rituximab	Prospective	28						

OC osteocalcin, *BALP* bone alkaline phosphatase, *PINP* N-terminal propeptide of type 1 procollagen, *CTX-I* C-terminal cross-linking telopeptide of type 1 collagen, *NTX-I* N-terminal cross-linking telopeptide of type I collagen, *ICTP* C-terminal cross-linking telopeptide of type collagen generated by matrix metalloproteinases, *DPD* deoxypyridinoline, *OPG* osteoprotegerin, *RANKL* receptor activator of nuclear kappa-B ligand, *Dkkl* Dickkopf-1

TNF-α inhibitors

TNF-α inhibitors were the first bDMARDs used in the treatment of RA, and they are still the most frequently prescribed. In clinical practice, there are five TNF-α antagonists approved for the treatment of RA: infliximab, adalimumab, etanercept, certolizumab, and golimumab.

There are compelling studies in animal models showing that TNF-α blockade impairs the development of osteoclast precursors and the activation of mature cells thus reducing the loss of bone [59]. In murine collagen-induced arthritis [60], and also in other models of murine autoimmune arthritis [61, 62], TNF-α deficiency or its inhibition prevented bone loss in parallel with the reduction of inflammation. In models of TNF-α transgenic mice, inflammation induced by TNF-α decreased new bone formation through Dkk-1 up-regulation and inhibition of insulin growth factor-1, Osterix, and Runx2. TNF-α inhibition leads to new bone formation [63]. The suppression of TNF-driven inflammation on bone metabolism has been described in several adult human cohorts and case-control studies. Infliximab has been the TNF antagonist most investigated regarding BMD and bone turnover markers.

The data on fractures are scarce, but three studies [64–66] using databases from health care, commercial insurance plan, and administrative health care organizations showed no difference in the risk of non-vertebral fractures in RA patients on treatment with TNF antagonists, methotrexate, or other non-biologic DMARDs.

Briefly In a population-based cohort study by Kim et al., using 12 years of health care data from a Canadian Province and a U.S. commercial insurance plan, 16,412 RA patients were divided into three treatment groups for comparison of the osteoporotic fracture risk: (1) TNF inhibitors with or without non-biologic DMARDs, (2) methotrexate without a TNF inhibitor, or (3) other non-biologic DMARDs without a TNF inhibitor or methotrexate. The study outcomes were hospitalizations for fractures of the hip, wrist, humerus, or pelvis. After a multivariate analysis, the adjusted risk of non-vertebral fractures was similar in RA patients starting a TNF inhibitor, methotrexate, or other non-biologic DMARDs [64].

Similar results were observed by Kawai et al. in a study analyzing retrospective cohorts in four large administrative databases including patients with RA, inflammatory bowel diseases, and a group composed by psoriasis, psoriatic arthritis, or ankylosing spondylitis. The results showed that the risk of combined fractures was very similar between patients treated with TNF inhibitors and non-biologic DMARDs for each disease. They also observed that among RA patients, the use of >10 mg/day of prednisone or equivalents at baseline was associated with an increase of fracture risk [65].

Coulson et al. reported data from 8419 female RA patients included in the Consortium of Rheumatology Researchers of

North America (CORRONA) registry. They analyzed many clinical factors regarding their possible influence on fracture risk and T-scores to determine if women with RA at risk for osteoporosis were adequately treated. The study results showed that postmenopausal status, low functional capacity, and prednisone use were associated with a higher risk of fracture; TNF-α monotherapy treatment was associated with decreased fracture risk. They also concluded that women with RA were inadequately treated for osteoporosis [66].

Effects of TNF-α inhibitors on BMD

Before the use of bDMARDs, a high rate of generalized bone loss was reported in RA patients. Haugeberg reported a BMD decrease of 1.7 % at the femoral neck and 2.7 % at the lumbar spine [13], and another study reported bone loss of 3.6 % at the femoral neck and 2.1 % at the lumbar spine [24].

Analysis of the available studies shows that the introduction of TNF inhibitors for the treatment of RA patients has been associated with decreases in generalized bone loss.

The effect of TNF blockade therapy on BMD was reported in an open-label prospective study with 50 RA patients on adalimumab treatment. After 1 year of follow-up, anti-TNF therapy arrested the generalized bone loss. A synergistic effect between adalimumab and prednisone was observed after a multivariate regression analysis showing that the concomitant use of prednisone explained 18.5 % of improvement on femur BMD [67].

In a cohort of 102 RA patients followed for 1 year on treatment with infliximab, clinical remission was associated with an arrest in bone loss at the lumbar spine and hip but with a 0.8 % BMD decrease at the hands, showing that, despite treatment, there was progressive localized metacarpal cortical bone loss [68]. In this regard, another study also demonstrated that despite inflammation control and preservation of BMD in the lumbar spine and hip, there was a continuous loss of bone in hands [69].

Two studies with adalimumab showed different results. Hoff et al. in a sub-analysis of the PREMIER trial showed that adalimumab plus MTX reduced metacarpal cortical bone loss independent of clinical response [70]. Krieckaert et al., in a cohort study of 184 RA patients taking adalimumab, showed that after 1 year of treatment, BMD of the hip and lumbar spine remained stable while BMD of the hands decreased significantly by 1.41 % [71]. Some studies suggest that TNF-α antagonists may induce clinical remission and halt generalized bone loss but are less effective in the absolute control of local joint inflammation associated with persistent joint damage and localized bone loss.

A 6-month study compared 20 RA patients taking anti-TNF-α therapy (etanercept or infliximab) with 10 patients taking MTX. For those on anti-TNF treatment, BMD increased by 0.2 % at lumbar spine and 0.1 % at the hip; for those not taking anti-TNF, there was a decrease by 0.8 and 0.6 % at lumbar spine and at the hip, respectively. The authors considered these BMD variations not significant. Probably it is likely that this study was underpowered to show a difference [72].

An open-label study including 36 RA patients treated for 1 year with infliximab reported a non-significant increase in the lumbar spine and a non-significant decrease in hip BMD [73].

A study compared 90 RA patients treated for 1 year with infliximab, who were non-responders to MTX, to an historical cohort of 99 RA patients (control group) who were treated with MTX in the prebiologic era. Results showed preservation of BMD at lumbar spine and at the hip in the anti-TNF-treated patients and loss of bone at both sites in the control group. The infliximab effect persisted even after models of stratification for confounding factors such as sex, age, menopause status, steroid, and/or bisphosphonate use. In this study, the protective effect of infliximab on bone was also observed in patients who did not exhibit a clinical response evaluated by the DAS-28, suggesting that the effects of TNF-α inhibition on bone metabolism may be partially independent of its action on RA activity [74]. The authors hypothesized that TNF-α antagonists, besides suppressing inflammation, may restore coupling of bone resorption and bone formation, previously disrupted in RA, halting systemic bone loss.

A recent prospective observational study analyzed bone loss in early RA patients followed for 10 years. In the first 2 years of disease activity, 18.5 % of patients were on bDMARDs and 91.3 % on synthetic DMARDs (sDMARDs). For the subsequent 8 years, 62.6 % were on bDMARDs and 89.2 % on sDMARDs. In the first 2 years, the annual rate of bone loss was significantly higher in patients under bDMARDs compared to those on sDMARDs at the femoral neck and total hip but not at the lumbar spine. For the whole 2–10-year period, no significant differences in bone loss at any site were found between the two groups. In multivariable models, the variables independently associated with BMD loss for the 0–2-year period were (1) use of bDMARDs for femoral neck, (2) cumulative dose of glucocorticoids for total hip, and (3) disease activity measured by DAS-28 for lumbar spine. For the 2–10 years' follow-up period, variables independently associated with bone loss were (1) menopause and smoking for the femoral neck and total hip and (2) female gender and rheumatoid factor for the lumbar spine. In the first 2 years, the association of bDMARDs and cumulative dose of glucocorticoids with bone loss can be interpreted as data coming from patients with more severe disease, taking into account that those two drugs behaved, in this observational study, as surrogate markers for disease activity. This study showed that a more effective suppression of inflammation, as seen in the 2–10-year period, including the use of bDMARDs, significantly reduced bone loss in RA patients [75].

The BeST study compared four different approaches to treat RA patients: (1) sequential monotherapy, (2) step up

combination therapy, (3) initial combined therapy with gluco-corticoids, and (4) initial combined therapy with infliximab. BMD measurements were performed in 342 patients with recent onset RA at baseline and after 1 year. Median BMD loss after 1 year was 0.8 and 1 % of baseline at the lumbar spine and at the hip, respectively, with no differences between treatment groups even for the infliximab patients. The determinants of BMD loss in this study were joint damage at baseline, joint damage progression, and no use of bisphosphonates. The tight control of inflammation in the four treatment strategies in patients with early and active RA did not allow difference among them in the preservation of bone mass. This was also achieved by the use of antiresorptive treatment. The conclusion of this study reinforces the concept that earlier suppression of inflammation with any aggressive effective treatment strategy may avoid joint destruction and preserves BMD in RA patients [76, 77].

Effects of TNF-α inhibitors on biochemical markers of bone turnover (BTMs)

BTMs are classified in two types: markers of bone formation and markers of bone resorption. The bone formation markers include osteocalcin (OC), bone alkaline phosphatase (BALP), N-terminal propeptide of type I procollagen (PINP), and C-terminal propeptide of type I procollagen (PICP). The bone resorption markers include C-terminal cross-linking telopeptide of type I collagen (CTX-I), N-terminal cross-linking telopeptide of type I collagen (NTX-I), C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases (CTP-MMP, ICTP), deoxypyridinoline (DPD), isoform 5b of tartrate-resistant acid phosphatase (TRACP5b), and helical peptide 620–633 of the alpha-1 chain [78]. Serum levels of OPG and RANKL can be measured and the RANKL/OPG ratio can be determined.

Baseline lower levels of RANKL and the RANKL/OPG ratio were described as predicting remission in RA patients treated with TNF inhibitors [79]. Anti-TNF treatment also increased synovial expression of OPG [80]. The BTMs, which were analyzed in the TNF-α antagonist RA studies, include PINP, OC, BALP, OPG, CTX-I, NTX-I, ICTP, DPD, and RANKL.

In a prospective study of 36 RA patients taking infliximab and methotrexate [81], OC, NTX-I, and DPD were measured at baseline and after 14 weeks, 6 months, and 12 months of treatment. Levels of OC and NTX-I lowered significantly at 14 weeks and levels of all BTMs were significantly lower at 6 and 12 months than the baseline levels. After a significant drop of NTX-I and DPD levels at 14 weeks, there was no further significant change for the rest of the treatment. Interestingly, the authors also measured the levels of different cytokines and found a significant correlation between levels of

IL-6 and of all BTMs at different time points, levels of IL-23 and OC before treatment and after 6 months, and levels of TNF-α and NTX at 14 weeks and DPD at 12 months. These results suggest that the changes induced by infliximab in the RA inflammatory process may promote changes in other cytokines, besides TNF, which can influence bone remodeling in different ways. In this regard, the effect in vitro of the pro-inflammatory cytokines TNF-α, IL-17, IL-6, IL-1, and IL-23 demonstrated that they have specific and characteristic properties on osteoclast development [82]. The initial decrease in the levels of bone-resorbing markers with no further drops may suggest a short-term positive effect of infliximab on bone remodeling. Chopin et al. also observed this positive effect in a study with 48 RA patients treated with infliximab for 1 year. There was an initial decrease in CTX-I at 6 and 22 weeks, returning to pretreatment levels at week 54 [83].

Vis et al. described an increase in the bone formation markers OC and PINP and a decrease of bone resorption marker ICTP after 6 weeks of treatment with infliximab in an open-label study with 68 RA patients [84]. In a follow-up of 102 RA patients taking infliximab (described before), the same authors, measuring BTMs at 14, 30, and 46 weeks, found an association between the clinical response and the decrease in the resorption markers CTX-I and RANKL [68].

After 1 year of treatment with infliximab, an increase in OC ($p < 0.01$) and a decrease in CTX-I ($p < 0.01$) compared to baseline levels were observed in a prospective open-label pilot study with 26 RA patients [85]. Similar results regarding persistent decrease in bone-resorbing markers and improvement in bone formation markers were reported in other studies [86, 87]. In a prospective study, the sera of 43 patients and 30 healthy individuals regarding OPG and RANKL levels were analyzed. For 21 patients under anti-TNF therapy, the high baseline serum levels of OPG and RANKL were normalized after 6 months of treatment [88].

Despite conflicting results, analysis of the existing data allows the conclusion that anti-TNF therapy is associated with a rapid decrease in bone resorption and a positive bone remodeling balance in RA patients. Many available studies are open label, with small sample sizes and are not controlled for confounding factors. The knowledge of differences on bone remodeling among the existing TNF inhibitors, adjustment for factors that may act on bone mass (steroids, smoking, comorbidities), and randomized long-term studies are needed for a better understanding of the impact of TNF antagonists on bone metabolism.

Interleukin-6 blockade

Interleukin-6 (IL-6) is a cytokine that has been associated with a large repertoire of functions. Classically, IL-6 is involved in protection from infection, but it is mainly associated with the

development of inflammatory process in many diseases. IL-6 promotes the liver production of acute phase reactants and has a prominent role in the maturation of B cells and plasma cells. In vitro observations showed that IL-6 induces osteoclastogenesis in a model of antigen-induced arthritis [89]. In RA patients, during the inflammatory process, cells of the monocyte/macrophage lineage differentiate into osteoclasts, which resorb bone, producing erosions. IL-6 in conjunction with IL-1 and TNF-α promotes the recruitment and proliferation of those inflammatory cells, enhancing the production of the *pannus* tissue with further destruction of cartilage and subchondral bone.

The relation of IL-6 with systemic bone loss was reported in an open study including 40 RA patients compared to 20 healthy controls matched by age and sex. IL-6 levels showed a significantly negative correlation with the T-score of spine and hip. A negative correlation was found between the T-scores and parameters of disease activity [90].

More recently, IL-6 involvement in inflammation-associated carcinogenesis and in the link between innate and adaptive immunity have been reviewed [91].

IL-6 stimulates target cells through its receptor in two ways: (1) *Classical binding*—IL-6 binds with its membrane bound receptor (IL-6R) on cell surface, or (b) *Trans-signaling*—IL-6 binds to a soluble form of IL-6R that further links to the cell membrane.

An IL-6 receptor-blocking agent (IL-6R), tocilizumab, has been used successfully to treat RA patients. This humanized anti-IL-6R has been effective in lowering the systemic and local signs of the inflammatory process measured by the reduced number of tender and swollen joints, normalization of the acute phase reactants, and reduction of the joint damage [92–94].

An in vitro study reported that murine anti-IL-6R reduced osteoclast differentiation and bone resorption in monocyte cultures stimulated with RANKL or RANKL plus TNF-α. In the same study, using human TNF-α transgenic mice as a model, IL-6R blockade strongly reduced osteoclast formation as well as bone erosion in vivo, but interestingly, it did not inhibit joint inflammation. This observation emphasizes the concept that the IL-6 inhibition of osteoclastogenesis is independent of its anti-inflammatory actions [95].

A micro-CT study analyzed bone erosions in the metacarpophalangeal joints of 20 RA patients treated with tocilizumab. Bone erosions were evaluated by the measurement of their maximal width and depth at baseline and after 1 year of treatment. Tocilizumab induced limited repair mainly in large lesions with sclerosis, reflecting its favorable action on local bone remodeling [96].

A 1-year randomized, controlled trial (SAMURAI study) showed that tocilizumab monotherapy in active RA patients reduced the progression of structural joint damage and promoted higher remission rates than the conventional DMARDs therapy [91]. In a sub-analysis of this prospective 1-year study, RA patients were divided into high-risk and low-risk groups according to four independent predictive markers for progressive joint damage (urinary CTX-II, urinary pyridinoline/deoxypyridinoine ratio, body mass index, and joint-space narrowing score). Tocilizumab monotherapy was more effective in reducing radiological progression in high-risk than in low-risk patients showing a better effectiveness in the first group [97].

In the multicenter, double-blind, placebo-controlled trial of tocilizumab in inadequate responders to methotrexate (OPTION study), patients with moderate-to-severe RA were randomized to receive tocilizumab (4 or 8 mg/kg subcutaneously) with methotrexate compared to methotrexate alone. From 623 patients included in the study, 416 were selected to investigate the effect on biochemical markers of bone and cartilage metabolism at 4, 16, and 24 weeks of treatment compared to baseline levels. Patients treated with tocilizumab showed (1) marked reduction, in a dose-dependent way, of cartilage metabolism markers (N-terminal propeptide of type IIA collagen, matrix metalloproteinase-3, and collagen helical peptide), (2) significant decreases in bone-resorbing markers (CTX-I and ICTP), and (3) an increase in the levels of bone formation markers that were significant only when the PINP levels were compared with placebo at 4 weeks. These results provided evidence for a beneficial effect on bone remodeling process in RA patients taking tocilizumab [98].

The changes in biochemical markers of bone metabolism were analyzed in a randomized, double-blind, placebo-controlled, parallel group trial including 299 anti-TNF refractory RA patients. They were randomly assigned to tocilizumab (4 or 8 mg/kg IV) plus methotrexate or placebo IV plus methotrexate once a month. Both tocilizumab doses significantly reduced the levels of biochemical markers of cathepsin K-mediated bone resorption. A significant decrease in the CTX-I/OC ratio was also observed indicating an improvement in bone balance [99].

Bone marrow histological changes in response to tocilizumab treatment were observed in a study including tissues extracted from 10 RA patients submitted to total knee arthroplasty. Samples from other 10 RA patients on MTX monotherapy were used for comparison. A significant increase in the expression of osteoprotegerin was demonstrated in the tocilizumab-treated patients after comparison with the control group [100].

The effects of IL-6 blockade on serum bone markers were reported in a pilot study comparing 22 active RA patients treated with tocilizumab and 22 healthy women. After 2 months of treatment, IL-6 blockade reduced the serum levels of Dkk-1 and significantly increased the ratio of OPG/RANKL. The change in the OPG/RANKL favoring bone formation was observed in 10 patients in remission or in low disease activity but not in 12 patients without control of the disease. These results indicate that the positive IL-6 blockade

effect on bone remodeling was mediated by the reduction of the Dkk-1 influence on the Wingless signaling pathway and in the rapid and effective suppression of inflammation [101].

Despite the good results obtained with anti-cytokine therapy in the management of RA, there are still a number of patients not responsive or who cannot tolerate it. A better understanding of the RA pathology, involving the interplay among cytokines and cells, allowed the development of new therapies offering a new opportunity for all those affected by the disease. Monoclonal antibodies directed against lymphocytes and co-stimulatory antagonists are part of this effort regarding new ways to reduce the activity of RA.

Biologic therapies that target the lymphocyte

B-Lymphocyte blockade

Experimental studies proposed that B-lymphocytes might synthesize and secrete RANKL. In a knockout mice model of ovariectomy-induced osteoporosis, the deletion of RANKL in B lymphocytes partially protected from cancellous bone loss [102]. A study using cytokine mRNA profiles in the analysis of RA synovial fluid cell populations reported that B cells are a major source of RANKL [103]. These observations coupled with the known involvement of B and T lymphocytes in the RA inflammatory process led to the hypothesis that the blockade of these cells could protect RA patients from bone loss.

Rituximab is a monoclonal antibody directed against the molecule CD20 on the surface of B lymphocytes. After binding to CD20, rituximab impairs the cell function leading to its apoptosis. Firstly utilized to treat lymphomas, this B-cell blocking agent was licensed for RA therapy in 2006 showing good efficacy as a primary agent after lack of response or intolerance to anti-TNF agents [104].

In a prospective study, the influence of rituximab on markers of bone metabolism was analyzed in 13 patients with a follow-up of 15 months after the beginning of treatment. A non-significant decrease in RANKL levels and a significant decrease in deoxypyridinoline levels were observed, showing a reduction in bone resorption [105]. Another prospective study analyzed expression of bone resorption markers in synovial biopsies of 28 patients with active RA, before and after 16 weeks of rituximab treatment. The results showed a decrease in synovial osteoclast precursors and RANKL expression. In the same period, an increase in serum OPG/RANKL ratio was observed [106]. Salvin et al. reported, in a small number of RA patients, an improvement in bone mineral density after rituximab treatment. Those results were better seen in patients with low activity, classified as clinical responders [107].

Co-stimulation blockade

The first molecular interaction in the initiation of RA synovitis occurs between an antigen-presenting cell (APC) and a Th1 lymphocyte. Arthritogenic antigens linked to the Major Histocompatibility Complex (MHC) molecule are exposed in the surface of an APC, and their binding to a Th1-cell receptor (TCR) makes what is called a tri-molecular complex. Although being the main step, this antigenic stimulation is not sufficient to start the T-cell activation, which further depends also on co-stimulatory signals. The binding of the T-cell molecule CD28 to the APC CD-80 (B7)/CD86 is an important positive co-stimulatory pathway. CTLA4, produced by cytotoxic T lymphocytes, is also a ligand of CD80/86 and a natural inhibitor of the T-cell activation.

Abatacept is a soluble fusion protein formed by the extracellular domain of human CTLA4 linked to a human IgG1 Fc portion. Abatacept (CTLA4-Ig) has been successfully used to treat RA patients. Its action decreased joint symptoms and signs and reduced RA radiological progression. It is currently indicated for the treatment of moderate-to-severe RA in patients not responsive to synthetic DMARDs or anti-TNF therapy [108].

In an experimental study with murine peripheral blood mononuclear cells, Axmann et al. showed that binding of CTLA4 to osteoclast precursor cells inhibited its differentiation and maturation having an anti-osteoclastogenic effect. They also showed that CTLA4 inhibited, dose-dependently, RANKL and TNF-mediated osteclastogenesis in vitro without the presence of T cells. These experiments explained, at least in part, the anti-erosive effect of abatacept [109]. It has also been reported that CTLA4-Ig may induce the down-regulation of key osteoclast genes as *c-Fos* and *NFATc1*, making a direct influence on the differentiation of osteoclasts and its bone-resorbing activity [110]. Bedi et al. showed, in an experiment in mice, that CTLA4-Ig prevented bone loss and bone resorption induced by PTH [111]. Recently, Bozec et al. reported that the binding of CTLA-4 to CD80/86 induced the activation of the enzyme idoleamine 2,3-dioxygenase in osteoclast precursors degrading tryptophan and promoting apoptosis. This molecular mechanism may also explain the direct action of abatacept on osteoclasts inducing its apoptosis and protecting bone mass [112].

Biologic anti-osteoclast treatment

Bone loss in RA, featured by erosions of subchondral bone, peri-articular, and systemic osteoporosis, is mainly mediated by RANKL. The blockade of its activity decreases the differentiation and development of osteoclasts preventing the resorbing process associated with inflammation.

Denosumab, a fully human monoclonal antibody anti-RANKL, has been successfully used to treat osteoporosis [113]. In the FREEDOM pivotal study, denosumab 60 mg SC every 6 months for 3 years reduced the risk of vertebral and non-vertebral fractures in postmenopausal women [114]. Deodhar et al. studied the effect of denosumab on bone loss in the hands of 56 individuals with erosive RA. Patients under methotrexate treatment received subcutaneous placebo, denosumab 60 mg, or denosumab 180 mg at 0 and 6 months. Evaluations were made regarding hand BMD, radiographs, and magnetic resonance images. Erosions were evaluated by the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring System of the metacarpophalangeal joints and wrists of both hands, modified with volume-based scoring of bone erosion for each joint from 0 to 10 with increments of 0.5 (total of 21points and maximum total erosion score of 500). After 1 year, an increase in hand BMD and a reduction in hand bone erosions were observed in the denosumab-treated patients compared to placebo. Low bone scores were also observed at 6 months on MRI for the same group of patients [115]. In a randomized prospective study post hoc analysis including 218 patients with active, erosive RA, the effects of denosumab on the metacarpal shaft cortical bone thickness were measured by digital x-ray radiogrammetry. Study subjects were given two injections of denosumab treatment or placebo at baseline and then repeated after 6 months, with continuous methotrexate treatment. Denosumab treatment prevented cortical bone loss, an effect observed for up to 12 months [116]. BMD and bone turnover markers were analyzed in a randomized, double-blind, placebo-controlled phase II study of denosumab in RA patients taking concurrent glucocorticoids or bisphosphonates. After 6 and 12 months, lumbar spine and total hip BMD increased significantly compared with placebo in denosumab-treated patients. sCTX-I and PINP were also reduced at 3 and 6 months in all subgroups of patients on denosumab. These results were observed regardless of baseline BMD and bone turnover markers or concomitant use of glucocorticoids or bisphosphonates [117].

These studies show beneficial effects of denosumab in the preservation of bone mass, particularly in juxta-articular osteoporosis. However, blockade of RANKL did not affect the inflammatory arthritis. In addition, recently Ferrari et al. reported that in preclinical and clinical RA studies, RANKL inhibitors did not significantly alter the inflammatory processes [118].

Clinicians are concerned that concomitant blockade with TNF and RANKL in the same patient may increase the risk of immunosuppression and/or infection. Curtis et al. evaluated all RA patients enrolled in Medicare during 2006–2012 for the risk of infection in those concurrently treated with a biologic agent and denosumab or zoledronic acid. The study concluded that the rate of hospitalized infection was not significantly increased for patients receiving denosumab compared with that receiving zoledronic acid [119].

Small molecules new agents

Although many new treatments have become available in the last 15 years improving RA treatment, there are patients not responsive to the available therapeutic agents and some who cannot use them because of adverse effects. In the search for new treatments, efforts have been made to find more selective immunosuppressive therapies such as those targeting cytokine intracellular signaling pathways. One of these targets, successfully inhibited, has been the Janus tyrosine kinases (JAKs) pathways.

Tofacitinib is a synthetic (not biologic) small molecule new oral drug, acting as a potent inhibitor of the JAK family of tyrosine kinases, with a high degree of selectivity or JAK 1 and JAK 3 [120]. Phase 3 studies showed that tofacitinib used alone [121] or in combination with methotrexate [122] reduced the progression of cartilage and bone destruction in RA patients. Although tofacitinib decreased the development of bone erosions, there are no, at this point, studies with primary outcomes of BMD, bone markers, or fractures.

Conclusion

We presented in this review the best evidence available regarding bone loss in RA patients. The more recent knowledge of the cytokines' interplay in the inflamed synovial membrane and its close relation to osteoclast development and activation demonstrated that the persistence of inflammation enhanced bone turnover, leading to bone erosions and systemic bone loss. Early and "aggressive" treatments were reported to be more effective in rapidly achieving a low level of inflammation and halting the progressive loss of bone.

Several new studies showed that therapies targeting specific cytokines and its signaling pathways with biologic DMARDs may protect the skeleton and should be introduced as soon as possible. Outcomes in these clinical studies were based mostly on changes in biological markers, and only a few of them reported modifications on BMD or localized osteoporosis. Only three retrospective studies reported reduction in fracture risk after anti-TNF therapy.

Some reported findings still need to be clarified.

The TNF blockade studies showed that even in RA patients not responsive to treatment, a protective effect on bone was observed suggesting the possibility that anti-TNF therapy may restore coupling of the bone remodeling independently of its anti-inflammatory action.

Another point was the lack of efficacy of TNF blockade on hand bone loss despite its preservation of BMD in lumbar spine and hip. Is it related to a lack of satisfactory local anti-inflammatory action? Interestingly, better results regarding localized bone loss were observed with anti-IL6 treatment.

Very few studies reported inhibition of bone loss after rituximab and abatacept treatment. Anti-RANKL therapy showed beneficial effects in the preservation of bone mass in RA, especially in juxta-articular osteoporosis, although this treatment cannot alter the inflammatory process. New non-biologic therapies but potent inhibitors of the cytokine network may offer future options for skeleton preservation in RA.

Although several studies reported favorable actions of biologic therapies on bone protection, there are still unmet needs for studies regarding their actions on the risk of bone fractures in RA patients. They will be developed in the near future or they are probably underway at this time.

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