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# Pathomechanisms of bone loss in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune disease, in which the inflammatory processes involve the skeletal system and there is marked destruction of the bones and the surrounding structures. In this review, we discuss the current concepts of osteoimmunology in RA, which represent the molecular crosstalk between the immune and skeletal systems, resulting in the disruption of bone remodeling. Bone loss in RA can be focal or generalized, leading to secondary osteoporosis. We have summarized the recent studies of bone loss in RA, which focused on the molecular aspects, such as cytokines, autoantibodies, receptor activator of nuclear kappa- $\beta$  ligand (RANKL) and osteoprotegerin (OPG). Apart from the above molecules, the role of aryl hydrocarbon receptor (Ahr), which is a potential key mediator in this process through the generation of the Th17 cells, is discussed. Hence, this review highlights the key insights into molecular mechanisms of bone loss in RA.

## KEYWORDS

rheumatoid arthritis, bone, osteoporosis, cytokines, ligands, autoantibodies

## Introduction

### Bone remodeling mechanisms in RA

Physiological bone remodeling occurs through cell-mediated processes in response to biomechanical signals. The initial phase of bone resorption which is mediated by osteoclasts is followed by the recruitment of osteoblasts which mediate bone formation (1). Under normal circumstances, there is a tight coupling of osteoclastic and osteoblastic activities. Transforming growth factor- $\beta$  (TGF- $\beta$ ) and bone morphogenetic proteins (BMPs) are bone growth factors which are sequestered into the bone matrix and have been strongly implicated in linking bone resorption and formation (2, 3). Besides, osteoclasts may independently modulate osteoblast differentiation and activity. There are other factors that influence physiologic bone remodeling include spingosine-1-phosphate, Wnt 10b and BMP6 that enhance bone formation (4) and semaphorin 4D that inhibits bone formation (5).

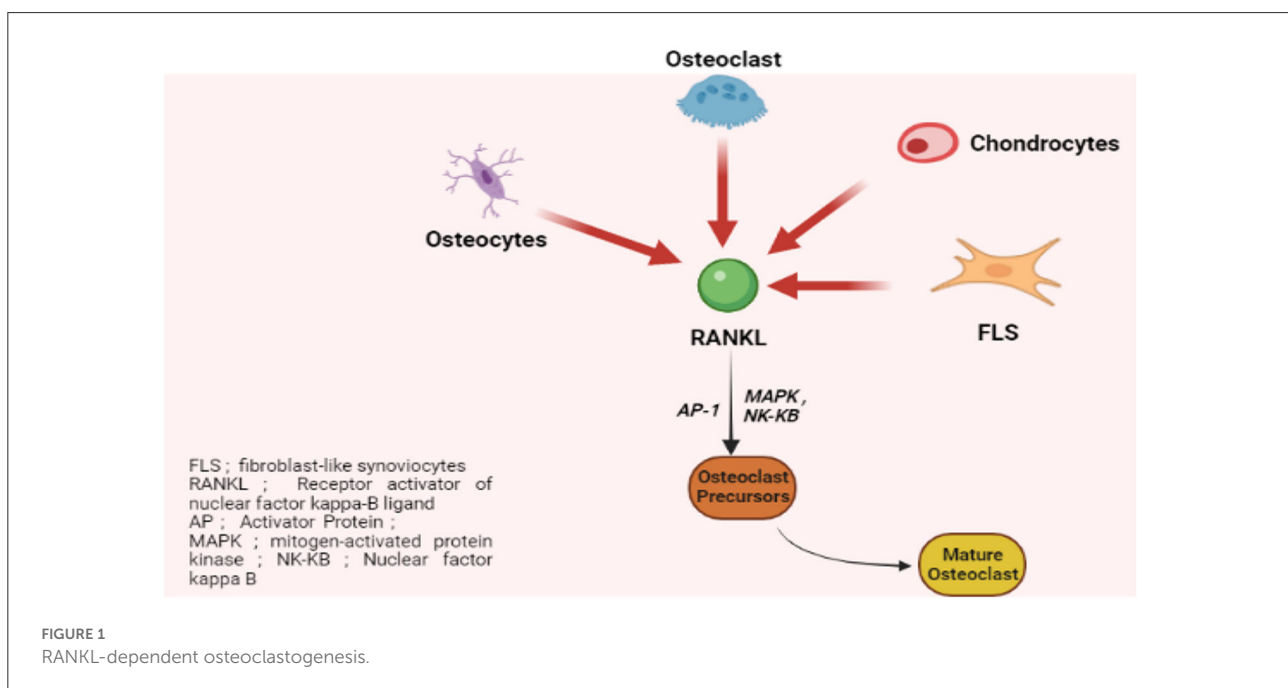
Rheumatoid arthritis (RA) is a form of chronic inflammatory arthritis with negative effects on skeletal remodeling. Periarticular bone erosions represent the radiographic hallmark of RA (6). Matrix metalloproteinases (MMPs) which are produced by synovial fibroblasts have been implicated in the degradation of extracellular matrix; mainly the

proteoglycans and collagens of the articular cartilage (7). MMP-1 and MMP-3 levels were found to correlate strongly with joint erosions but there is a paucity of published data on the effects of MMPs on osteoclastogenesis (8–10). In active RA, disruptions in physiologic bone remodeling occur, whereby a mismatch in osteoclastic-osteoblastic activity results in enhanced bone resorption with a lack of compensatory bone formation. The processes that regulate the coupling of bone resorption and formation which usually occur under physiologic conditions. There is a mismatch in osteoclastic-osteoblastic activity resulting in enhanced bone resorption with a lack of compensatory bone formation. Beyond the disordered focal bone remodeling associated with the active synovitis of the affected joints, there is generalized axial and appendicular bone loss at distant sites which are not inflamed. Hence, there are 3 types of bone loss i.e., local, juxta-articular and systemic. While all these forms are related to inflammation, the underlying mechanisms for each type of bone loss vary. Systemic bone loss or osteoporosis in RA is caused by systemic inflammation, the use of glucocorticoid therapy and physical impairment while local bone loss in the form of joint erosions due to the effects of the synovial immune cells and cytokines (11). In RA, studies have consistently pointed out that biochemical markers of bone turnover have a significant positive correlation with the disease activity. The systemic bone loss is mediated by synovial cytokines with osteoclastogenic activity that enter the blood circulation and adversely affect the generalized bone remodeling (12).

The bone pannus interface in patients with RA exhibits a heterogeneous population of cells including osteoclasts.

Animal models have implicated osteoclasts for the marginal joint erosions in RA. The rheumatoid synovium contains abundant osteoclast precursors and factors with potent pro-osteoclast differentiation and activation activity. Receptor activator of nuclear kappa- $\beta$  ligand (RANKL), a member of the tumor necrosis factor ligand family, plays a vital role in osteoclastogenesis. RANKL is expressed by synovial fibroblasts and activated T cells in the joints of RA patients. Hence, treatment with osteoprotegerin (the soluble receptor that inhibits RANKL activity) results in marked inhibition of joint erosions (Figure 1).

In RA, an additional mechanism of bone adaptation involves the process of endochondral bone formation whereby there is replacement of cartilaginous matrix with mineralised bone. Local production of bone growth factors, including TGF- $\beta$  and BMPs have been implicated in this process (13). Multiple cytokines, lipid mediators and growth factors have been shown to function as anabolic mediators of bone formation either *via* effects on osteoblasts and their precursors or *via* chondrogenesis. Of these mediators, particular attention has focused more recently on the wingless (WNT) signaling pathway (14). The Wnt ligands regulate bone formation *via* several distinct pathways, including the WNT/b-catenin pathway, the non-canonical WNT/planar cell polarity pathway and the WNT/calcium pathway. Signaling *via* the canonical pathway is initiated by the binding of WNT ligands to a dual receptor complex comprising low-density lipoprotein receptor-related protein (LRP) 5 or LRP6. This leads to the inactivation of a multiprotein  $\beta$ -catenin complex that targets  $\beta$ -catenin



to proteosomal degradation.  $\beta$ -Catenin then accumulates in the cytoplasm and translocates to the nucleus, where it stimulates osteoblast differentiation. Activation of the canonical  $\beta$ -catenin pathway results in transcriptional activation of the osteoprotegerin (OPG) gene (15). OPG is a potent inhibitor of RANK ligand (RANKL), which is the key regulator of osteoclast differentiation and activation. The WNT signaling pathway is regulated by families of both activators and inhibitors. Sclerostin, is one of the most potent inhibitors of this pathway (16). The Dickkopf (DKK) proteins and sclerostin, as well as soluble frizzled related protein play a role in modulating the pattern of bone repair and regeneration in RA (17).

## Receptor activator of nuclear kappa- $\beta$ ligand (RANKL)/osteoprotegerin (OPG)

The bone remodeling process is delicately orchestrated by osteoclasts and osteoblasts through multiple signaling pathways. The RANKL/OPG is the most recognized axis in mediating the formation of osteoclasts (18). Osteoblasts secrete RANKL that binds with RANK on the osteoclasts precursors to promote their differentiation into mature osteoclasts with bone-resorption ability. Apart from osteoblasts, hypertrophic chondrocytes and osteocytes are the major sources of RANKL during growth and maturation (19). In patients with RA and animals with experimentally induced inflammatory arthritis, fibroblast-like synoviocytes (FLS) represent a major source of RANKL (20, 21). Briefly, the binding of RANKL to RANK recruits adaptor protein tumor necrosis factor receptor and activates mitogen-activated protein kinases, nuclear factor-kappa  $\beta$  and activator protein-1. Coupled with immunoreceptor tyrosine-based activation motif-mediated calcium ion signaling, this eventually leads to activation and amplification nuclear factor of activated T-cells cytoplasmic 1, which transcribes genes that stimulate osteoclast differentiation and bone resorption (22, 23). OPG acts as a decoy receptor to bind with RANKL, thus preventing the activation of RANK-RANKL signaling (23, 24) (Figure 2).

The expression of RANKL and OPG is triggered by various factors, including inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, IL-6, IL-17 present in high levels among patients with RA (25). Of note, IL-6 and soluble IL-6 receptor induce RANKL expression in FLS and they are vital for the TNF- $\alpha$  and IL-17 mediated RANKL expression (26). A recent study revealed that the level of lymphotoxin- $\alpha$ , a close homolog of TNF- $\alpha$ , is higher in patients with RA compared to those with OA. It can induce RANKL expression in chondrocytes in a manner like TNF- $\alpha$ , which subsequently promotes the differentiation of peripheral mononuclear cells into osteoclast-like cells *in vitro* (27). Thus, these factors could contribute to increased RANKL levels leading to bone erosions; observed in RA patients. In tissues samples of patients with

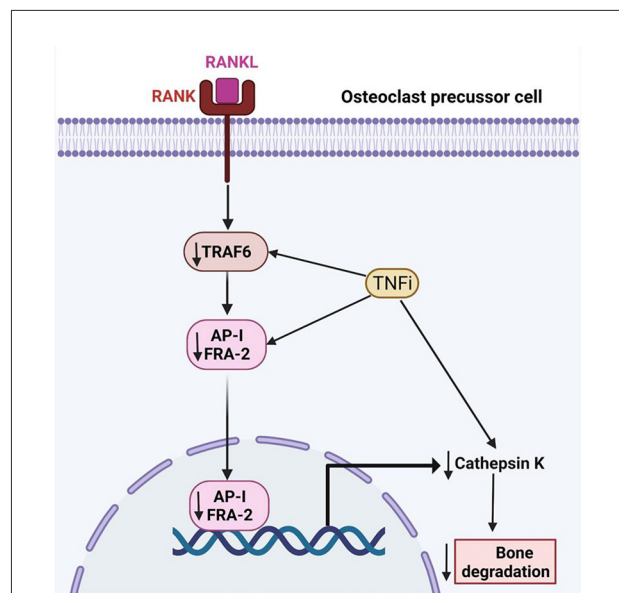


FIGURE 2

The effects of tumor necrosis factor inhibitors on RA-related bone loss. TRAF6, TNF-receptor associated factor-6; FRA-2, Fos-related antigen-2; AP-1, Activator protein 1; RANK, receptor activator of nuclear factor kappa-B; RANKL, Receptor activator of nuclear factor kappa-B ligand.

RA, RANKL and OPG are co-expressed at the sites of articular bone erosion, with RANK-positive multinucleated cells (28). In addition, RANKL expression by FLS is shown to play a more significant role in RA-associated bone erosion based on a study using cell-specific RANKL knockout animals (29). Conventional anti-RA agents like methotrexate have been shown to prevent bone resorption by reducing serum soluble RANKL levels (30).

Epidemiological studies have reported that RANKL level is higher in patients with RA compared to normal controls (31), and correlates with Larsen score and radiological progression (31). A meta-analysis showed that RANKL gene rs2277438 polymorphism increases RA risk, while RANK gene rs1805034, OPG gene rs3102735, rs2073618, rs3134069 polymorphism were not related to RA risk (32). A Polish study revealed that RANK rs8086340 single nucleotide polymorphism affects disease susceptibility while RANK rs8086340 and RANKL rs7325635 GG were associated with lower C-reactive protein levels. RANKL rs8086340 and rs1805034 CC homozygotes were associated with higher alkaline phosphatase levels compared to other phenotypes whereas RANKL rs7988338 GG homozygotes were associated with a lower number of swollen joints compared to the A allele (33). A meta-analysis of three French cohorts found that the OPG gene G allele of rs2073618 is associated with bone erosion defined by the Sharp score, but not RANKL rs7325635 gene allele (34).

Given the important role of RANKL in RA, denosumab, an antibody against RANKL, could ameliorate the effects of

RANKL on bone damage. Individual randomized controlled trials have demonstrated the efficacy of denosumab in reducing new bone erosion and promoting bone erosion healing in patients with RA (35, 36). Discontinuation of denosumab among patients with RA receiving glucocorticoids leads to a gradually increased bone remodeling and decreased bone mineral density (BMD) to baseline level after 12 months (37). These data show that inhibition of RANKL activity is a promising approach to reduce bone damage in patients with RA. A study on Fc-osteoprotegerin infusion in animals with collagen-induced arthritis showed that the treatment can prevent bone erosion by inhibiting osteoclast formation, but not cartilage erosion (38). Table 1 summarizes the recent studies on RANKL/OPG in RA.

## The central role of cytokines

The RANKL/OPG ratio determines the physiological balance of bone formation and resorption, with a higher ratio favoring increased bone resorption. A higher RANKL/OPG ratio is associated with increased radiographic damage in RA patients (39). Various proinflammatory cytokines regulate the expression of RANKL and OPG including TNF $\alpha$ , IL-6 and IL-1 (40). Table 2 summarizes the findings of recent studies on cytokine related bone loss in RA.

### TNF $\alpha$

TNF $\alpha$  promotes osteoclastogenesis by promoting the expression of the essential osteoclast differentiation factor, RANKL, and/or OPG, by bone marrow stromal cells (41, 42). Jura-Póltorak et al. demonstrated that the 15-month anti-inflammatory treatment with TNF $\alpha$  inhibitors was associated with increased bone formation markers i.e., C- and N-terminal propeptides of type I procollagen (PICP, PINP) and reduced bone resorption markers i.e., C- and N-terminal cross-linking telopeptides of type I collagen (CTX-I, NTX-I) (43). In keeping with these findings, Perpetuo et al. disclosed that after TNF $\alpha$  inhibitor therapy, patients had reduced RANKL surface expression in B-lymphocytes and the frequency of circulating classical CD14<sup>bright</sup>CD16<sup>-</sup> monocytes. Apart from the serum levels of RANKL, RANKL/OPG ratio, CTX-I, TRAF6 and cathepsin K showed a reduction with TNF $\alpha$  inhibitors (44).

### IL-6

IL-6 has a wide variety of biological effects in RA such as induction of proliferation of B cells and plasma cells, enhancement of production of acute-phase protein by hepatocytes and stimulation of differentiation of T helper

cells. IL-6 is one of the culprit cytokines responsible for joint destruction in RA.

In murine bone marrow cells, IL-6 suppresses osteoclast differentiation through inhibition of the nuclear factor of activated T cells cytoplasmic 1 (NFATc1) (45). Besides, animal studies have suggested that IL-6 is essential for the differentiation from naïve T cells to Th17 cells (46, 47). Th17 cells, a subset of T-helper cells, are also known to express RANKL (48). Following treatment with anti-IL-6 receptor antibodies in murine models of arthritis, osteoclasts tend to transform into non-resorbing cells (49). Recent studies have also reported that co-stimulation of IL-6 and TNF $\alpha$  may trigger osteoclast differentiation from bone marrow-derived macrophages. This form of osteoclastogenesis is considered to occur in a RANKL-independent manner, given that it is not inhibited by OPG (50).

### IL-1

The IL-1 family of ligands includes 11 members, with IL-1 $\beta$  as the main culprit in many inflammatory conditions. The inactive IL-1 $\beta$  precursor is cleaved by caspase-1 into an active cytokine (51), which binds to type I (IL-1RI) and type II (IL-1RII) specific receptors. IL-1 $\beta$  is a strong stimulator of osteoclastogenesis (52) through upregulation of the production of RANKL and downregulation of OPG (53). Besides, IL-1 $\beta$  increases prostaglandin synthesis (54), which is a potent resorption stimulus (10). Prostaglandins, such as prostaglandin E2 (PGE2), may mediate the upregulation of RANKL by activating cell-surface receptors (55). Furthermore, IL-1 $\beta$  also stimulates osteoclast activity by increasing the production of macrophage colony-stimulating factor (M-CSF) and inhibiting osteoclast apoptosis. IL-1 $\beta$  has an intimate relationship with TNF- $\alpha$  and experimental evidence suggests that blocking IL1 $\beta$  and TNF- $\alpha$  may completely halt bone resorption (56).

Apart from enhancing osteoclastogenesis, IL-1 $\beta$  strongly inhibits osteoblastogenesis. The inhibition of osteoblast activity is modulated *via* mitogen-activated protein kinase (MAPK), by activated signal transducers and activators of transcription (STATs). IL-1 $\beta$  also upregulates DKK1- and sclerostin, which may inhibit the production of osteoblasts (57). In a Chinese population of patients with postmenopausal osteoporosis, there was a strong association between the Taq I IL-1 $\beta$  exon 5 gene polymorphism and a reduced BMD (58) (Figure 3).

### Other cytokines

Many other cytokines such as IL-22, IL-9, and IL-17A have been implicated to a lesser extent in RA-related bone loss (59–61). IL-17A which has a well-established role in the pathogenesis of enthesitis in spondyloarthritides. It exerts its pathogenic effects on FLS *via* IL-17/IL-17RA/STAT-3 signaling.

TABLE 1 Recent evidence on the association between RANKL/OPG and RA.

| Study                       | Study design   | Key findings   |
|-----------------------------|--|--|
| Komatsu et al. (21)         | Animal study   | RANKL from bone marrow plasma cells contributed to periarticular bone loss   |
| Perpetuo et al. (44)        | Interventional study<br>Subjects: Patients with RA and DAS28 > 3.2<br>Treatment: Methotrexate for at least 6 months.   | RANKL from synovial fibroblasts contribute to joint erosion<br>↓ RANK and monocyte activation markers with methotrexate ± low-dose prednisolone treatment.<br>↓ bone resorption from osteoclast-like cells differentiated from peripheral blood mononuclear cells <i>in vitro</i> after treatment.<br>↓ serum RANKL level after treatment. Serum OPG level was not changed.                    |
| Boman et al. (31)           | Longitudinal study<br>Subjects: Patients with early RA and were symptomatic <1 year.<br>Followed up for 24 months  | ↑ RANKL in patients compared to controls.<br>↑ RANKL in anti-CCP-positive compared to seronegative patients<br>RANKL level correlated positively with Larsen score at baseline & 24 months<br>RANKL level correlated positively with radiological progression at 24 months   |
| Yang et al. (32)            | Case-control study<br>Case: 574 patients with RA: 804 controls.<br>Meta-analysis<br>9 studies involving Asians and Caucasians  | RANK gene rs1805034 was not related to risk of developing RA.<br><br>RANKL gene rs2277438: ↑ RA risk.<br>RANK gene rs1805034: not related to RA risk.<br>OPG gene rs3102735, rs2073618, rs1314069: not related to RA.  |
| Wielńska et al. (33)        | Longitudinal studies<br>Patients with RA, DAS28 > 5.1, qualified for TNF $\alpha$ inhibitor therapy<br>Followed up for 12 weeks  | ↑ RANK rs8086340-G allele in patients than controls.<br>↓ CRP levels in RANK rs8086340 and RANKL rs7325635 GG homozygotes vs. other phenotypes.<br>↑ alkaline phosphatase level in RANKL rs8086340 and rs1805034 CC allele homozygotes vs. other phenotypes.<br>↓ number of swollen joints in RANKL rs7988338 GG homozygotes compared to A allele.<br>↑ bone erosion in OPG rs2073618 G allele |
| Ruyssen-Witrand et al. (34) | Meta-analysis<br>Three Frech cohorts: Etude de Suivi des PolyArthrites Indifférenciées Récentes (ESPOIR) ( <i>n</i> = 632)<br>Rangueil Midi-Pyrénées (RMP) ( <i>n</i> = 249)<br>French Rheumatoid Arthritis Genetic Consortium (FRAGC) ( <i>n</i> = 590) |  |
| Hu et al. (89)              | Meta-analysis<br>10 studies with 1,758 patients  | ↓ modified total Sharp score and erosion score in denosumab-treated group.   |
| So et al. (35)              | Randomized controlled trial<br>Subjects: Patients with RA and DAS28 ≤ 5.1.<br>Treatment: subcutaneous denosumab 60 mg or placebo once every 6 months for 24 months   | ↑ new erosion and erosion progression in the placebo group after 24 months.<br>↑ erosion healing in the denosumab group at 24 months.<br>No significant changes in joint space parameters, van der Heijde-Sharp erosion score, DAS28 and HAQ-DI between the two groups.  |
| Takeuchi et al. (36)        | Randomized controlled trial<br>Subjects: Patients with RA receiving conventional synthetic disease-modifying antirheumatic drugs.<br>Treatment: denosumab 60 mg every 3 months, denosumab every 6 months or placebo for 12 months.                       | ↓ modified total Sharp score in denosumab-treated group.<br>↓ bone erosion score in denosumab-treated group.<br>No difference in joint space narrowing score.  |
| Saag et al. (37)            | Randomized controlled trial<br>Patients: Patients with RA<br>Treatment: Denosumab 60 or 180 mg or placebo, every 6 months for 12 months. Subjects were followed up for an additional 12 months after discontinuation                                     | ↓ CTX and PINP in both denosumab groups (vs baseline).<br>↑ CTX following denosumab discontinuation, but not significantly different from level during treatment.  |

RANKL, Receptor activator of nuclear factor kappa- $\beta$  ligand; CTX 1, carboxy-terminal crosslinked telopeptide of type 1 collagen; CRP, C-reactive protein; CCP, citrullinated cyclic peptide; ↓, reduced; ↑, increased; DAS 28, 28-joints based disease activity score.

TABLE 2 Recent studies on cytokines-related bone loss in RA.

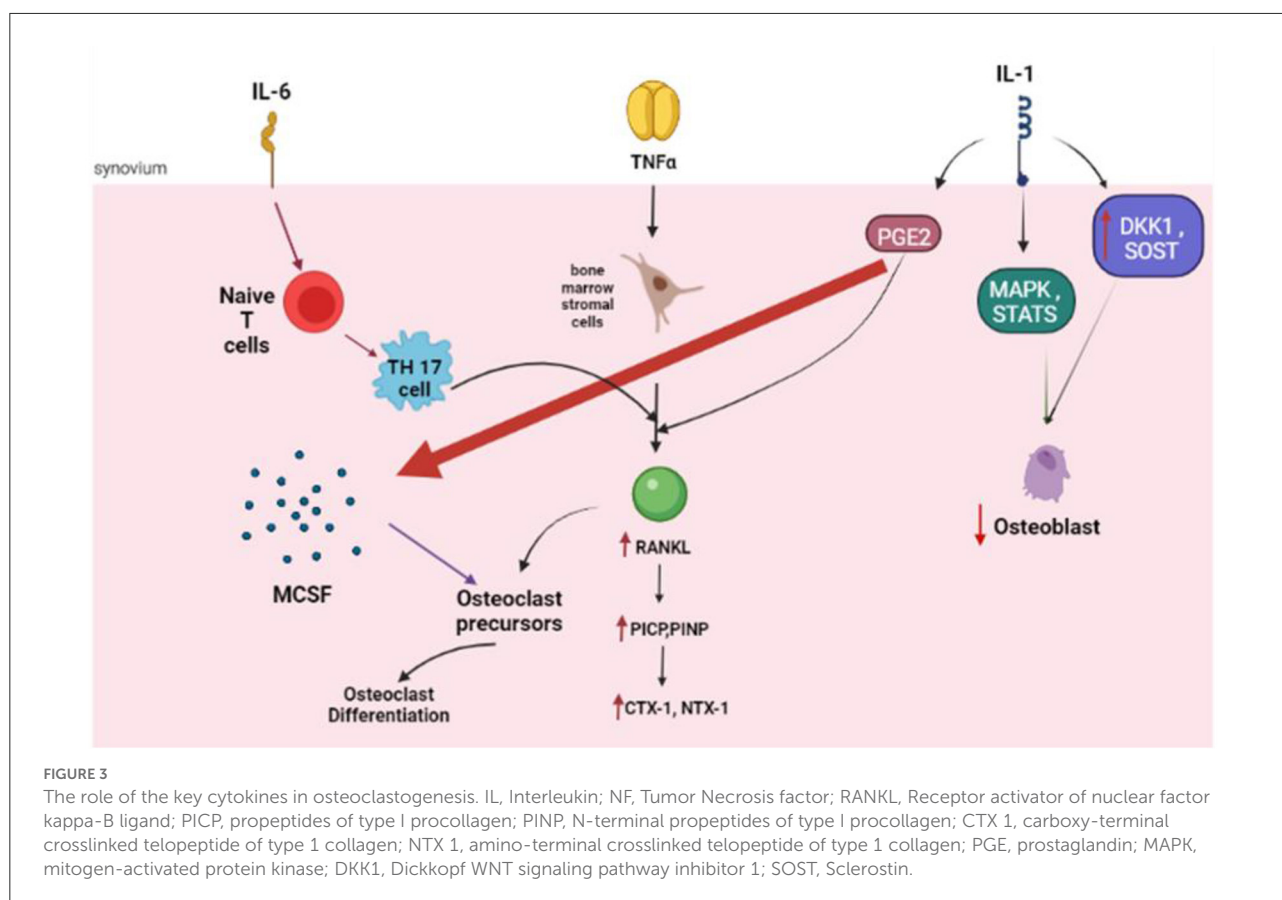
| Article                          | Study design   | Key findings  |
|----------------------------------|--|---|
| Jura-Poltorak et al. (43)        | Cross sectional<br>50 female RA patients   | In a 15-months anti-inflammatory treatment with TNF $\alpha$ blockers, increased bone formation markers i.e., C- and N-terminal propeptides of type I procollagen (PICP, PINP) and reduced bone resorption markers i.e., C- and N-terminal cross-linking telopeptides of type I collagen (CTX-I, NTX-I) were observed.  |
| Perpetuo et al. (44)             | Cross sectional<br>17 patients with RA, evaluated before and after starting TNF inhibitors therapy, were included in this study.   | With TNF $\alpha$ inhibitors therapy, patients had reduced RANKL surface expression in B-lymphocytes and the frequency of circulating classical CD14 <sup>bright</sup> CD16 <sup>-</sup> monocytes. Apart from serum levels of RANKL, RANKL/OPG ratio, CTX-I, TRAF6 and cathepsin K showed reduction with TNF $\alpha$ inhibitors.  |
| Matsuura et al. (49)             | <i>In vivo</i> animal study<br>Lipopolysaccharide (LPS) was injected into the calvarial periosteum of mice to induce inflammatory bone destruction. The sample size was not available  | Anti-IL-6 receptor antibody and anti-TNF $\alpha$ antibody therapy affected mature osteoclasts and switched bone-resorbing osteoclasts to non-resorbing cells.  |
| O'Brien et al. (50)              | Longitudinal<br>24 mice were treated with either RANKL, or TNF $\alpha$ plus IL-6. Osteoprotegerin, anti-IL-6 receptor antibody and hydroxyurea were used to block RANKL, the IL6 receptor and cell proliferation, respectively.   | Reduction of bone erosion and osteoclast formation in arthritic mice with inducible deficiency of RANK. TNF $\alpha$ , IL-6, but not RANKL, induced osteoclast formation in bone marrow and synovial cultures from RANK-deficient animals.  |
| Polzer et al. (90)               | <i>In vitro</i> studies<br>Human TNF $\alpha$ mice (hTNFtg) were crossed with mice lacking IL-1. 32 animals were included in clinical, histological and cellular analyses and animals were killed at 12 weeks of age by cervical dislocation.  | Lack of IL-1 completely reversed increased osteoclast formation and bone resorption in hTNFtg mice and the increased levels of RANKL in these mice. These data shows that IL-1 is essential for TNF-mediated bone loss. Despite TNF-mediated inflammatory arthritis, systemic bone is fully protected by the absence of IL-1.   |
| Saidenberg-Kermanach et al. (25) | <i>In vivo</i> Study<br>DBA/1 mice ( $n = 28$ ) were immunized with bovine type II collagen to induce arthritis and subsequently treated with OPG-Fc or anti-TNF $\alpha$ antibody or both.  | Systemic OPG and anti-TNF $\alpha$ antibody therapy prevented bone loss in arthritic mice through distinct mechanisms involving decreased bone resorption and preserved bone formation.   |
| Gulyás et al. (91)               | Longitudinal Study<br>36 RA and 17 Ankylosing Spondylitis patients undergoing 1-year etanercept or certolizumab-pegol therapy were studied.  | Anti-TNF $\alpha$ antibody therapy halted further bone loss over 1 year. In general, anti-TNF antibody therapy significantly increased PINP, SOST levels, and the PINP/ $\beta$ CTX ratios, while decreased DKK-1 and CathK production at different time points in most patient subsets. Bone erosion was effectively blocked by anti-TNF antibody (-79%) and OPG (-60%), but not by IL-1 receptor antagonist monotherapy. The combination of anti-TNF with IL-1 receptor antagonist however, completely blocked bone erosion (-98%). Inhibition of bone erosion was accompanied by a reduction of osteoclast numbers in the synovial tissue. |
| Zwerina et al. (92)              | <i>In vivo</i> Study<br>A total of 64 mice were examined in this study. Human TNF-transgenic (hTNFtg) mice were treated with anti-TNF antibody (infliximab), IL-1 receptor antagonist (IL-1Ra; anakinra), or OPG either alone or in combinations of 2 agents or all 3 agents.  | Bone erosion was effectively blocked by anti-TNF antibody (-79%) and OPG (-60%), but not by IL-1 receptor antagonist monotherapy. The combination of anti-TNF with IL-1 receptor antagonist however, completely blocked bone erosion (-98%). Inhibition of bone erosion was accompanied by a reduction of osteoclast numbers in the synovial tissue.  |
| Binder et al. (93)               | <i>In vivo</i> and <i>in vitro</i> Study<br>The effects of TNF inhibition on osteoclast precursors as well as local bone destruction <i>in vivo</i> were assessed by treating TNF-transgenic mice with different doses of adalimumab.<br>Sample size not available.  | TNF stimulated osteoclastogenesis mainly by increasing the number of osteoclast precursor cells <i>in vitro</i> .<br>In the hTNF-transgenic mouse model of destructive arthritis, low-dose TNF-inhibiting therapy with adalimumab had no effect on synovial inflammation but significantly inhibited local bone destruction and the generation of osteoclasts.  |
| Axmann et al. (94)               | <i>In vivo</i> and <i>in vitro</i> Study<br>The efficacy of a murine antibody against IL-6 in blocking osteoclast differentiation of mononuclear cells stimulated with RANKL was tested. In addition, arthritic human TNF $\alpha$ -transgenic mice were treated with anti-IL-6 antibody, and osteoclast formation and bone erosion were assessed in arthritic paws. Each group consists of 8 animals. | Blockade of IL-6 dose dependently reduced osteoclast differentiation and bone resorption in monocyte cultures stimulated with RANKL or RANKL plus TNF. In human TNF-transgenic mice, IL-6 blockade did not inhibit joint inflammation, but it strongly reduced osteoclast formation in inflamed joints as well as bone erosions.  |

(Continued)

TABLE 2 Continued

| Article           | Study design  | Key findings  |
|-------------------|---|---|
| Lange et al. (95) | Open-label prospective study<br>26 patients with persistently active RA were treated with infliximab. | After 12 months of infliximab therapy, there was a significant increase in BMD in the spine and the femoral neck. There was a significant increase in osteocalcin serum levels between baseline and after 12 months ( $P < 0.01$ ) and a significant decrease in the marker for bone resorption ( $P < 0.01$ ) but no change in serum calcium was observed. |

RANKL, Receptor activator of nuclear factor kappa-B ligand; BMD, bone mineral density; TNF, tumor necrosis factor; CTX 1, carboxy-terminal crosslinked telopeptide of type 1 collagen; PINP, procollagen type I N-propeptide (PINP); SOST, sclerostin; DKK1, Dickkopf WNT signaling pathway inhibitor 1; CathK, cathepsin K.



IL-17 was found to have osteoclastogenic potential through the upregulation of RANKL (59). In a similar manner, IL-9 stimulation was reported to significantly enhance M-CSF - RANKL-mediated osteoclast formation and differentiation. IL-9 regulates the expression of genes in the metabolic pathways and the expression of matrix metalloproteinases (MMPs), which are responsible for bone degradation (60).

## Autoantibodies and bone resorption

Autoantibodies, namely rheumatoid factor (RF) and anti-citrullinated cyclic peptide (anti-CCP), play pivotal roles in RA

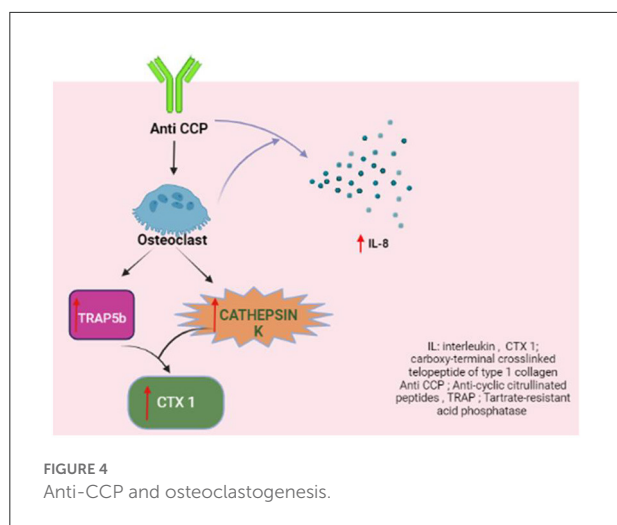
for diagnostic and prognostic purposes. Seropositive disease is associated with a more severe and aggressive disease course (62). Emerging evidence suggests an association between anti-CCP and bone resorption. Clinical studies have consistently revealed that anti-CCP positivity was related to an increased risk of major fracture, lower BMD and osteopenia (63, 64). Bugatti et al. reported that anti-CCP positivity negatively affected the Z-scores of the spine and hip. The above association was observed even at low levels of RF [adjusted OR (95 % CI) 2.65 (1.01 to 7.24)], but was further increased by concomitant high RF [adjusted OR (95 % CI) 3.38 (1.11 to 10.34)] (64). This finding lends credence to the notion that autoantibodies may have a direct causative role in bone

TABLE 3 Recent studies on the association between anti-CCP and bone mineral density.

| Article              | Study design  | Key findings   |
|----------------------|---|--|
| Cheng et al. (96)    | Cross sectional<br>The participants were categorized into two groups according to anti-CCP-positive (anti-CCP+) or anti-CCP- status and into four groups (I–IV) according to the anti-CCP level quartiles.                                    | Compared with anti-CCP- patients, anti-CCP+ patients had a significantly higher 10-year probability of major fracture and a significantly lower BMD of the femoral neck ( $p = 0.0196$ ). The rates of osteoporosis and previous fracture were comparable. The BMD and 10-year probability of major fracture among the groups were significantly different.  |
| Hafstrom et al. (97) | Retrospective<br>Baseline data from the BARFOT (Better Anti-Rheumatic Pharmacotherapy) cohort, which consists of patients with RA with a disease duration of 1 year or less.  | Patients positive for anti-CCP had significantly more frequent osteopenia in the femoral neck and Ward's triangle compared with anti-CCP-negative patients ( $p = 0.016$ and $0.003$ , respectively). This difference was found in men at any anti-CCP titer, but in women, osteopenia in these hip locations was found only in those with high anti-CCP titers ( $> 500$ IU/ml). Anti-CCP was not associated with osteopenia in the lumbar spine or the metacarpal bones. In multiple logistic regression analyses, anti-CCP was independently associated with osteopenia in the femoral neck and/or Ward's triangle. |
| Wysham et al. (98)   | Cross sectional<br>Demographic, clinical, laboratory and functional variables were collected at study visits.   | Age and high anti-CCP positivity were negatively associated with BMD after controlling for other variables ( $\beta = -0.003$ and $-0.055$ , respectively, $p < 0.05$ ).<br>In highly-positive anti-CCP participants, increasing anti-CCP levels were associated with a negative linear trend in BMD ( $\beta = -0.011$ , $p = 0.026$ ).   |
| Kurowska et al. (63) | Cross sectional.<br>Bone marrow samples taken from the femur during a hip replacement surgery performed as part of normal clinical care. Paired peripheral blood samples were collected 1–1.5 h before the hip joint prosthesis implantation. | Anti-CCP present in RA bone marrow was associated with increased amounts of TRAP5b, cathepsin K and CTX-I in this location. Levels of IL-8, the key mediator of anti-CCP-induced bone resorption, were also elevated in bone marrow containing anti-CCP antibodies and positively correlated with TRAP5b and cathepsin K concentrations. Higher levels of TRAP5b, cathepsin K, CTX-I and IL-8 in bone marrow compared to peripheral blood indicate local generation of these molecules.  |
| Amkreutz et al. (99) | Longitudinal<br>Dual x-ray absorptiometry of the lumbar spine and left hip was performed in 408 Dutch patients with early RA during 5 years of follow-up and in 198 Swedish patients with early RA during 10 years of follow-up.              | In the Dutch cohort, significantly lower BMD at baseline was observed in anti CCP-positive patients compared to anti CCP-negative patients. In the Swedish cohort, anti-CCP-positive patients tended to have a higher prevalence of osteopenia at baseline ( $P = 0.04$ ).   |
| Ahmad et al. (100)   | Cross sectional<br>Eligible patients had known BMD, as measured by digital X-ray radiogrammetry (DXR-BMD), and anti-CCP2 antibody measurements at the same time point or within 6 months.   | DXR-BMD was lower in the anti-CCP2 + ve vs. the anti-CCP2-ve groups. DXR-BMD decreased with increasing anti-CCP2 titer ( $P < 0.001$ for left and right hands).  |
| Bugatti et al. (64)  | Cross sectional<br>Systemic bone mineral density (BMD) was measured in the lumbar spine and the hip in 155 consecutive treatment-naïve patients with early RA (median symptom duration 13 weeks).   | The anti-CCP positivity negatively affected the Z-scores of the spine and hip. The above association was observed even at low levels of RF [adjusted OR (95 % CI) 2.65 (1.01 to 7.24)], but was further increased by concomitant high RF [adjusted OR (95 % CI) 3.38 (1.11 to 10.34)].   |
| Llorente et al. (65) | Cross sectional<br>BMD was measured using dual X-ray absorptiometry. Anti-CCP titers were determined through enzyme immunoassay   | Anti-CCP positivity remained significantly associated with lower bone density at the lumbar spine, femoral neck, and hip but not at the metacarpophalangeal joints despite adjustment for gender, age and body mass index  |

BMD, bone mineral density; CCP, citrullinated cyclic peptide; TRAP5b, Tartrate-resistant acid phosphatase 5b; CTX-I, carboxy-terminal crosslinked telopeptide of type 1 collagen; RF, Rheumatoid Factor.





remodeling. In a Spanish cohort of patients with early arthritis and a median disease duration of 5.1 months, anti-CCP positivity remained significantly associated with lower BMD at the lumbar spine, femoral neck, and hip but not at the metacarpophalangeal joints despite adjustment for gender, age and body mass index (65). Taken together, these results may suggest that autoantibodies tend to induce systemic bone loss earlier than local or articular bone loss. Table 3 summarizes the findings of recent studies on anti-CCP and bone loss. However, there is a lack of data on the relationship between RF and bone loss.

The levels of the active TRAP5b isoform, which represent the number and activity of osteoclasts (35, 36), and cathepsin K, which degrades the bone matrix proteins (37, 38), were both found to be significantly higher in the bone marrow samples with anti-CCP. The anti-CCP-positive bone marrow samples also contained increased concentrations of CTX-I, which is produced as a result of the enzymatic activity of cathepsin K (66, 67).

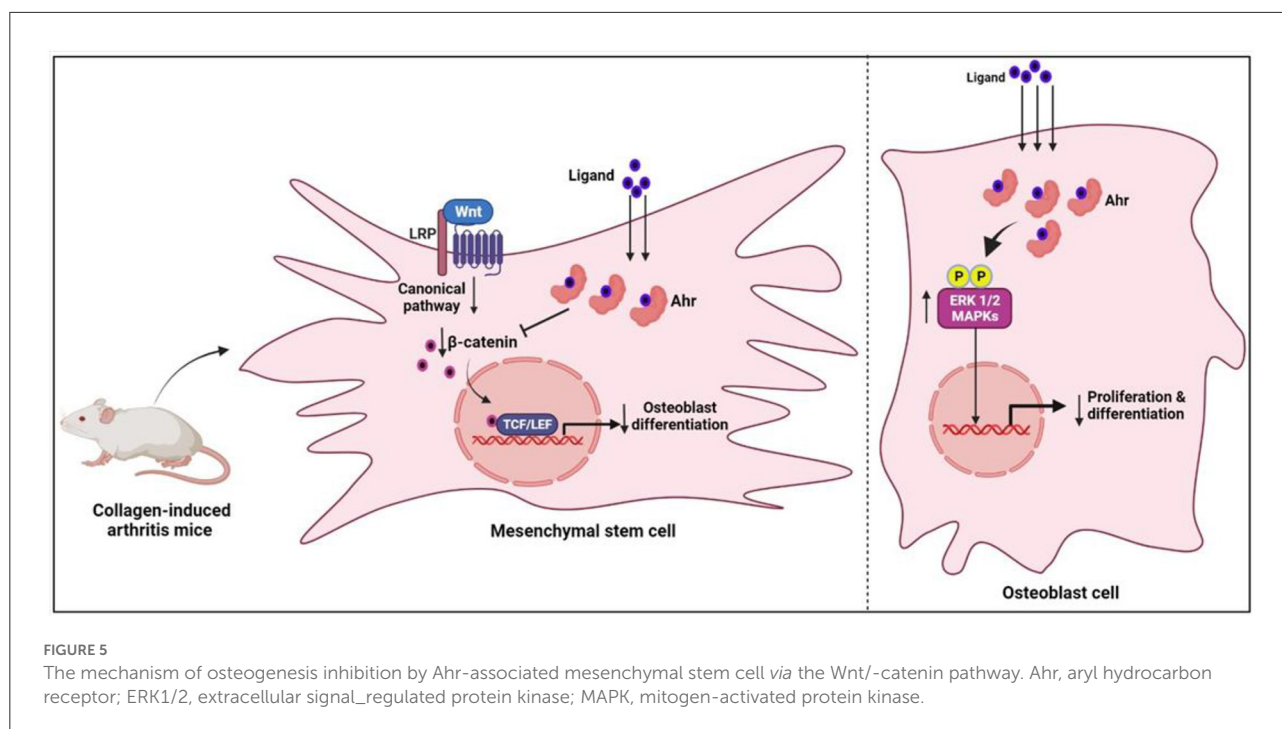
The key orchestrator of anti-CCP-dependent osteoclastogenesis is IL-8 (68). The concentration of this cytokine increases during osteoclast differentiation and in the presence of anti-CCP. Bone marrow samples from RA patients revealed increased IL-8 levels in anti-CCP positive bone marrow. TRAP5b and cathepsin K levels were found to correlate positively with IL-8 concentration (63).

Apart from RF and anti-CCP, anti-carbamylated protein antibodies, which are not routinely tested in clinical practice, were found to have an independent association with lower BMD in the spine and hip but not the metacarpophalangeal joint (69) (Figure 4).

## Role of aryl hydrocarbon receptor in RA-associated bone erosion

Aryl hydrocarbon receptor (Ahr) is a ligand-activated transcription factor that belongs to the Per-Arnt-Sim protein superfamily. It is a nuclear receptor and usually stays inactive in the cytosol due to its binding with cochaperones. When it binds to a specific ligand, its structure transforms and it is translocated to the nucleus, where it forms heterodimerization with aromatic hydrocarbon receptor nuclear transfer protein (ARNT). Subsequently, the Ahr/ARNT complex regulates biological functions, including toxicity, biological evolution, bone remodeling, and immune response by promoting gene transcription of various prototypic genes (70). Increasing evidence confirms its close association with the pathogenesis of immune diseases, including RA. Ahr activation significantly contributes to the development of RA (71). In experimental studies, Ahr activation with its agonists contributed to RA disease progression, bone damage and osteoclast differentiation (72). Ahr expression was found to be 2-fold higher in RA patients when compared to controls. Furthermore, a positive association between cigarette smoking and Ahr activation in RA patients has been demonstrated (73) (Figure 5).

Maintaining homeostasis in bone remodeling requires a balance between the relative activity of bone-forming osteoblasts and bone-resorbing osteoclasts. The modulation of Ahr signaling *via* the NF- $\kappa$ B, Wnt, and MAP kinase pathways has been linked to changes in bone remodeling (74–78). The activated Ahr may affect bone remodeling by interfering with the functional differentiation of osteoblasts and osteoclasts (79). The influence of Ahr signaling on osteoblast function is well-established in recent studies. The bone erosion in arthritic mice was found to be positively associated with high Ahr expression, and these highly expressed Ahr levels were relatively localized to the osteoblast cells (80). Additionally, in *in vitro* conditions, the activated Ahr inhibited the proliferation and differentiation of osteoblast cells by upregulating the ERK/MAPK signaling pathway (80). In further investigations, it was concluded that the activated Ahr in mesenchymal stem cells, derived from collagen-induced arthritis mice, inhibited osteogenesis by downregulating  $\beta$ -catenin (81). On the other hand, the influence of Ahr signaling on osteoclastogenesis is not clearly understood. In *in vivo* and *in vitro* studies of rodents, there were inconsistent results regarding the effects of Ahr on osteoclast differentiation (82). However, findings from a recent study on human osteoclast cells revealed that Ahr activation through kynurenine inhibited osteoclast differentiation by downregulating NFATc1 protein expression (83). The same study concluded that Ahr activation could be a potential target to treat the bone loss in RA. In support of this, natural products derived Ahr ligands/agonists have been shown to prevent bone erosions in RA (84, 85). Overall, the influence of Ahr



signaling on RA-associated bone erosion is still controversial as it inhibits the osteoblast cells and may either inhibit or promote osteoclast differentiation.

## Disease-modifying anti-rheumatic drugs (DMARDs) and bone loss

Disease-modifying anti-rheumatic drugs (DMARDs) are the mainstay of treatment in RA. There are conventional (methotrexate, leflunomide, sulfasalazine), biologic (adalimumab, etanercept, golimumab, tocilizumab etc) and targeted synthetic DMARDs (tofacitinib, baricitinib, upadacitib). Apart from suppression of inflammation, there is ample evidence that all of these classes of drugs prevent articular erosions and radiographic progression of joint damage. Chen et al. reported that after 3 years of biologic or targeted synthetic DMARD therapy, BMD remained stable at the femoral neck, hip and lumbar vertebra (86). These findings were echoed by other studies (87, 88).

## Conclusions

There is compelling evidence that the interplay of various pathways and different mechanisms contribute to bone loss and secondary osteoporosis in RA. Osteoclastogenesis may occur *via* RANKL-dependent and RANKL-independent processes. TNF $\alpha$ , IL-1, IL-6 anti-CCP which are the key orchestrators of inflammation tend to affect bone remodeling adversely.

## Author contributions

RS, RU, K-YC, and SD contributed to the literature search and manuscript composition. SS contributed to creation of the figures and manuscript composition. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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