



Osteoporosis in the view of osteoimmunology: common feature underlined by different pathogenic mechanisms

DARJA FLEGAR^{1,2}
ALAN ŠUČUR^{1,2}
ANTONIO MARKOTIĆ^{1,2,3}
NATAŠA KOVAČIĆ^{2,4}
DANKA GRČEVIĆ^{1,2}

¹Department of Physiology and Immunology,
University of Zagreb School of Medicine, Šalata 3b,
Zagreb-HR 10000, Croatia

²Laboratory for Molecular Immunology, University of
Zagreb School of Medicine,
Šalata 12, Zagreb-HR 10000, Croatia

³Department of Physiology, University of Mostar
School of Medicine, Bijeli Brijeg bb, 88000 Mostar,
Bosnia and Herzegovina

⁴Department of Anatomy, University of Zagreb School
of Medicine, Šalata 3b,
Zagreb-HR 10000, Croatia

Correspondence:

Danka Grčević
Department of Physiology and Immunology
University of Zagreb School of Medicine
Šalata 3, 10000 Zagreb, Croatia
E-mail address: danka.grcevic@mef.hr

Abstract

Osteoporosis is a skeletal disorder characterized by low bone mineral density and microarchitectural changes with increased susceptibility to fractures, resulting in significant morbidity and mortality. Although it predominantly affects postmenopausal women, it is now well known that systemic bone loss is a common underlying feature of different metabolic, endocrine and inflammatory diseases. Investigations of osteoporosis as a complication of chronic inflammatory conditions revealed immune mechanisms behind the increased osteoclast bone resorption and impaired osteoblast bone formation. This concept was particularly emphasized after the research field of osteoimmunology emerged, focusing on the interaction between the immune system and bone. It is increasingly becoming evident that immune cells and mediators critically regulate osteoclast and osteoblast development, function and coupling activity. Among other mediators, receptor activator of nuclear factor- κ B ligand (RANKL), receptor activator of nuclear factor- κ B (RANK) and soluble decoy receptor osteoprotegerin (OPG) form a key functional link between the immune system and bone, regulating both osteoclast formation and activity as well as immune cell functions. Excessive production of inflammatory mediators exerts autocrine, paracrine and endocrine signaling effects on bone remodeling with the net increase in bone resorption locally, in diseases primarily affecting joints, bones or surrounding tissues, and systemically, causing osteoporosis in various chronic inflammatory

Abbreviations:

BMD	– bone mineral density
BME	– bone marrow edema
DKK-1	– Dickkopf-1
IL	– interleukin
M-CSF	– macrophage colony-stimulating factor
MRI	– magnetic resonance imaging
ODF	– osteoclast differentiation factor
OPG	– osteoprotegerin
PGE ₂	– prostaglandin E ₂
RANK	– receptor activator of nuclear factor – κ B
RANKL	– receptor activator of nuclear factor – κ B ligand
TNF	– tumor necrosis factor
TNFSF11	– tumor necrosis factor (ligand) superfamily, member 11
TNFRSF11A	– tumor necrosis factor receptor superfamily, member 11
TRAIL	– TNF-related ligand
TRANCE	– TNF-related activation-induced cytokine
TRAP	– tartrate-resistant acid phosphatase

diseases. This brief review particularly focuses on bone pathology in rheumatoid arthritis, as one of the most extensively studied conditions accompanied by local and systemic inflammation-induced bone loss.

1. INTRODUCTION

Osteoporosis is a systemic skeletal disease which affects more than 200 million people and is one of the major public health problems (1). It is characterized by low bone mass and microarchitectural changes, both leading to enhanced susceptibility to bone fractures and increased mortality risk (2, 3). Estimated occurrence of osteoporosis is 1 in 3 women and 1 in 12 men over the age of 50 worldwide, being one of the main risk factors for pathologic fractures, mostly involving the lumbar vertebrae, hip, and wrist (4). Primary osteoporosis affects postmenopausal women due to estrogen deficiency, and elderly women and men through vitamin D insufficiency and secondary hyperparathyroidism which contribute to bone loss (3). On the other hand, secondary osteoporosis is outlined as low bone mineral density (BMD) with increased risk of fractures caused by various factors other than aging or postmenopause (5). Therefore, it affects men and premenopausal women who have not been typically considered at high risk for primary osteoporosis (6). Increased understanding of bone metabolism and factors that influence bone turnover leads to a better characterization of diseases, disorders, treatments and medications that decrease bone quality in women and men of all ages (2, 5). These include various endocrine disorders (such as type I and II diabetes, Cushing's syndrome, hyperthyroidism, hyperparathyroidism, hypogonadism and others), metabolic disorders (Marfan syndrome, Gaucher's disease, galactosemia), rheumatologic and inflammatory diseases (systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease), chronic diseases (chronic obstructive pulmonary disease, celiac disease), neurologic, hematologic and oncologic diseases (2-6). It also includes medications such as glucocorticoids, anticonvulsants, glitazones, heparine, cyclosporine and others. Iatrogenic secondary osteoporosis thus became a long-term complication of diseases treated with these medications (4, 5). In this brief review we will particularly focus on mechanisms of bone loss associated with inflammatory arthritides, characterized by chronic inflammatory response as well as periarticular and generalized bone loss due to deregulation of bone metabolism – bone resorption by osteoclasts and bone formation by osteoblasts (7-11).

2. BONE PATHOLOGY IN OSTEOPOROSIS

Bone remodeling is a well balanced, continuously ongoing homeostatic process of osteoclast bone resorption and osteoblast bone formation, ensuring and adjusting

bone mechanical properties and its response to metabolic needs. Hence, it is regulated by numerous factors like hormones, cytokines, chemokines and biochemical stimuli, and therefore easily disrupted in different pathological conditions (5, 12). Well-coordinated differentiation and activation as well as synchronized function of two bone cell types, osteoclasts and osteoblasts, is crucial for bone homeostasis maintenance.

Osteoblasts are mature, cuboidal, non-proliferating cells that synthesize and mineralize bone matrix, and influence osteoclast differentiation (13, 14). Osteoblast progenitors arise from mesenchymal stem cells found in periosteum, bone marrow and circulation, which alongside of osteoblasts can produce a variety of lineages such as adipocytes, chondrocytes, myocytes and hematopoiesis supporting cells (15-19). Osteoblastogenesis includes proliferation and differentiation of osteoblast progenitors through series of maturational stages to functional, bone matrix-secreting osteoblasts. By producing macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor- κ B ligand (RANKL) and osteoprotegerin (OPG), osteoblasts regulate osteoclast progenitor pool size, their differentiation and bone-resorbing activity (14, 20).

Osteoclasts are large, multinucleated, tartrate-resistant acid phosphatase (TRAP)-positive, unique bone-resorbing cells (21-26). They originate from hematopoietic stem cells found in bone marrow and circulation. Belonging to myeloid monocyte/macrophage lineage, osteoclast progenitors express M-CSF receptor (CD115, cFms) and receptor activator of nuclear factor- κ B (RANK; also known as tumor necrosis factor receptor superfamily, member 11, TNFRSF11A), activation of which is crucial for their differentiation and maturation (27). Excessive bone resorption is an exclusive consequence of increased osteoclast activation, since the ability of macrophages and fibroblasts to degrade bone is weak (28). Osteoclasts are critically involved in skeletal functions such as bone remodeling, fracture repair and pathological bone resorption associated with inflammatory conditions (29).

Osteoporosis results from an imbalance in bone remodeling, where bone resorption predominates over bone formation (5, 28). Subsequent changes in bone architecture and a net loss of bone mass make bones weak and incapable of bearing physiological load. In the most severe cases of osteoporosis even normal physical activities lead to pathologic fractures (30). Due to most common forms of osteoporosis, the postmenopausal osteoporosis and senile osteoporosis, and well described effects of vitamin D₃, calcium and other nutritional deficiencies on bone structure, the disease is usually classified as an endocrine or metabolic disorder (4). Studies investigating secondary osteoporosis, where chronic inflammatory diseases, regardless of the cause and primarily affected sites, are accompanied by systemic bone loss and increased risk of

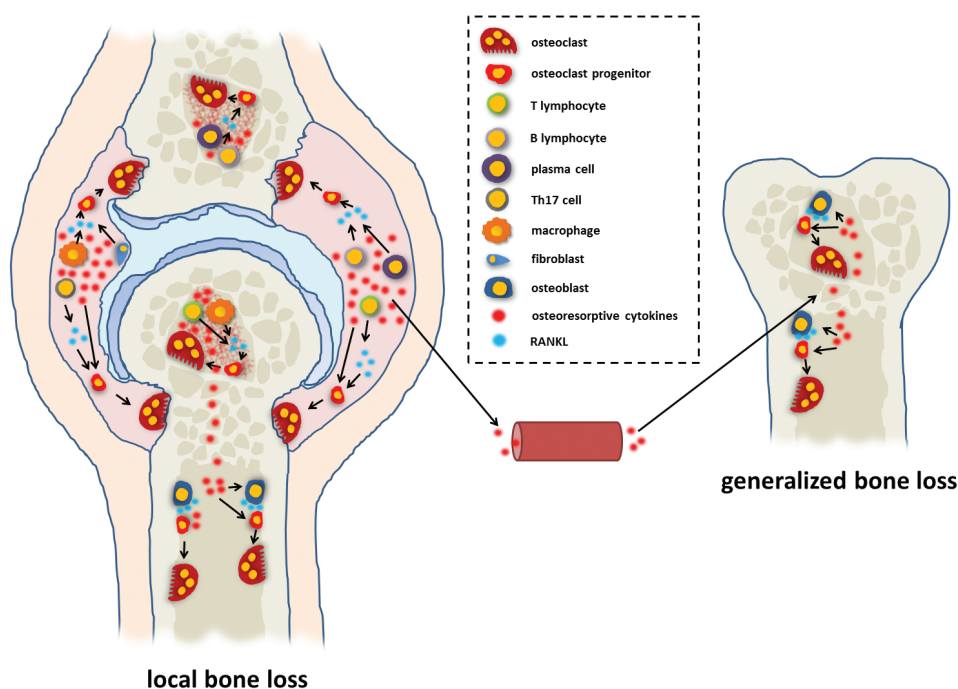


Figure 1. Bone loss in rheumatoid arthritis is caused by the complex interplay between immune and bone cells. Immune cells, including macrophages, neutrophils, dendritic cells, and T and B lymphocytes, infiltrate the rheumatoid synovium that is constantly inflamed and invades adjacent tissue, resulting in joint destruction. These innate and adaptive immune cells vigorously drive differentiation of osteoclast progenitors by producing proinflammatory cytokines and RANKL. In addition, synovial fibroblasts and osteoblasts comprise the cellular components of synovial and bone matrix, and contribute to osteoclast formation under inflammatory conditions. The osteoclast-rich portion of hyperplastic and highly vascularized synovial membrane, called pannus, destroys bone, whereas enzymes, secreted by neutrophils, synoviocytes and chondrocytes, degrade cartilage. Mature osteoclasts, as the final effectors of bone loss, exert their bone-resorbing activity at local and systemic levels, which include marginal erosions, subchondral bone loss, periarticular bone loss as well as systemic osteoporosis.

fractures, demonstrated the role of the immune system in bone remodeling (12, 27). The notion that the plurality of etiological causes of bone loss shares a final step of impairing osteoclast and osteoblast coupled activity, which is mediated by immune cells and cytokines, created the research area of osteoimmunology (4, 31, 32). This novel approach offers a new perspective in disease pathogenesis considering it an inflammatory disease (4).

3. OSTEOIMMUNOLOGY AND RANKL/RANK/OPG SYSTEM

The field of osteoimmunology emerged with the discovery that T lymphocytes participate in the regulation of osteoclast formation, either by stimulatory or inhibitory effects. As such, the research area of osteoimmunology focuses on the functional crosstalk between the immune system and bone, making it a new interdisciplinary field with a potential to indicate novel targeted therapies in diseases accompanied with inflammation-induced bone loss (4).

It has been well demonstrated that osteoclasts could be derived from immature macrophages and early hema-

topoietic progenitors (33). Osteoclasts share many histochemical and functional similarities with both tissue and inflammatory macrophages, including the property of tissue degradation followed by reparation carried out by cells of mesenchymal origin, osteoblasts or fibroblasts respectively (34). However, osteoclasts express low levels of class II major histocompatibility complex molecules, CD14, Fc and complement receptors, and do not serve as antigen presenting cells (33).

During recent times, TNF-superfamily ligands and their respective receptors have been shown to be a crucial link between the immune system and other organ systems, including bone. One of the major examples that has significantly contributed to the development of the field of osteoimmunology is the cytokine RANKL, additionally identified as TNF-related activation-induced cytokine (TRANCE), osteoclast differentiation factor (ODF), and TNFSF11. RANKL, together with its signaling receptor RANK and soluble decoy receptor OPG, comprises the crucial paracrine system, which regulates osteoclast formation and function (35, 36). The RANKL/RANK/OPG system was originally identified as having an important role in immunity, mostly mediated through

dendritic cells, and, in parallel, as being a crucial factor in the regulation of osteoclast formation and therefore bone homeostasis. Mouse knockout models for the RANKL/RANK/OPG axis components have emphasized and clarified its role and importance in both bone and immunity during development, homeostasis and disease, thereby advancing the field of osteoimmunology (27).

RANKL potently stimulates both the formation of osteoclasts from progenitor cells and the bone-resorbing activity of mature osteoclasts. It is predominantly expressed as a transmembrane protein on the surface of stromal cells, osteoblasts, osteocytes, hypertrophying chondrocytes and synovial fibroblasts, but can also be secreted in soluble form by other cells like activated T lymphocytes. RANKL knockout mice have no osteoclasts and exhibit osteopetrosis, but have a normal number of monocyte/macrophage lineage cells. They also exhibit altered lymph node development, with impaired early B- and T-lymphoid cell differentiation, pointing to its important role in both, bone and immune system homeostasis. A number of hormones, cytokines and inflammatory mediators, including vitamin D₃, parathyroid hormone, prostaglandin E₂ (PGE₂), interleukin(IL)-1, IL-6, IL-7, IL-15, IL-17, IL-21 and IL-22 appear to exert their osteoresorptive effect by inducing RANKL expression in mesenchymal lineage cells (33, 35, 37).

RANK (designated as TNFRSF11A), the biologically active signaling receptor for RANKL, is expressed on osteoclast progenitors and mature osteoclasts, but also on dendritic cells. It has been detected at the RNA level in a variety of cell types and tissues (including thymus, mammary glands, prostate, bone marrow, lung, brain, liver and skin). RANK is strongly induced by M-CSF, especially on osteoclast progenitors. As expected, RANK knockout mice exhibit the same phenotype as RANKL knockouts (33, 37). Osteoclast differentiation is physiologically triggered by RANKL/RANK binding, in the presence of M-CSF, which serves to promote proliferation and survival of osteoclast progenitor cells (20, 33).

OPG, as a soluble receptor, inhibits osteoclast differentiation by binding RANKL and thus preventing RANKL/RANK interaction. Additionally, OPG can also bind TNF-related ligand (TRAIL). It is produced by bone marrow stromal cells, but also expressed by B lymphocytes and dendritic cells. It is proposed that OPG is constantly produced in physiological conditions, thereby suppressing RANKL activity, which is supported by the finding of severe osteoporosis in OPG knockout mice (33, 36).

Factors that regulate bone remodeling often act by affecting the balance of RANKL and OPG as a key determinant of the rate of osteoclastogenesis and bone resorption. Consequently, an increased RANKL/OPG ratio results in enhanced osteoclast differentiation and forma-

tion of bone erosions in various pathologic conditions such as bone cancers and metastases, rheumatoid arthritis and osteoporosis. Cytokines and growth factors which are produced at sites of inflammation or released from matrix during bone turnover often enhance or modulate the response of osteoclasts and their progenitors to RANKL/RANK stimulation (20, 33).

4. CHRONIC INFLAMMATORY DISEASES AND BONE DESTRUCTION

Bone loss as a consequence of chronic inflammation represents a serious complication associated with arthritis as well as other inflammatory processes such as chronic bacterial and viral infections, inflammatory bowel diseases and certain metabolic disorders (38-40). Through the production of osteoresorptive mediators (IL-1, IL-6, IL-11, IL-17, TNF- α , RANKL, CCL2, CXCL12, etc) by inflammatory and immune cells, inflammation creates conditions that promote osteoclast differentiation and structural damage in arthritis (15, 41-44). Three major forms of chronic inflammatory joint diseases are classified in clinical practice, depending on their pathogenic mechanisms and primary target tissue: osteoarthritis, rheumatoid arthritis and spondyloarthritis. The latter includes ankylosing spondylitis, psoriatic arthritis, reactive arthritis, arthritis associated with inflammatory bowel disease, and juvenile and undifferentiated forms (45, 46). In addition to arthritis, bone loss as a consequence of chronic inflammation represents a key feature of localized bacterial infections of bones or adjacent tissues, including caries, periodontitis or osteomyelitis (31).

Rheumatoid arthritis and periodontal disease are representative examples of chronic inflammatory diseases with bone loss occurring locally at inflamed sites as well as systemically in the form of osteopenia and osteoporosis. Chronic inflammation in rheumatoid arthritis is a consequence of an autoimmune disorder primarily affecting synovial joints, whereas in periodontal disease the persistent bacterial gingival infection repeatedly triggers immune response. Central to the pathogenesis of rheumatoid arthritis is the inflammation of synovial tissue followed by joint destruction and ankylosis (45, 46). Periodontitis implies chronic inflammation of tooth supporting structures leading to progressive alveolar bone resorption and tooth loss (4). Unlike rheumatoid arthritis, in ankylosing spondylitis inflammation occurs at areas of ligament and tendon insertions to bone (entheses), followed by progression to joint synovium and, finally, joint destruction (45). Periarticular bone mineralization is usually maintained and accompanied by ossification of inflamed entheses and syndesmophite formation, suggesting a disorder pattern of enhanced bone remodeling in contrast to the predominance of bone resorption seen in rheumatoid arthritis (14, 47).

In other conditions hallmarked by chronic inflammation not present near bones, such as inflammatory bowel diseases (i.e. Crohn's disease and ulcerative colitis), celiac disease, cystic fibrosis and chronic obstructive pulmonary disease, the occurring bone loss is only systemic (12). Different factors, such as bed rest, immobilization, malnutrition and medications (especially glucocorticoid treatment) contribute to pathogenesis of generalized and localized osteopenia by separate mechanisms. In parallel, inflammation activated immune mechanisms disturb the homeostasis of bone remodeling by acting directly on bone cells, resulting in increased osteoclast activity and excess bone resorption as well as impaired osteoblast function with insufficient bone formation (1-3, 48).

5. PATHOGENESIS OF INFLAMMATION-INDUCED BONE LOSS

During inflammatory response, cells of innate and adaptive immunity produce various mediators, many of which, by autocrine, paracrine and endocrine signaling, affect osteoclast and osteoblast differentiation, function and migration, leading to an imbalance between bone resorption and formation (32). A wide range of proinflammatory cytokines (IL-1, IL-6, IL-17, IL-18, TNF- α), chemokines (such as CCL2, CCL3, CCL4, CCL5, CXCL12), growth factors (vascular endothelial growth factor, hypoxia-inducible factors), and apoptotic mediators (Fas ligand, TRAIL, ligand for herpesvirus entry mediator) are disturbed in arthritis, which directly or indirectly, through the RANK/RANKL/OPG system, promote osteoclastogenesis (49-52). It has been shown that even a small rise in the level of systemic inflammation can precipitate osteodestruction, leading to fractures and disabilities related to arthritis (53). Moreover, the presence of generalized bone loss, manifested as lower BMD and a higher rate of pathologic fractures, has been observed in different forms of arthritides and is associated with disease severity (20). Although generalized osteoporosis in arthritis was usually attributed to immobility and glucocorticoid treatment, more recent studies suggest that systemic osteopenia, as well as periarticular bone loss and marginal erosions, are caused at least in part by a common pathogenic mechanism that involves inflammation-induced increase in osteoclast number and activity (Figure 1) (54-57).

Apart from systemic bone loss, patients with rheumatoid arthritis have significant destruction of affected joints. It was traditionally postulated that inflammation of synovial tissue, with increased vascularity, hyperplasia and accumulation of macrophages, plasma cells, T and B lymphocytes, dendritic cells, natural killer cells and mast cells, is the major pathogenic event as it invades adjacent structures (articular cartilage, cortical bone surface and underlying bone marrow) and leads to typical signs and symptoms of rheumatoid arthritis (joint pain, stiffness,

swelling and structural changes) (58, 59). However, 'synovio-centric' model of rheumatoid arthritis has been refined after findings that joint disease spreads well beyond synovial tissue inflammation involving other, neighboring anatomic compartments (including subchondral and periarticular bone) (60).

With the advance in magnetic resonance imaging (MRI), subchondral bone has been recognized as a site of pivotal importance for the inflammatory processes in rheumatoid arthritis. Bone marrow involvement in the form of MRI-visualized bone marrow edema (BME) is a common feature at all stages of the disease, and is associated with disease activity and, most importantly, with future development of bone erosions and poor functional outcomes. MRI scans show structural changes, which extend into the bone marrow cavity and are linked either to cortical bone destruction (bone erosion) or to more diffuse changes in the bone marrow space (BME or osteitis). The latter lesions have also been described in osteoarthritis, spondyloarthritis and systemic lupus erythematosus (54, 62-63).

5. 1. LOCAL BONE LOSS IN RHEUMATOID ARTHRITIS

Inflammation in rheumatoid arthritis comprises all joint structures, beginning with the synovial membrane and propagating to joint capsule, cartilage and juxta-articular bone. Increased osteoclast differentiation in rheumatoid arthritis occurs at the bone surface/pannus interface as well as within the inflamed synovial tissue (64). Due to proximity of inflamed synovial pannus and paracrine diffusion of proinflammatory cytokines, two shapes of bone loss occur locally: focal erosions and subchondral bone loss as well as periarticular bone loss (Figure 1).

Most prominent changes of the subchondral compartment include cortical bone erosions, caused by activated osteoclasts that migrate from pannus and invade subchondral bone, and diffuse BME adjacent to inflamed joints. Focal bone erosions (marginal erosions) develop early in disease pathogenesis due to direct contact of pannus and cortical bone surface at the margins of the joint. Bone erosions and following joint destruction are hallmarks of rheumatoid arthritis but also occur in spondyloarthritis and the erosive form of osteoarthritis (20). The pathohistological correlate of BME is osteitis, with increased vascularization and inflammatory infiltrate (comprised of T and B lymphocytes, plasma cells and macrophages) replacing marrow fat adjacent to juxta-articular trabecular bone. Rheumatoid bone marrow is the site of active pathology with histological findings similar to what is found within the synovial membrane but with the addition of active osteoclasts closely juxtaposed to trabecular bone and likely to be mediating the erosive processes (52, 60, 65).

Periarticular osteopenia is characterized by a decrease in trabecular size and number in the metaphyseal regions adjacent to the affected joint (66). Periarticular bone loss occurs at sites close to inflamed joints but not in direct contact with synovial tissue and subchondral lesions. Presumably, it develops due to an increased local production of osteoclast-activating cytokines and their diffusion over Haversian and Volkmann canals, leading to excessive bone resorption. Decreased loading and reduced motion of the limb due to pain and disability contribute to reduced BMD. Periarticular demineralization appears earlier than systemic osteoporosis, sometimes even before erosions, and can serve as a predictor of greater joint damage (32).

The seronegative spondyloarthropathies (such as psoriatic arthritis, ankylosing spondylitis, reactive arthritis and arthritis associated with inflammatory bowel diseases) share similarities with some of the local findings in rheumatoid arthritis, although distal joints and axial skeleton may also be affected. The onset of inflammation is at the entheses, where calcification and ossification may occur. With the progression of inflammation, bone erosions and bone loss first occur at tendon and ligament insertions, and then spread to joint margins, although less severe than in rheumatoid arthritis (12).

Rheumatoid synovium, as a rich source of immunomodulatory and proinflammatory mediators with osteoclastogenic activity, represents the major driving force of local bone destruction. The joint structure is invaded by the pannus containing a massive infiltrate of immune cells, proliferative vessels and an increased number of osteoclasts, causing bone degradation. Cytokines abundantly produced by synovial tissue include IL-1, IL-6, IL-17 and TNF- α , all of which enhance RANKL-driven maturation of osteoclasts from monocyte/macrophage progenitors. Therefore, the key factor driving inflammatory bone loss is over-expression of RANKL on synovial fibroblasts and infiltrating T lymphocytes. In particular, the highly osteoclastogenic Th17 subset of T lymphocytes stimulates bone destruction by inducing RANKL expression on synovial fibroblasts and by, *per se*, producing RANKL in response to IL-6 secreted by synovial fibroblasts. In rheumatoid arthritis, RANKL is also produced by macrophages, dendritic cells and activated B lymphocytes (12, 20, 26, 27). In addition to RANKL upregulation, proinflammatory cytokines enhance osteoclastogenesis by enhancing RANK expression on osteoclast progenitors and by inducing other proresorptive mediators (including M-CSF, PGE2 and other inflammatory molecules). Several cytokines (i.e. TNF- α , IL-17, IL-32, IL-33) act directly on osteoclast progenitors, thereby inducing osteoclastogenesis by activating crucial differentiation pathways independently of RANKL. By all these mechanisms, cytokines induce self-amplifying autocrine and paracrine cycles in osteoclast progenitors and mature

osteoclasts leading to increased osteoclast activation and aggressive bone loss (67).

In rheumatoid arthritis, osteoblasts are present at sites of bone destruction, but bone repair is absent since their low activity fails to compensate for excessive bone resorption (32). Proinflammatory cytokines (including IL-1, IL-6 and TNF- α) are known to suppress osteoblast differentiation and bone-matrix production, resulting in reduced active mineralizing surfaces in areas of inflammation. Moreover, TNF- α increases production of Dickkopf-1 (DKK-1) by synovial fibroblasts, endothelial cells and chondrocytes in contact with invading pannus. DKK-1 is an inhibitor of Wntless (Wnt) pathway, a signaling cascade involved in osteoblastogenesis. When inhibited by DKK-1, osteoblast differentiation is stopped at a preosteoblast stage, which impairs bone formation. Insufficient osteogenesis is further reinforced by inflammation-induced activation of nuclear factor- κ B in synovial mesenchymal cells, promoting their proliferation but inhibiting osteoblast/chondroblast lineage differentiation (68). Hyperplastic synovial fibroblasts and immature osteoblasts highly express RANKL, thus contributing to osteoclast differentiation and bone resorption (14).

5.2. GENERALIZED BONE LOSS IN RHEUMATOID ARTHRITIS

In addition to local bone loss, rheumatoid arthritis is often complicated with generalized osteoporosis in the axial and appendicular skeleton (Figure 1). This bone loss progresses throughout the disease and correlates with disease severity, eventually leading to joint deformity and increased susceptibility to fractures (14). The presence of rheumatoid arthritis increases the risk of fracture independently of BMD, even after excluding glucocorticoid-treated patients, estimated to be approximately 30% higher for major osteoporotic fracture (hip, spine, wrist, humerus). The causes of increased fractures risk are multifactorial and include, besides lower BMD, systemic effects of inflammation on bone remodeling rate and reduced physical activity, which leads to impaired mechanic stimulation of bone formation and muscle weakness (64-66).

Systemic bone loss in the shape of generalized osteoporosis is similar in all chronic inflammatory conditions. Inflammatory factors such as IL-6 and TNF- α from disease-affected sites spill into the circulation proportionally to the intensity of the disease, and affect osteoblast and osteoclast differentiation and function. It is of particular importance that cells with osteoclastogenic potential, besides bone marrow and synovial compartments, also exist in blood and peripheral hematopoietic organs. It has been proposed that these peripheral osteoclast progenitors are able to progress into multiple terminally differentiated monocyte/macrophage lineage populations, and that, under pathological osteoresorptive conditions,

they could efficiently home to bone surfaces and mature into bone-resorbing osteoclasts (29).

Proinflammatory cytokines such as IL-1, IL-6, TNF- α and IL-17, released from activated immune cells, target osteogenic cells and change their expression profile causing further production of proinflammatory cytokines and chemokines, up-regulation of RANKL expression and stimulation of matrix metalloproteinases (64-67). In addition, some TNF-superfamily members initiate death pathways causing increased apoptosis of osteogenic cells, resulting in the impaired osteoblast bone formation (4, 68). Furthermore, proinflammatory cytokines can perturb the Wnt and bone morphogenetic protein signaling pathways, by the production of DKK-1 and sclerostin, leading to aberrant osteoblast differentiation and activity (14, 48). In the case of osteoporosis which accompanies chronic bacterial infections, circulating bacterial products from infection sites affect osteoblasts and osteoclasts by interacting with Toll-like and NOD-like receptors, resulting in stimulation of osteoclasts and inhibition of osteoblasts (4, 66, 69, 70).

Factors that contribute to the development of osteoporosis are, besides chronic inflammation, duration of the disease, age of the patient, malnutrition and vitamin deficiency, immobility and pharmacological treatment (glucocorticoids, nonsteroidal-anti-inflammatory drugs, disease modifying therapy, biological therapy), which can have both positive and negative effects on BMD (71). Specifically glucocorticoids, the most common cause of iatrogenic osteoporosis, remain a common treatment option for rheumatologic diseases. Glucocorticoids have the negative impact on bone by both direct effects on bone cells and indirect effect on calcium absorption, resulting in reduction of bone formation coupled with persistent bone destruction, and increased osteocyte and osteoblast apoptosis. Despite a beneficial suppression inflammation, the detrimental effects on bone predominate leading to increased fragility and fracture risk especially at sites rich in trabecular bone (such as the femoral neck and lumbar spine). Fracture risk should, therefore, be considered in all glucocorticoid-treated patients since it increases within the first three months of supraphysiological doses of glucocorticoids and is further heightened by the classical osteoporosis risk factors (such as increased age, female sex, thin stature, tobacco and alcohol use, and lower BMD) (14, 48).

6. CONCLUSION

Studies investigating different chronic inflammatory diseases demonstrated the presence of local and systemic bone loss, leading to osteoporosis and increased risk of fractures and confirming the role of immune system in bone remodeling. Bone loss in rheumatoid arthritis, as the most studied example of inflammation-induced bone loss, is caused by the complex interactions between bone and

immune cells at the local and systemic levels. Recent studies suggest that the major pathogenic mechanism, causing systemic osteoporosis as well as periarticular bone loss and marginal erosions, involves inflammation-induced increase in osteoclast number and activity paralleled by suppressed osteoblast differentiation and function. Although it is known that a number of systemic and local stimuli, such as hormones and cytokines/chemokines, promote osteoresorption by enhancing osteoclast differentiation, activation, lifespan and function in inflammatory conditions, further dissection of the molecular mechanisms involved in the interactions between immune and bone cells is crucial to reveal new findings that could translate into effective targeted therapies for inflammatory osteoporosis.

Acknowledgement: This work was supported in part by the Croatian Science Foundation under the project 5699.

REFERENCES

1. REGINSTER J Y, BURLET N 2006 Osteoporosis: a still increasing prevalence. *Bone* 38(2): S4-9
2. MONTALCINI T, ROMEO S, FERRO Y, MIGLIACCIO V, GAZZARUSO C, PUJIA A 2013 Osteoporosis in chronic inflammatory disease: the role of malnutrition. *Endocrine* 43(1): 59-64
3. FERRARI S, BIANCHI M L, EISMAN J A, FOLDES A J, ADAMI S, WAHL D A, STEPAN J J, DE VERNEJOU M C, KAUFMAN J M 2012 Osteoporosis in young adults: pathophysiology, diagnosis, and management. *Osteoporos Int* 23(12): 2735-48
4. GRUBER R 2010 Cell biology of osteoimmunology. *Wien Med Wochenschr* 160/17-18:438-445
5. EMKEY G R, EPSTEIN S 2014 Secondary osteoporosis: pathophysiology & diagnosis. *Best Pract Res Clin Endocrinol Metab* 28(6): 911-3
6. HUDEC S M, CAMACHO P M 2013 Secondary causes of osteoporosis. *Endocr Pract* 19(1): 120-8
7. THEILL L E, BOYLE W J, PENNINGER J M 2002 RANK-L and RANK: T Cells, Bone Loss, and Mammalian Evolution. *Annu Rev Immunol* 20: 795-823
8. WALSH N C, GRAVALLESE E M 2010 Bone remodeling in rheumatic disease: a question of balance. *Immunol Rev* 233(1): 301-12
9. SCHETT G 2009 Osteoimmunology in rheumatic diseases. *Arthritis Res Ther* 11: 210
10. TAKAYANAGI H 2009 Osteoimmunology and the effects of the immune system on bone. *Nat Rev Rheumatol* 5(12): 667-76
11. GOLDRING S R, PURDUE P E, CROTTI T N, SHEN Z, FLANNERY M R, BINDER N B, ROSS F P, MCHUGH K P 2013 Bone remodelling in inflammatory arthritis. *Ann Rheum Dis* 72: 52-5
12. REDLICH K, SMOLEN JS 2012 Inflammatory bone loss: pathogenesis and therapeutic intervention. *Nature Reviews* 11: 234-250
13. LIAN J B, GRAVALLESE E M, STEIN G S 2011 Osteoblasts and their Signaling Pathways: New Frontiers for Linkage to the Immune System. In: Lorenzo J, Choi Y, Horowitz M, Takayanagi H (ed) *Osteoimmunology: Interactions of the Immune and Skeletal Systems*. Academic Press Elsevier, London, p 102-128

14. BAUM R, GRAVALLESE E M 2014 Impact of Inflammation on the Osteoblast in Rheumatic Diseases. *Curr Osteoporos Rep* 12(1): 9–16
15. MENDEZ-FERRER S, MICHURINA TV, FERRARO F, MAZLOOM A R, MACARTHUR B D, LIRA S A, SCADDEN D T, MA'AYAN A, ENIKOLOPOV G N, FRENETTE P S 2010 Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 466(7308): 829–34
16. PITTENGER M F, MACKAY A M, BECK S C, JAISWAL R K, DOUGLAS R, MOSCA J D, MOORMAN M A, SIMONETTI D W, CRAIG S, MARSHAK D R 1999 Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411): 143–7
17. CRISAN M, YAP S, CASTEILLA L, CHEN C W, CORSELLI M, PARK T S, ANDRIOLO G, SUN B, ZHENG B, ZHANG L, NOROTTE C, TENG P N, TRAAS J, SCHUGAR R, DEASY B M, BADYLAK S, BUHRING H J, GIACOBINO J P, LAZZARI L, HUARD J, PÉAULT B 2008 A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3: 301–13
18. NISHIKAWA K, NAKASHIMA T, TAKEDA S, ISOGAI M, HAMADA M, KIMURA A, KODAMA T, YAMAGUCHI A, OWEN M J, TAKAHASHI S, TAKAYANAGI H 2010 Maf promotes osteoblast differentiation in mice by mediating the age-related switch in mesenchymal cell differentiation. *J Clin Invest* 120: 3455–65
19. OHISHI M, ONO W, ONO N, KHATRI R, MARZIA M, BAKER EK, ROOT SH, WILSON T L, IWAMOTO Y, KRONENBERG H M, AGUILA H L, PURTON L E, SCHIPANI E 2012 A novel population of cells expressing both hematopoietic and mesenchymal markers is present in the normal adult bone marrow and is augmented in a murine model of marrow fibrosis. *Am J Pathol* 180: 811–8
20. ŠUČUR A, KATAVIĆ V, KELAVA T, JAJIĆ Z, KOVAČIĆ N, GRČEVIĆ D 2014 Induction of osteoclast progenitors in inflammatory conditions: key to bone destruction in arthritis. *Int Orthop* 38: 1893–1903
21. HOROWITZ M C, LORENZO J A 2004 The origins of osteoclasts. *Curr Opin Rheumatol* 16: 464–8
22. LACEY D L, TIMMS E, TAN H L, KELLEY M J, DUNSTAN C R, BURGESS T, ELLIOTT R, COLOMBERO A, ELLIOTT G, SCULLY S, HSU H, SULLIVAN J, HAWKINS N, DAVY E, CAPPARELLI C, ELI A, QIAN Y X, KAUFMAN S, SAROSI I, SHALHOUB V, SENALDI G, GUO J, DELANEY J, BOYLE W J 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93: 165–76
23. BOYLE W J, SIMONET W S, LACEY D L 2003 Osteoclast differentiation and activation. *Nature* 423(6937): 337–42
24. ASAGIRI M, TAKAYANAGI H 2007 The molecular understanding of osteoclast differentiation. *Bone* 40: 251–64
25. YASUDA H, SHIMA N, NAKAGAWA N, YAMAGUCHI K, KINOSAKI M, MOCHIZUKI S, TOMOYASU A, YANO K, GOTO M, MURAKAMI A, TSUDA E, MORINAGA T, HIGASHIO K, UDAGAWA N, TAKAHASHI N, SUDA T 1998 Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 95: 3597–602
26. WALSH M C, CHOI Y 2003 Biology of the TRANCE axis. *Cytokine Growth Factor Rev* 14: 251–63
27. WALSH M C, CHOI Y 2014 Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond. *Front Immunol* 5: 511
28. GOLDRING S R, SCHETT G 2011 The Role of the Immune System in the Bone Loss of Inflammatory Arthritis. In: Lorenzo J, Choi Y, Horowitz M, Takayanagi H (ed) *Osteoimmunology: Interactions of the Immune and Skeletal Systems*. Academic Press Elsevier, London, p 302–315
29. JACOME-GALARZA C E, LEE S K, LORENZO J A, AGUILA H L 2013 Identification, characterization, and isolation of a common progenitor for osteoclasts, macrophages, and dendritic cells from murine bone marrow and periphery. *J Bone Miner Res* 28(5): 1203–13
30. HERNLUND E, SVEDBOMA A, IVERGÅRD M, COMPSTON J, COOPER C, STENMARK J, MCCLOSKEY E V, JÖNSSON B, KANIS J A 2013 Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos* 8(1–2): 136
31. KELAVA T, ŠUČUR A, KUZMAC S, KATAVIĆ V 2014 Interactions between bone and immune systems: A focus on the role of inflammation in bone resorption and fracture healing. *Period Biol* 116: 45–52
32. LORENZO J, CHOI Y, HOROWITZ M, TAKAYANAGI H 2011 Overview: The Developing Field of Osteoimmunology. In: Lorenzo J, Choi Y, Horowitz M, Takayanagi H (ed) *Osteoimmunology: Interactions of the Immune and Skeletal Systems*. Academic Press Elsevier, London, p 1–5
33. FACCIO R, CHOI Y, TEITELBAUM S L, TAKAYANAGI H 2011 The Osteoclast: The Pioneer of Osteoimmunology. In: Lorenzo J, Choi Y, Horowitz M, Takayanagi H (ed) *Osteoimmunology: Interactions of the Immune and Skeletal Systems*. Academic Press Elsevier, London, p 142–175
34. GRČEVIĆ D, KATAVIĆ V, LUKIĆ I K, KOVAČIĆ N, LORENZO J A, MARUŠIĆ A 2001 Cellular and molecular interactions between immune system and bone. *Croat Med J* 42(4): 384–92
35. FULLER K, WONG B, FOX S, CHOI Y, CHAMBERS T J 1998 TRANCE is necessary and sufficient for osteoblast mediated activation of bone resorption in osteoclasts. *J Exp Med* 188(5): 997–1001
36. SIMONET W S, LACEY D L, DUNSTAN C R, KELLEY M, CHANG M S, LÜTHY R, NGUYEN H Q, WOODEN S, BENNETT L, BOONE T, SHIMAMOTO G, DEROSE M, ELLIOTT R, COLOMBERO A, TAN H L, TRAIL G, SULLIVAN J, DAVY E, BUCAY N, RENSHAW-GEGG L, HUGHES T M, HILL D, PATTISON W, CAMPBELL P, SANDER S, VAN G, TARPLEY J, DERBY P, LEE R, BOYLE W J 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 89(2): 309–19
37. DOUGALL W C, GLACCUM M, CHARRIER K, ROHRBACH K, BRASEL K, DE SMEDT T, DARO E, SMITH J, TOMETSKO M E, MALISZEWSKI C R, ARMSTRONG A, SHEN V, BAIN S, COSMAN D, ANDERSON D, MORRISSEY P J, PESCHON J J, SCHUH J 1999 RANK is essential for osteoclast and lymph node development. *Genes Dev* 13(18): 2412–24
38. SATO K, TAKAYANAGI H 2006 Osteoclasts, rheumatoid arthritis, and osteoimmunology. *Curr Opin Rheumatol* 18(4): 419–26
39. OCHI S, SHINOHARA M, SATO K, GOBER H J, KOGA T, KODAMA T, TAKAI T, MIYASAKA N, TAKAYANAGI H 2007 Pathological role of osteoclast costimulation in arthritis-induced bone loss. *Proc Natl Acad Sci USA* 104(27): 11394–9
40. TAKAYANAGI H 2007 Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 7(4): 292–304
41. BRENNAN F M, MCINNES I B 2008 Evidence that cytokines play a role in rheumatoid arthritis. *J Clin Invest* 118(11): 3537–45
42. GALLIERA E, LOCATI M, MANTOVANI A, CORSI M M 2008 Chemokines and bone remodeling. *Int J Immunopathol Pharmacol* 21(3): 485–91

43. BRADLEY J R 2008 TNF-mediated inflammatory disease. *J Pathol* 214(2): 149-60
44. TAKAYANAGI H, IIZUKA H, JUJI T, NAKAGAWA T, YAMAMOTO A, MIYAZAKI T, KOSHIHARA Y, ODA H, NAKAMURA K, TANAKA S 2000 Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. *Arthritis Rheum* 43(2): 259-69
45. LORIES R J, LUYTEN F P 2005 Bone morphogenetic protein signaling in joint homeostasis and disease. *Cytokine Growth Factor Rev* 16(3): 287-98
46. SANGHA O 2000 Epidemiology of rheumatic diseases. *Rheumatology (Oxford)* 39 (Suppl 2): 3-12
47. IKIĆ M, JAJIĆ Z, LAZIĆ E, IVČEVIĆ S, GRUBIŠIĆ F, MARUŠIĆ A, KOVAČIĆ N, GRČEVIĆ D 2014 Association of systemic and intra-articular osteoclastogenic potential, pro-inflammatory mediators and disease activity with the form of inflammatory arthritis. *Int Orthop* 38(1): 183-92
48. WARRINER A H, SAAG K G 2013 Prevention and treatment of bone changes associated with exposure to glucocorticoids. *Curr Osteoporos Rep* 11(4): 341-7
49. MCINNES I B, SCHETT G 2007 Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 7(6): 429-42
50. GRČEVIĆ D, JAJIĆ Z, KOVAČIĆ N, LUKIĆ I K, VELAGIĆ V, GRUBIŠIĆ F, IVČEVIĆ S, MARUŠIĆ A 2010 Peripheral blood expression profiles of bone morphogenetic proteins, tumor necrosis factor-superfamily molecules, and transcription factor Runx2 could be used as markers of the form of arthritis, disease activity, and therapeutic responsiveness. *J Rheumatol* 37(2): 246-56
51. DALBETH N, POOL B, SMITH T, CALLON K E, LOBO M, TAYLOR W J, JONES P B, CORNISH J, MCQUEEN F M 2010 Circulating mediators of bone remodeling in psoriatic arthritis: implications for disordered osteoclastogenesis and bone erosion. *Arthritis Res Ther* 12(4): R164
52. BUGATTI S, MANZO A, BOMBARDIERI M, VITOLO B, HUMBY F, KELLY S, MONTECUCCO C, PITZALIS C 2011 Synovial tissue heterogeneity and peripheral blood biomarkers. *Curr Rheumatol Rep* 13(5): 440-8
53. KIM M S, MAGNO C L, DAY C J, MORRISON N A 2006 Induction of chemokines and chemokine receptors CCR2b and CCR4 in authentic human osteoclasts differentiated with RANKL and osteoclast like cells differentiated by MCP-1 and RANTES. *J Cell Biochem* 15;97(3): 512-8
54. SCHETT G 2009 Bone marrow edema. *Ann N Y Acad Sci* 1154: 35-40
55. CHOI Y, ARRON J R, TOWNSEND M J 2009 Promising bone-related therapeutic targets for rheumatoid arthritis. *Nat Rev Rheumatol* 5 (10): 543-548
56. CORRADO A, NEVE A, MARUOTTI N, CANTATORE F P 2013 Bone effects of biologic drugs in rheumatoid arthritis. *Clin Dev Immunol* 2013: 945945
57. HAYNES D R 2007 Inflammatory cells and bone loss in rheumatoid arthritis. *Arthritis Res Ther* 9 (3): 104
58. KINNE R W, STUHLMÜLLER B, BURMESTER G R 2007 Cells of the synovium in rheumatoid arthritis. Macrophages. *Arthritis Res Ther* 9(6): 224
59. BARTOK B, FIRESTEIN G S 2010 Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev* 233(1): 233-55
60. BUGATTI S, MANZO A, CAPORALI R, MONTECUCCO C 2012 Inflammatory lesions in the bone marrow of rheumatoid arthritis patients: a morphological perspective. *Arthritis Res Ther* 14: 229
61. WALSH N C, REINWALD S, MANNING C A, CONDON K W, IWATA K, BURR D B, GRAVALLESE E M 2009 Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J Bone Miner Res* 24(9): 1572-85
62. DUER-JENSEN A, HØRSLEV-PETERSEN K, HETLAND M L, BAK L, EJBJERG B J, HANSEN M S, JOHANSEN J S, LINDEGAARD H M, VINTERBERG H, MØLLER J M, ØSTERGAARD M 2011 Bone edema on magnetic resonance imaging is an independent predictor of rheumatoid arthritis development in patients with early undifferentiated arthritis. *Arthritis Rheum* 63(8): 2192-202
63. MCQUEEN F M 2012 Bone marrow edema and osteitis in rheumatoid arthritis: the imaging perspective. *Arthritis Res Ther* 14: 224
64. GUERRINI M M, TAKAYANAGI H 2014 The immune system, bone and RANKL. *Arch Biochem Biophys* 561: 118-123
65. FINZEL S, RECH J, SCHMIDT S, ENGELKE K, ENGBRECHT M, STACH C, SCHETT G 2011 Repair of bone erosions in rheumatoid arthritis treated with tumour necrosis factor inhibitors is based on bone apposition at the base of the erosion. *Ann Rheum Dis* 70(9): 1587-93
66. DEAL C 2012 Bone loss in rheumatoid arthritis: systemic, periarthicular, and focal. *Curr Rheumatol Rep* 14(3): 231-7
67. BOYCE B F, XING L 2008 Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 473(2): 139-46
68. MOSLER LAZIĆ E, JELUŠIĆ M, GRČEVIĆ D, MARUŠIĆ A, KOVAČIĆ N 2012 Osteoblastogenesis from synovial fluid-derived cells is related to the type and severity of juvenile idiopathic arthritis. *Arthritis Res Ther* 14(3): R139
69. HARDY R, COOPER M S 2009 Bone loss in inflammatory disorders. *J Endocrinol* 201(3): 309-20
70. CVIJA H, KOVAČIĆ N, KATAVIĆ V, IVČEVIĆ S, AGUILA H L, MARUŠIĆ A, GRČEVIĆ D 2012 Chemotactic and immunoregulatory properties of bone cells are modulated by endotoxin-stimulated lymphocytes. *Inflammation* 35(5): 1618-31
71. TAK P P, KALDEN J R 2011 Advances in rheumatology: new targeted therapeutics. *Arthritis Res Ther* 13(Suppl 1): S5