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Osteoprotegerin and receptor activator of NF-kappaB ligand as biomarkers predicting bone resorption-related disorders

Lalotra T *et al*. RANK/RANKL/OPG axis

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Abstract

Osteoclastic bone diseases represent a significant health burden, especially in an ageing population, due to imbalances in bone homeostasis that result in postmenopausal osteoporosis, inflammatory arthritis, malignant-induced bone disease and secondary metabolic conditions, which result in fragility fractures, disability and death. Although advances in imaging and pharmacology have enhanced management, existing diagnostic strategies have poor sensitivity in detecting early-stage pathological changes or fracture risk. Key to bone turnover is the receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand (RANKL)/osteoprotegerin (OPG) pathway, which is dysregulated in bone degradation leading to increased osteoclast numbers and bone degradation. Circular levels of RANKL and OPG, in particular the RANKL/OPG ratio, have shown potential as biomarkers for early diagnosis, monitoring, and evaluating therapeutic response, with the RANKL/OPG ratio reflecting the balance of the osteoclastogenic activity in the bone microenvironment and correlating with bone mineral density, fracture risk, and bone pathology. It exhibits lower biological variability and enhanced sensitivity for early disease detection compared with individual markers. This review assesses the experimental, translational and clinical evidence for the predictive and monitoring potential of RANKL and OPG, and correlations between various imaging techniques and circulating biomarkers to gain insight into disease progression. We explore and discuss the limitations with regards to methodological issues, assay variability and biological variability, and the therapeutic implications of modulating the RANKL/OPG axis, highlighting its potential in informing treatment approaches and improving outcomes in bone resorptive diseases.

Key Words: Receptor activator of NF-kappaB; Receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand; Osteoprotegerin axis; Osteoclast; Osteoporosis

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Core Tip: The receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand (RANKL)/osteoprotegerin (OPG) pathway is a pivotal bone remodelling pathway and its disruption is responsible for the osteoclast-driven bone resorption in the disease states of osteoporosis, arthritis and bone-related cancer. Plasma RANKL and OPG, and their ratio, may be more sensitive indicators for diagnosis, fracture prediction and therapy assessment. While assays are variable, there is evidence that the RANKL/OPG ratio could be a better predictor than either marker alone - and has the potential to improve diagnosis, treatment and outcomes in bone resorption disease.

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INTRODUCTION

Bone remodelling is a dynamic, well-coordinated and highly regulated life-long process essential for maintaining skeletal integrity, structure and metabolism[1]. Throughout life, bone undergoes a continuous bone remodelling process through coordinated cycles of resorption and formation, facilitating the repair of microstructural damage, adaptation to mechanical stress, and plays a key role in regulating calcium and phosphate metabolism[2]. Bone remodelling is tightly regulated by spatial and temporal coordination between osteoblast and osteoclast mediated bone formation and resorption activity, respectively, dependent on direct cellular communication[3]. Bone is typically constituted by type 1 collagen fibres, which are intricately woven and arranged together with crystals of calcium hydroxyapatite, conferring strength and flexibility to the bone[4]. Under physiological conditions, the equilibrium between the formation and resorption preserves bone mass and microarchitecture. However, even subtle disruptions in this tightly regulated process can progressively impair skeletal integrity, leading to reduced

bone mineral density, making it fragile and increasing fracture susceptibility[5]. Significantly, bone degradation often occurs gradually over time and is often undiagnosed until a fracture or deformity[6]. This process is normal during the ageing process and occurs in both males and females after peak bone mass[7]. Bone resorption disorders, such as postmenopausal osteoporosis, inflammatory arthritis (IA) and bone diseases in cancer, are a significant public health burden globally[8]. Any studies have demonstrated that patients with a low bone mass concentration are at a higher risk of all types of fractures, and it was estimated that by 2050 the number of hip fractures will rise by 240% in women and 310% in men[9]. These diseases are the major causes of fragility fracture, pain, disability and morbidity[10,11]. Fractures also cause a rise in mortality, loss of independence and the significant global healthcare burden[12]. Osteoporosis is a major global burden affecting more than 200 million people with 8.9 million fractures every year[13]. The clinical and socioeconomic impact is significant. With an ageing population around the world, it's expected that these conditions will become more prevalent, putting a greater burden on the health care system and highlighting the need for better approaches to early detection, risk assessment and management of these conditions[14].

Despite improvements in diagnostic imaging and drug therapies, existing clinical methods still have limited capacity to detect pathological changes in bone remodelling at an early stage[15]. The World Health Organisation considers dual-energy X-ray absorptiometry to be the gold standard method for measuring bone mineral density. However, it measures total bone loss, rather than the active bone remodelling or the molecular events that lead to disease[16]. Further to this, bone mineral density fails to reflect bone quality, microarchitectural changes and fracture risk, especially in secondary bone diseases and inflammatory conditions. These factors have created a need for molecular biomarkers that may complement the diagnostic tools, help in monitoring disease progression, shed light on disease activity, and allow early and accurate intervention[17]. Biomarkers of bone turnover, mostly proteins or their fragments, are

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released during bone remodelling and are essential to diagnose and treat various metabolic bone diseases[18].

The discovery of the receptor activator of NF-kappaB (RANK) receptor and the identification of its ligand, RANK ligand (RANKL), as the major osteoclastogenic factor in 1999, and the identification of osteoprotegerin (OPG) as a soluble decoy receptor, ushered in a new era in understanding the biology of osteoclast and bone remodelling[19]. These proteins are new members of the tumour necrosis factor (TNF) receptor superfamily and their discovery has greatly facilitated our understanding of how to block the osteoclastogenic potential, thus preserving the bone from degradation[20]. Bone mesenchymal cells are important players in bone remodelling as they express RANKL, which controls osteoclastogenesis and bone degradation[21]. Overall, the RANK/RANKL/OPG signalling pathway is the main regulatory pathway that leads to differentiation, activation and survival of osteoclasts, and provides a common molecular mechanism for the development of bone resorptive diseases[22]. Notably, RANK/RANKL/OPG system brings together endocrine, immune and mechanical factors and is at the intersection of skeletal and systemic physiology[23]. This pathway has been implicated in a variety of bone-related diseases, including postmenopausal and age-related osteoporosis, IA, and malignant bone erosion, where RANKL has been linked with cancers such as bone metastasis, breast cancer, multiple myeloma, urological cancers and lung carcinoma[24].

Unlike traditional molecular markers of bone turnover, which largely signal downstream metabolic end-products of bone matrix formation or resorption, components of the RANK/RANKL/OPG system might offer additional upstream regulatory message that regulate osteoclastogenesis, for therapeutic monitoring[25]. Therefore, circulating serum levels of RANKL and OPG, especially their ratio, have gained attention as promising biomarkers that may more accurately reflect the balance in the bone remodelling process[26]. Increasing evidence suggests that these biomarkers hold potential value not only for disease prediction and risk stratification but also for monitoring disease progression and therapeutic response[27]. In recent times, the

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therapeutics for skeletal disorders targeting the RANKL and OPG pathways have been studied widely[28]. Despite extensive research already done on this signalling pathway, existing literature mainly focuses on its molecular interactions and its disease pathology[29].

In contrast to studies done prior, this narrative review provides more insight into the prognostic and diagnostic value of these individual biomarkers, as well as their ratio in certain diseases. This review also provides information regarding the comparative value of the RANKL/OPG ratio over individual biomarkers, and also evaluates the inconsistencies occurring across the studies due to variability in the methodological sampling techniques. It aims to comprehensively analyse current research and data available on circulating OPG and RANKL as predictive biomarkers in bone resorption disorders, with particular emphasis on serum ratios, correlations with imaging biomarkers, and clinical applications in treatment monitoring[30]. Therefore, this review aims to provide a more detailed understanding of the potential and limitations of these biomarkers in clinical practice. Several narrative reviews have examined the RANK/RANKL/OPG axis from mechanistic, disease-specific, and therapeutic perspectives. But, most lack this integration of circulating biomarkers, different methodological approaches and clinical applications. A comparative table is presented highlighting the differences in Table 1[26,31,32].

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SEARCH STRATEGY

A search strategy was made, and a comprehensive literature search was conducted across databases such as PubMed, Web of Science, Google Scholar, and Scopus. Relevant studies were selected for the study using the keywords such as “RANKL”, “Osteoprotegerin”, “RANKL/OPG ratio”, “bone remodelling”, “osteoporosis”, and “biomarkers”. Both clinical and experimental trials, along with reviews, were considered for the study that focused on the role of RANKL, OPG, and their ratio in bone metabolism and disease pathology. The reference list for each article was also considered and reviewed to identify additional studies for this study. Since it’s a narrative review, there are no systematic

inclusion and exclusion criteria made, but only peer-reviewed, relevant, and highly valued articles were selected for the study.

MOLECULAR BASIS OF THE RANK/RANKL/OPG AXIS

RANKL expression and osteoclastogenesis

RANKL, encoded by the *TNFSF11* gene, is a cytokine of the TNF superfamily that serves as a central mediator of osteoclastogenesis and is expressed across multiple bone-related cell types, including hypertrophic chondrocytes, osteoblasts, osteoclasts, and immature osteocytes[33]. Both membrane and soluble RANKL are involved in the regulation of osteoclast maturation and activation in normal and pathological conditions[34]. It is essential for bone metabolism, and is now a promising therapeutic target in diseases such as osteoporosis, arthritis and osteolytic metastasis[35]. The membrane-bound form is particularly potent, as it facilitates sustained cell-to-cell signalling within the bone microenvironment[36]. Under normal conditions, RANKL is predominantly produced by osteoblasts and osteocytes, with osteocytes being the major source of RANKL, now recognized as the principal regulators of osteoclast differentiation in adult bone remodelling[37]. Hence, osteocytes are the key to the bone remodelling process due to the action of RANKL and sclerostin as produced by them[38]. However, in pathological states such as IA, bone resorptive lesions, periodontal diseases, and malignancies, immune cells such as T cells, B cells, and synovial fibroblasts become additional sources of RANKL[39,40].

The binding of RANK receptor with its ligand on osteoclast precursors expressed *via* macrophage-colony stimulating factor stimulation initiates a series of intracellular signalling events responsible for osteoclastogenesis and subsequent bone resorption[41]. Upon activation, RANK mediates intracellular signal transduction *via* molecules such as tumor necrosis factor receptor-associated factors, which results in activation of nuclear factor-κB, mitogen-activated protein kinases, c-Jun N-terminal kinase, and calcium-dependent signalling pathways, that together drive osteoclast differentiation[42,43]. These signals converge on nuclear factor of activated T cells cytoplasmic 1, the master

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transcription factor governing osteoclastogenesis by regulating several osteoclast-specific genes such as tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, and osteoclast-associated receptor through cooperation with microphthalmia-associated transcription factor and c-Fos[44,45]. Nuclear factor of activated T cells cytoplasmic 1 is responsible for the regulation of gene expression required for osteoclast fusion, cytoskeletal reorganization, acid secretion, and degradation of bone matrix[46]. Persistent RANKL signalling not only increases osteoclast number but also enhances osteoclast survival, thereby amplifying cumulative bone resorption and contributing to progressive skeletal deterioration in chronic disease states such as malignancy, as shown in Figure 1[47].

OPG as an endogenous inhibitor

OPG, encoded by *TNFRSF11B*, is a soluble glycoprotein and is a member of the TNF receptor family[48]. It is secreted mainly by osteoblast lineage cells, endothelial cells, and immune cells, playing a major role in bone remodelling, immunoregulation, vascular function, carcinogenesis, central thermoregulation, and fibrosis[20,49,50]. OPG inhibits the activation and differentiation of osteoclasts by binding with RANKL, preventing its interaction with RANK[51]. Through this mechanism, OPG regulates the bone resorption process, maintaining skeletal integrity[52].

Beyond skeletal regulation, OPG exhibits pleiotropic biological functions by binding to TNF-related apoptosis-inducing ligand, which further binds to its death domain-containing receptors, DR4 and DR5, inducing apoptosis[53]. Due to this interaction, OPG has been associated with cardiovascular disorders and is also involved in immune regulation, vascular homeostasis, and endothelial biology[54,55]. OPG is associated with speeding up the atherosclerotic process, explaining the reason behind poor clinical outcomes in patients with high concentrations of OPG[56]. These extra-skeletal effects are clinically relevant when interpreting circulating OPG concentrations, as elevated levels may reflect systemic inflammation or vascular pathology rather than purely skeletal responses[57,58].

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Physiological regulation and pathological imbalance

Physiological bone remodelling depends on a finely regulated equilibrium between RANKL and OPG expression[3]. The equilibrium between osteoblastic and osteoclastic activity is essential for maintaining the bone remodelling process that occurs through expression of RANKL, OPG, and certain hormones that occur over weeks[59]. Hormonal regulators, including oestrogen, testosterone, parathyroid hormone, glucocorticoids, growth hormone, erythropoietin, insulin-like growth factor 1, vitamin D, intestinal and adipocyte hormones, modulate this balance at transcriptional and post-transcriptional levels[60]. Oestrogen deficiency is often associated with accelerated bone loss, enhanced osteoclastogenesis, and fragile bones due to increased RANKL and decreased OPG expression[61]. Parathyroid hormone, insulin-like growth factor 1, and testosterone upregulate bone formation, increasing bone mineral density and preventing bone resorption[62].

This balance is supported by the evidence from animal models. Mice lacking OPG suffer from severe osteoporosis and spontaneous fractures, while osteopetrosis is observed in transgenic mice as a result of increased OPG levels, which inhibits osteoclastogenesis[63,64]. Bone turnover is a highly regulated process that is controlled by the RANKL/OPG ratio, with a relative increase in RANKL favouring bone degradation[65,66]. In humans, pathological changes in the RANKL/OPG ratio have been reported in a number of bone-resorptive diseases, suggesting the potential diagnostic use of this ratio as a composite biomarker[32,67].

Analytical and methodological considerations of circulating OPG and RANKL as biomarkers

Circulating OPG and RANKL measurements have gained attention as minimally invasive biomarkers that provide insight into systemic osteoclastogenic activity[68]. The clinical interpretation of circulating RANKL and OPG levels is significantly influenced by methodological variability in their measurement[69,70]. In most studies, enzyme-

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linked immunosorbent assays are employed to quantify serum or plasma concentrations[71]. However, all these commercially available assays differ considerably in their design, including differences in antibody specificity, detection sensitivity, calibration standards, and the ability to distinguish between free and bound RANKL. These variations contribute to wide inter-study variability in reported biomarker levels, hindering the definition of standardized reference ranges suitable for routine clinical use[72-74].

Preanalytical variables further influence biomarker measurements and include sample type, whether serum or plasma is collected, processing time, storage conditions, freeze-thaw cycles, and diurnal variation[75]. In addition, circulating levels of OPG and RANKL may be affected by age, sex, renal function, inflammatory status, and comorbid cardiovascular disease[76,77]. For example, a serious imbalance has been found in multiple myeloma, where OPG was profoundly decreased, and RANKL concentration was found to be significantly higher[78]. Importantly, circulating concentrations do not fully reflect local RANKL and OPG activity within the bone microenvironment, where paracrine and cell-cell interactions predominate, and their concentrations vary with pregnancy, age, inflammation, and renal failure[79,80]. This lack of a generalised system and a protocol for detecting these biomarkers across studies places limitations on routinely using them for clinical application[81]. Despite these limitations, systemic measurements provide valuable integrative information in diseases with generalized skeletal involvement[82]. OPG binds with an affinity that is 500 times stronger than RANK to RANKL at unique sites, inhibiting osteoclastogenesis effectively[41]. Accumulating evidence indicates that the OPG/RANKL ratio offers greater biological relevance than absolute concentrations alone by capturing the net balance between pro-resorptive and anti-resorptive signalling forces[30,83].

Osteoporosis and age-associated bone loss

Osteoporosis is a skeletal disorder predominant in the elderly population that is characterised by a progressive decrease in bone mass and alteration in its architectural

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integrity that requires proper treatment and management[84]. Osteoporosis is more commonly seen in elderly females, where numerous studies have shown 40%-50% of women over 60 years old being affected by osteoporosis[85]. Postmenopausal osteoporosis represents the most extensively studied clinical paradigm of RANK/RANKL/OPG dysregulation and provides a mechanistic framework for understanding age-related bone loss[86,87]. Oestrogen is critical in bone remodelling that acts through two major receptors, oestrogen receptor-alpha and oestrogen receptor-beta, modulating the RANKL/OPG axis[88]. Post menopause, oestrogen deficiency results in a persistent increase in osteoclast activity and impaired bone remodelling process because of upregulation of RANKL expression and downregulation of OPG expression[89]. Dysregulated RANKL/OPG ratio causes an increase in uncoupled bone remodelling, which in turn causes bone mass and integrity loss[90].

Studies evaluating circulating OPG and RANKL in postmenopausal women have produced variable results due to the characteristics of study populations, stage of disease and methodological variations[64,91-93]. But interpretation of these results in clinical practice is limited by study variability and their utility as diagnostic markers is still to be determined. However, when assessed over time or in large populations, consistent trends are evident showing that a decreased OPG/RANKL ratio is associated with lower bone mineral density and incidence of fragility fractures. Measurements of circulating OPG and RANKL have been proposed as clinically useful, non-invasive biomarkers of systemic osteoclastogenesis[68]. Analytical variations in circulating RANKL and OPG measurements have a profound impact on their clinical interpretations[94-96].

Bone loss associated with rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease, which is characterised by inflammatory bone destruction, focal erosions in the joints and systemic bone fragility[97]. It has recently been determined that joint and bone erosions seen in RA patients result from RANKL over-expression in the synovial tissue of joints[98-100]. The chronic inflammation in the synovium results in the infiltration of activated T cells,

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macrophages, and fibroblast-like synoviocytes. These cells show increased production of RANKL and promote the differentiation of osteoclasts at the bone/pannus junction. This osteoclast activation leads to the formation of marginal erosions seen in RA[101].

In addition to local bone destruction, systemic inflammation in RA results in altered bone metabolism and systemic osteoporosis and fractures[102]. In patients with RA, increased systemic levels of RANKL, and decreased OPG/RANKL ratios, have been linked to disease activity, radiographic joint damage and systemic bone degradation[103]. TNF-α cytokine plays a key role in inflammation and bone erosion in RA. Critical to this process, successful anti-inflammatory treatment with biological agents, especially TNF-α inhibitors, results in a decrease in RANKL levels, alleviation of symptoms, partial restoration of the biomarker profile and slowing of the rate of progression of damage[104,105]. These observations underscore the utility of RANK/RANKL/OPG biomarkers not only as indicators of inflammatory bone destruction but also as tools for monitoring therapeutic response[106]. While these findings suggest a role in disease activity, their utility as reliable clinical biomarkers remain uncertain due to heterogeneity across studies.

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Cancer-associated bone disease

Cancer is a rising global burden affecting nearly 12.7 million people worldwide, resulting in mortality of over 7 million as per data studies in 2008[107]. Tumour cells can directly alter the bone microenvironment, favouring bone resorption, or the therapies targeting the tumour can further suppress the formation of bone and accelerate bone resorption, resulting in cancer-induced bone disease[17]. Cancer-associated bone diseases result in alteration of the microenvironment due to the complex interaction between them, resulting in excessive osteoclast activation and skeletal destruction triggered by pro-inflammatory cytokines, which are overactivated in pathological conditions[108,109]. Tumour cells secrete cytokines, growth factors, and extracellular vesicles that stimulate RANKL expression by osteoblasts, stromal cells, and immune cells, thereby promoting osteoclastogenesis, which further releases growth factors such as tumour growth factor

β and insulin-like growth factor 1 from the bone matrix, which in turn stimulates parathyroid hormone-related peptide production and promotes tumour growth[110]. This process establishes a self-perpetuating "vicious cycle" in which tumour growth and survival are established due to growth factors and cytokines released in bone resorption[111].

Clinical studies have frequently demonstrated elevated circulating RANKL levels and reduced OPG concentrations in patients with renal cancer, prostate cancer, oral cancer, breast cancer, and multiple myeloma having bone metastases[112]. Alterations in the RANKL/OPG ratio have been associated with higher risk of skeletal-related pathological states, disease burden, and poor clinical outcomes[83,113]. As a result, this ratio gives integrated results and serves as a potential biomarker that can help in evaluating the disease progression and therapeutic monitoring to antiresorptive therapies such as denosumab and bisphosphonates[26,114]. But these changes may be affected by a variety of systemic factors and may lack clinical value as a single factor[115]. Overall, there is a clear rationale to include RANK/RANKL/OPG axis profiling in the clinical assessment of patients with oncologic bone disease[116-118].

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Secondary and rare bone disorders

The RANK/RANKL/OPG axis plays a key role in bone remodelling and bone homeostasis, and is therefore involved in a wide range of secondary and rare bone diseases[119]. In glucocorticoid-induced osteoporosis, glucocorticoids directly up-regulate RANKL expression, facilitating differentiation of bone-marrow derived macrophages to osteoclasts, stimulating bone degradation while down-regulating OPG expression, resulting in severe and rapid bone degradation[120]. Adverse effects seen in patients due to prolonged glucocorticoid use through this mechanism include increased fracture risk, cortical porosity and reduced bone mineral density, resulting in an increased burden and reduced quality of life[121,122].

Elevated circulating OPG levels have been reported in chronic kidney disease which is a mineral and bone disorder and may reflect a complex interplay between skeletal

pathology, systemic inflammation, and vascular calcification[123]. Immobilization-induced bone loss following spinal cord injury, stroke, multiple sclerosis, amyotrophic lateral sclerosis, or prolonged bed rest is similarly characterized by increased osteoclast activity and altered RANKL/OPG dynamics, resulting in rapid skeletal deterioration[124,125]. In fibrous dysplasia, excessive RANKL expression drives abnormal osteoclast activation that results in bone resorption by forming resorption pits, making RANKL a potential therapeutic target in this disorder[126,127].

Critical analysis of evidence

Although numerous studies have been conducted showing the association between RANKL, OPG, and disease states, the results are still not consistent with each other. These discrepancies majorly occur due to the heterogeneity in patient's characteristics such as age, sex, ethnicity, and disease severity varying across different studies[96,128]. As discussed above, the variations in methodology, and differences in study design, being cross sectional, or longitudinal further result in this difference[98]. Bone remodelling is also an intricately highly regulated process, also influenced strongly by hormones and other systemic conditions that may in turn aid in these inconsistencies[26]. Hence, these biomarkers, though having high clinical utility, keeping in mind these discrepancies, more extensive, large-scale, and longitudinal research needs to be carried out to understand their validity completely.

Imaging biomarkers and correlation with serum markers

While dual-energy X-ray absorptiometry is considered the gold standard method for checking bone mineral density, it has certain limitations as it does not provide adequate details regarding the quality of bone and details about its microarchitecture[129]. Bone mineral density does not provide us with complete information about bone strength, quality, and fragility in the evaluation of bone resorption disorders that may not predict future fracture risk, giving rise to the need for advanced imaging modalities[130]. One such new imaging modality is high-resolution peripheral quantitative computed

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tomography, helping in detailed assessment of trabecular and cortical microarchitecture of bone helping in integrating the deterioration results in imaging with the biochemical markers in serum for evaluating skeletal disorders[131,132].

Magnetic resonance imaging has particular relevance in IA, where it can detect any active inflammation, as well as detect bone marrow edema reflecting active osteitis, and also serves as a strong predictor of subsequent erosion development[133]. Magnetic resonance imaging (MRI) is the preferred imaging technique as it has the capacity to detect early disease and is also useful in monitoring treatment[134]. Significantly, biopsy-proven inflammation at MRI sites is associated with local increases in RANKL, demonstrating a connection between imaging and molecular pathways of osteoclast activation[135]. Combining the latest imaging techniques with serum levels of RANK/RANKL/OPG offers a comprehensive evaluation of disease activity and progression[136]. Yet, more research needs to be done on the clinical integration of these imaging modalities and biochemical markers for clinical decision-making.

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TREATMENT MONITORING AND THERAPEUTIC IMPLICATIONS

RANKL inhibition

RANKL is central to the bone resorption disorders, giving rise to denosumab, which is a major therapeutic advancement in the treatment of the disorders, a human monoclonal antibody that targets RANKL[137]. By functionally mimicking the action of OPG, denosumab potently suppresses osteoclast formation, activity, and survival, increasing the bone mass, strength, and mineral density, further reducing the risk of fractures in both osteoporosis and malignancy-associated bone disease[138-140]. Side effects associated with denosumab therapy are low calcium and phosphate in the blood, giving rise to muscle cramps, and rarely cellulitis and numbness, with hypocalcemia being the most common[141]. Because denosumab directly targets the RANK/RANKL/OPG axis, serial monitoring of circulating RANKL and OPG levels reflects pharmacodynamic effects and may assist in evaluating treatment adherence, efficacy, and the risk of rebound bone loss following discontinuation[142,143].

Other antiresorptive and anabolic therapies

Bisphosphonates, selective oestrogen receptor modulators, calcitonin, and anabolic agents such as parathyroid hormone analogues exert their skeletal effects through indirect modulation of osteoblast-osteoclast coupling and remodelling dynamics[144]. These therapies influence the RANK/RANKL/OPG axis by altering osteoblast function, inflammatory signalling, and bone turnover rates[145,146]. Biomarker profiling may therefore support individualized therapeutic decision making, optimization of treatment duration, and strategic sequencing of these therapeutic agents, especially in patients with complex or refractory disease[147]. The functional treatment options involved in preventing osteoclast stimulation from the pathological activation of the RANK/RANKL/OPG axis are given in Figure 2.

Limitations and challenges

Despite strong biological rationale and emerging clinical evidence, there are inconsistencies in the results due to a wide range of factors that limit the routine clinical application of RANK/RANKL/OPG biomarkers[69,148]. These include the time of sampling, sample storage, assay variability, methods of collection, and confounding effects of age, gender, inflammation, renal dysfunction, and cardiovascular disease[82,149-152]. Additionally, circadian rhythms and seasonal variation may influence the circulating biomarker levels, underscoring the need for standardised sampling protocols and careful clinical interpretation[153]. Targeting the RANK/RANKL/OPG pathway has shown significant therapeutic potential, but it still has its limitations where different patients have different responses to the therapies, with some showing suboptimal responses[24]. Discontinuing anti-RANKL therapy is associated with a rebound increase in bone resorption and an increased susceptibility to fractures, posing a great limitation to the therapy[154]. Also, the potential development of resistance in long-term management and the influence of systemic conditions on these therapies affecting the outcome are other limitations that need to be evaluated properly.

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Hence, this initiates the requirement for an individualised treatment approach and the need for biomarkers that can reliably predict therapeutic response[155]. The regulations and limitations of RANK/RANKL/OPG axis are tabulated in Table 2[156,157].

Future perspectives

Subsequent studies in this area should aim to enhance consistency in laboratory methods and develop robust reference data from large, well-defined populations that reflect differences in age, sex, and ethnicity, amounting to the confounding factors. Future studies should strive to standardise the protocols for the assays and set up the normal reference ranges for RANKL and OPG. Combining the RANK/RANKL/OPG biomarkers with imaging, artificial intelligence-based models, and conventional clinical assessment may enhance the accuracy of predicting early disease onset, fracture risks and disease progression. Knowledge of systemic biology and new tools such as single-cell sequencing may also help us better understand how the various regulatory networks such as hormones, inflammatory factors, genetic factors work in concert with RANKL/OPG to regulate bone remodelling and help us better define the clinical significance of these biomarkers. Large-scale studies, multicentre and longitudinal studies need to be conducted to validate their use and to translate them to the clinical setting[16,132,134,136,158-160].

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CONCLUSION

The RANK/RANKL/OPG pathway plays a critical role in bone turnover, activating and driving osteoclast differentiation. Abnormalities lead to bone homeostasis imbalance, promoting osteoporosis, inflammatory joint conditions, bone destruction in cancer and rare bone diseases. Serum concentrations of RANKL and OPG are variable, but their ratio is more informative for net osteoclastogenic activity because it combines opposing signals. When used alongside innovative imaging technologies, including high-resolution peripheral quantitative computed tomography and MRI, these biomarkers improve evaluation of bone mineral and microarchitecture, fracture risk, and disease

progression. That enhances assessment of disease staging and response to treatment, as seen with denosumab, which acts on this pathway and has biomarker changes in line with structural and clinical changes. But issues persist: Assay precision, lack of reference ranges and confounding effects of ageing, kidney disease, systemic inflammation and heart disease. To advance this, multi-disciplinary translational research is needed to enhance the accuracy of assays, provide reliable reference ranges in a broad population, and combine biomarkers with imaging and clinical measures. This will facilitate risk assessment, early diagnosis and tailored therapies. As bone biology progresses, the RANK/RANKL/OPG pathway will continue to play a pivotal role in the diagnosis and treatment of bone resorption diseases.

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Footnotes

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Figures and Tables

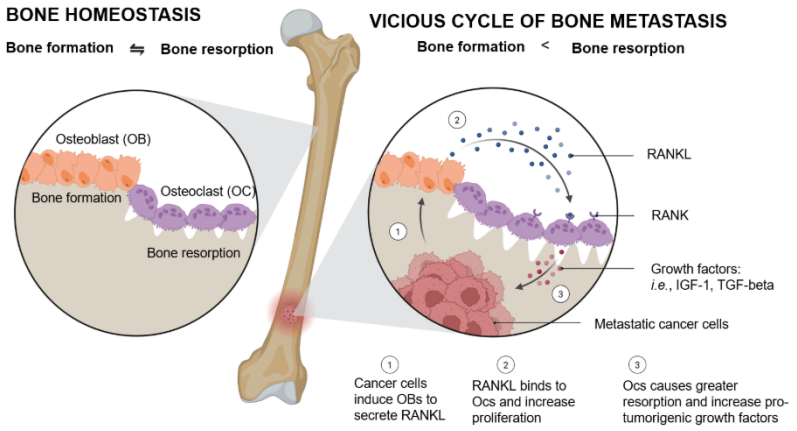

Figure 1 Normal bone homeostasis and abnormal activation of osteoclasts by receptor activator of NF-kappaB ligand from metastatic cancer cells, resulting in bone resorption and increased pro-tumorigenic growth factors. RANKL: Receptor activator of NF-kappaB ligand; RANK: Receptor activator of NF-kappaB; OC: Osteoclast; OB: Osteoblast; IGF: Insulin-like growth factor; TGF: Transforming growth factor. Created in BioRender (Supplementary material).

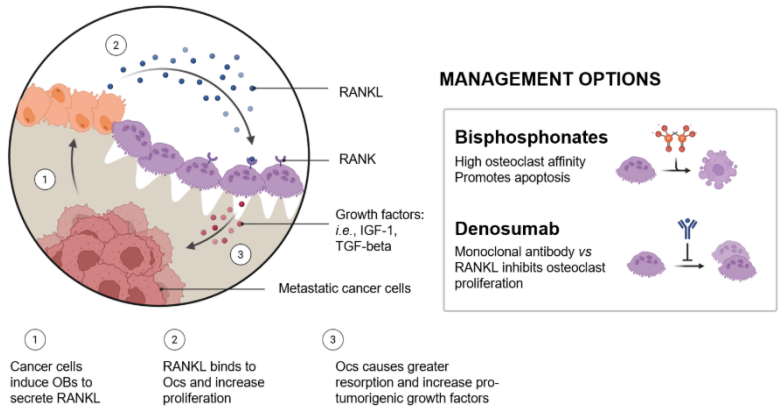

Figure 2 Management options involved in preventing osteoclast stimulation from the pathological activation of the receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand/osteoprotegerin axis. RANKL: Receptor activator of NF-kappaB ligand; RANK: Receptor activator of NF-kappaB; OC: Osteoclast; OB: Osteoblast; IGF: Insulin-like growth factor; TGF: Transforming growth factor. Created in BioRender (Supplementary material).

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Table 1 Comparative literature analysis of receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand/osteoprotegerin axis

Ref.	Focus	Limitations	Contribution of present review
Boyce and Xing[26]	Overview of RANK/RANKL/OPG pathway in bone biology	Primarily mechanistic focus with limited discussion of clinical biomarker applications	Extends molecular insights to clinical biomarker relevance across diseases
Khosla[31]	Role of RANKL/OPG in osteoporosis pathophysiology	Focused on osteoporosis with limited multi-disease perspective and no discussion on circulating biomarkers	Provides multi-disease perspective and emphasizes circulating biomarker utility
Di Cicco *et al*[32]	Role of RANK/RANKL/OPG signaling in osteoarthritis and targeted therapies	Disease-specific focus with limited emphasis on circulating biomarkers and methodological variability	Expands to multi-disease biomarker applications and includes methodological considerations
Present review	Integrated molecular, clinical, and biomarker-focused approach		Combines molecular mechanisms, biomarker evaluation, methodological considerations, and clinical applicability

RANKL: Receptor activator of NF-kappaB ligand; RANK: Receptor activator of NF-kappaB; OPG: Osteoprotegerin.

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Table 2 Summary of regulations and limitations of markers of receptor activator of NF-kappaB/receptor activator of NF-kappaB ligand/osteoprotegerin axis

Marker	Type	Clinical use	Variability	Limitations
RANKL	Regulatory	Experimental	High	Assay variability, not standardized
OPG	Regulatory	Experimental	Moderate	Influenced by multiple systemic factors
RANKL/OPG ratio	Integrated marker	Emerging	Moderate	Lack of standardized cut-offs
PINP[156]	Bone formation marker	Established	Low	Reflects slower changes
β-CTX[157]	Bone resorption marker	Established	Moderate	Diurnal variation

RANKL: Receptor activator of NF-kappaB ligand; OPG: Osteoprotegerin; PINP: Procollagen type I N-terminal propeptide; CTX: C-terminal telopeptide of type I collagen.

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