REVIEW: Circulating Osteoprotegerin and Receptor Activator for Nuclear Factor κB Ligand: Clinical Utility in Metabolic Bone Disease Assessment

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Context: The discovery of the receptor activator for nuclear factor κB (RANK) ligand (RANKL)/RANK signaling pathway has marked a major advance in our understanding of the mechanisms controlling osteoclastogenesis. RANKL, expressed by preosteoblasts and stromal cells, binds to RANK, expressed by cells of the osteoclast lineage, inducing a signaling cascade leading to the differentiation and fusion of osteoclast precursor cells and stimulating the activity of the mature osteoclast. The effects of RANKL are counteracted by osteoprotegerin (OPG), a soluble neutralizing decoy receptor.

Evidence: This paper reviews the literature surrounding the use of circulating OPG and soluble RANKL (sRANKL) measurements and assesses their potential as markers of bone disease. Original clinical and basic research articles and reviews were identified using a Pubmed search strategy (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and cover the time period up until January 2005. Search terms osteoprotegerin, OPG, RANK, RANKL, and RANK ligand were used alone and in combination with bone, osteoporosis, and disease.

Evidence Synthesis: Assays for detecting OPG and sRANKL in the circulation in humans have been developed, and differences in the circulating concentrations of OPG and sRANKL have been observed in different disease states. There are, however, some inconsistencies in study outcome. These may relate to differences in study design, methodology, and other unknown factors influencing the variability of these measurements.

Conclusions: The clinical utility of serum OPG and sRANKL measurements as markers of disease activity requires additional investigation. In particular, rigorous testing of assays and identification of the sources of measurement variability are required. (*J Clin Endocrinol Metab* **90: 6323–6331, 2005**)

RESEARCHERS IN THE field of bone biology have, for a long time, sought to understand the mechanisms responsible for the cross-talk between osteoblasts and osteoclasts. A major step toward answering this question was provided by the discovery of osteoprotegerin (OPG). OPG was identified in 1997 by three different groups working in different areas (1–4). Although dendritic cells may express a cellular form, OPG is generally considered to be a secreted soluble receptor and is produced by many different tissues and cell types including osteoblasts. It functions as a decoy receptor for the receptor activator for nuclear factor κB (RANK) ligand (RANKL) and is thus an inhibitor of osteoclastogenesis (3) (Fig. 1C). In addition, OPG neutralizes the apoptosis inducing factor TNF-related apoptosis-inducing ligand (TRAIL) (5, 6). Studies in mice have revealed that the OPG knockout mouse develops severe osteoporosis, whereas the overexpression of OPG in transgenic mouse models and OPG treatment of normal mice leads to osteopetrosis (1, 4).

RANKL was discovered during the search for a ligand for OPG. It was found to be identical to another TNF receptor superfamily member that had already been characterized by two other groups (3, 7, 8). Many cell types express RANKL, including osteoblasts and endothelial cells. The cell-bound form of RANKL is the most common, although a soluble form created by the cleavage of a truncated ectodomain by a TNF-α converting enzyme-like protease and a primary secreted form also exist (3, 7).

By binding to RANK, and in the presence of macrophage colony-stimulating factor (M-CSF), RANKL promotes osteoclast differentiation, activation, survival, and also adherence to bone surface (9–12). *In vivo* experiments showed that when soluble (sRANKL) was administered to mice, an increase in osteoclast formation and activation was observed that led to osteoporosis and hypercalcemia (7). RANKL knockout mice on the other hand revealed an increased bone mass (osteopetrosis) and impaired tooth eruption because of a lack of mature osteoclasts (13).

RANK is the receptor for RANKL (14). Its expression has been detected mainly in osteoclast and dendritic cells. C-fms, the receptor for M-CSF, and RANK are sequentially expressed during the development of the mature osteoclast (15). Administration of sRANK, or RANK neutralizing antibodies, block osteoclastogenesis, whereas RANK stimulatory antibodies promote the osteoclastogenesis of progenitor cell lines (14, 16). RANK knockout mice have a phenotype similar to RANKL knockout mice, *i.e.* osteopetrosis, impaired dental eruption, and a lack of lymph nodes (17).

The signaling cascade following RANKL/RANK binding is not fully understood. TNF receptor-associated factors, especially TNF receptor-associated factor-6, and c-*src* may be crucial in this pathway (18). Details of RANK signaling pathways have been described previously (19–23).
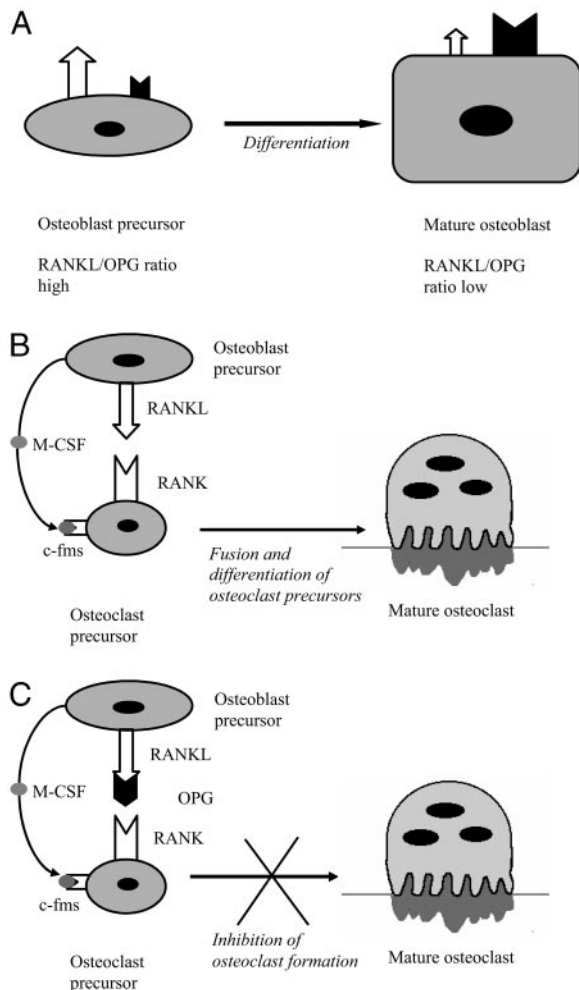
FIG. 1. A, The relative production of RANKL (*block arrows*) and OPG (*chevrons*) changes as the osteoblast precursor matures. B, RANKL binds to RANK expressed by osteoclast precursor cells inducing osteoclast differentiation and maturation; C, OPG acts as a decoy receptor by binding to RANKL and thus blocking the RANKL/RANK pathway and inhibiting osteoclast formation.

The relative expression of RANKL and OPG is critical in the regulation of osteoclast activity and in the perpetuation of the bone remodeling cycle (Fig. 1A). Preosteoblast cells and mature osteoblasts express RANKL, which binds to RANK expressed on the surface of osteoclast precursor cells. This signal leads to the differentiation and fusion of precursors and formation of the mature osteoclast (Fig. 1B). OPG acts as a decoy receptor by binding and neutralizing RANKL, inhibiting osteoclastogenesis, osteoclast activity and inducing osteoclast apoptosis (Fig. 1C). As the osteoblast differentiates, the relative production of OPG compared with RANKL increases, thus inhibiting osteoclast activity and eventually leading to osteoclast apoptosis allowing the mature osteoblast to refill the remodeling space (Fig. 1A) (24).

Factors Regulating RANKL and OPG Expression *in Vitro*

In vitro experiments using stromal and osteoblast cell models have revealed several hormonal and autocrine/paracrine factors, which have the ability to regulate RANKL and OPG production (Table 1). However, as will become apparent, these data are not always consistent with *in vivo* findings. Factors that increase RANKL mRNA expression include the proinflammatory cytokines TNFα, IL-1, IL-6, IL-11, and prostaglandin E$_2$. IL-1 and TNFα stimulate M-CSF production, which increases the pool of osteoclast precursor cells as well as directly increasing RANKL production (25). PTH, glucocorticoids, and 1,25 (OH)$_2$ vitamin D$_3$ also stimulate RANKL production (26–28). Factors that stimulate OPG production include TGF-β and 17β-estradiol (29, 30), whereas PTH and glucocorticoids decrease OPG production (26, 31). Regulators of RANKL and OPG production have been reviewed extensively elsewhere (32–35) and are summarized in Table 1.

Assays for Serum OPG and sRANKL

Serum OPG

Commercial assays for serum OPG have a used a sandwich assay ELISA format with a monoclonal capture and polyclonal detection antibodies. Some researchers have also developed in-house assays using a similar format. As yet, normal reference ranges for serum OPG have not been independently established. One company reports a median value for serum OPG of 1.8 pmol/liter (Biomedica, Vienna, Austria), whereas another quotes a mean value of 4.1 ± 0.33 (SEM) pmol/liter (Biovendor, Brno, Czech Republic). Differences in reported reference ranges may reflect the use of different OPG standards and reference populations by these manufacturers.

Assay manufacturers claim that OPG is stable at −20 C in serum and in EDTA, citrate, and heparin plasma, and also at 4 C for up to 14 d (Biovendor, ELISA version 07 131204). Three freeze-thaw cycles did not affect recovery of sample from serum, EDTA plasma, or citrate plasma, although recovery was significantly reduced in heparinized plasma (Biovendor). However, one independent report asserts that storage of sample for over 6 months at −70 C leads to significant reduction in serum OPG (36). In view of these discrepancies, it is recommended that samples should be processed immediately or stored for short periods at −70 C and not be subjected to repeated freeze-thaw cycles. If possible, inves-

TABLE 1. Factors regulating OPG and RANKL production *in vitro*

Factor	OPG	RANKL	Refs.
1,25 (OH)$_2$ vitamin D$_3$	↑	↑	27, 111
17β-Estradiol	↑	?	29, 30
Bone morphogenetic protein 2	↑	?	111
Calcium	↑	↑	3
Immunosuppressants	↓	↑	112
Glucocorticoids	↓	↑	28, 113
ICI 182,780	↓	?	29
IL-1β	↑	↑	25
IL-6	?	↑	114
Prostaglandin E$_2$	↓	↑	115
PTH	↓	↑	26
TGF-β	↑	↓	116
TNF-α	↑	↑	25
Vasoactive intestinal peptide	↑	↓	117

↑, Stimulatory; ↓, inhibitory; ?, no published data.

tigators should carry out their own assessment of sample stability.

Manufacturer's estimates of assay precision, where stated, are acceptable; Biovendor intraassay coefficient of variation (CV), 2.4–7.0%, and interassay CV, 3.4–7.4%; Biomedica intraassay CV, 4–10%, and interassay CV, 7–8%. Independent investigators have not always reported their own estimates of assay precision. In one study, an intraassay CV of over 20% was used as a cutoff for repeating the measurement (37). Investigators are advised to determine their own data on precision and normal reference ranges.

One research group has used RANKL-coated microtiter plates to capture OPG in serum, along with a monoclonal detection antibody (38, 39). This assay was found to be more sensitive than antibody-based assays with a lower detection limit of 0.244 μg/liter (~0.01 pmol/liter). The latest generation of commercial OPG assays has lower detection limits of 0.4 pmol/liter (Biovendor) and 0.14 pmol/liter (Biomedica).

OPG circulates as a monomer, a homodimer, and bound to RANKL. Commercial OPG assays are designed to detect all forms of circulating OPG. One independent research group has designed an immuno-PCR assay to specifically detect homodimeric OPG (40). Using this assay, the concentration of homodimeric OPG was 3.3 times higher in osteoporotic patients (n = 22) compared with controls (n = 11), whereas total OPG was 1.5 times higher in the osteoporotic patients compared with controls. This method of detection has not been widely used and is not commercially available.

Assays for serum sRANKL

A commercial assay for sRANKL uses an ELISA format with OPG as a capture molecule and a polyclonal detection antibody (Biomedica). The design and performance of this assay has been reported in detail (41). The sensitivity of the original assays produced by this manufacturer was insufficient to detect sRANKL in a proportion of normal individuals (42). Assay sensitivity has since been improved with the use of a different secondary antibody and a modified detection method. The lower limit of detection for the current assay is 0.08 pmol/liter.

There is some disagreement over the stability of sRANKL in serum and plasma. In one study, sRANKL was stable at up to four freeze-thaw cycles in serum and heparinized plasma (41). Another study showed that collection of sample on Li-heparin and storage for over 6 months at −70 C led to significant loss of recovery of sRANKL. Investigators are advised to perform their own assessment of sample stability.

Manufacturer's estimates of precision for sRANKL (Biomedica) are intraassay CV from 3% (at 3.2 pmol/liter) to 5% (at 1.0 pmol/liter) and interassay CV from 6% (at 1.78 pmol/liter) to 9% (at 0.80 pmol/liter).

Independent reference ranges for sRANKL using large well-characterized populations have not been established.

Preanalytical variability of serum OPG and sRANKL

Aside from assay performance, there are other potential sources of variability (preanalytical) that should be addressed when interpreting estimates of circulating OPG and sRANKL. Although these factors have been studied extensively for established markers of bone turnover, little is known about which factors influence serum OPG and sRANKL measures (43). These sources of variability may include factors that can be controlled, such as circadian rhythm, menstrual cycle, and exercise effects. Other factors may be uncontrollable, such as age, sex, and menopausal status. Some uncontrollable factors have been shown to influence serum OPG concentration, such as age, gender, and menopause (see below) (37, 44). Less is known about which preanalytical factors influence serum sRANKL levels.

Age and Gender Effects on Serum OPG and sRANKL

Epidemiological studies have assessed relationships between serum OPG and bone turnover or bone density. Both negative and positive associations between serum OPG and bone turnover have been described (45–48). In men, urinary total deoxypyridinoline, a marker of bone resorption, and PTH were inversely associated with serum OPG (45). In contrast, in postmenopausal women, significant negative associations were observed between serum markers of bone resorption and formation and serum OPG but not with urinary resorption markers or PTH (46). Both studies (45, 46) observed weak positive associations (r ~ 0.2) between serum OPG and serum estradiol. OPG also correlated positively with bone density in the women's study but not in the men's.

Later larger studies of both men and women reported strong positive associations between serum OPG and age but not with bone density (37, 48). In one study, a weak negative association between serum OPG and estradiol was observed in contrast to earlier findings (37). In a study of Icelandic men and women, positive associations between serum OPG and the bone formation marker osteocalcin were observed, whereas an inverse relationship with the bone resorption marker TRAP-5b (the 5b isoform of tartrate-resistant acid phosphatase) was seen in women only (48).

One study has shown that premenopausal women had higher serum OPG than men of a similar age. This difference was not observed when postmenopausal women were compared with older men (44). These findings suggest that estrogen status may influence the relationship between serum OPG and bone turnover. Indeed, Fahrleitner-Pammer *et al.* (47) showed a positive correlation between serum OPG and bone turnover but only in postmenopausal and not in premenopausal women.

Serum OPG and sRANKL in Metabolic Bone Disease

A summary of factors influencing serum OPG and sRANKL can be found in Table 2.

Osteoporosis

Several studies have assessed the clinical importance of serum concentration of OPG and latterly of serum sRANKL in relation to postmenopausal osteoporosis. The first of these studies by Yano *et al.* (49) performed an evaluation of immunoassays for the measurement of serum OPG. They showed that OPG circulates mainly as a monomer and not in the active dimeric form. They observed as in almost all

TABLE 2. Factors influencing serum OPG and sRANKL in humans

Parameter	Serum OPG	Serum RANKL	Refs.
Age	↑	?	37, 44, 45, 48, 49
Arthritis	↑	↓	94, 95
Bisphosphonate therapy	↓ ↔	↔	68, 102
Estrogen therapy	↑ ↓	↓ ↔	4, 97, 99
Fracture healing	↓	?	74
Glucocorticoid therapy	↓	?	76, 104, 105
Multiple myeloma	↓	↑	63–65
Paget's disease	↑	↔	68
PTH	↑	↑	107
Parathyroidectomy	↔	?	108
Phytoestrogens	↔	↓	99
Postmenopausal osteoporosis	↑ ↓	↑ ↓	49–51
Pregnancy	↑	?	42, 70
Primary biliary cirrhosis	↑	↓	79
Prostate cancer	↑	↑	39, 57, 118
Renal failure	↑	?	75–77
Risk of osteoporotic fracture	↑ ↓	↑ ↓	47, 52–54
Vascular disease	↑	?	53, 84–86, 87–90, 119

↑, Increase in serum concentration; ↓, decrease in serum concentration; ↑ ↓, both an increase and a decrease have been reported; ↔, no significant change in serum concentration; ?, no published data.

subsequent studies that serum OPG increases with age. They also showed that serum OPG was significantly higher in osteoporotic women compared with age-matched controls and that higher serum OPG was observed in women with higher bone turnover. This finding has been confirmed in subsequent studies (50, 51). It has been suggested that these observations may represent a compensatory response to the enhanced osteoclastic bone resorption observed in osteoporosis.

The association between serum OPG/sRANKL and osteoporotic fracture risk is uncertain. Low serum OPG has been associated with prevalent vertebral fracture in one study of osteoporotic postmenopausal women (47) other studies have demonstrated an increased risk of wrist and hip fracture in women with higher serum OPG (52, 53).

A recent publication by Schett *et al.* (54) showed, somewhat paradoxically, that low levels of serum sRANKL and high levels of serum OPG were associated with incidence of nontraumatic fracture.

Malignant disease

Skeletal metastases are commonly observed in patients with prostate and breast cancer. Interestingly, the bone metastases in prostate cancer are predominantly sclerotic rather than lytic. *In vitro*, Brown *et al.* (55) have reported that prostate cancer cells from bone metastases express higher levels of OPG and sRANKL than nonosseous metastases. The same group observed that patients with advanced prostate cancer had significantly higher levels of serum OPG than those with benign prostatic hyperplasia and clinically localized prostate cancer (39). These and other studies (56, 57) suggest that there may be a potential role for serum OPG as a marker of disease progression. Using *in vitro* methods, Holen *et al.* (6) concluded that a possible mechanism for the relationship between OPG and prostate cancer disease severity may be the ability of OPG to neutralize the apoptosis-inducing ligand

TRAIL, thus acting as a survival factor for prostate cancer cells. Recently, the same group has shown that OPG will also promote the survival of two different breast cancer cell lines *in vitro* (58). Serum OPG concentrations do not, however, appear to be related to the presence of bone metastases associated with breast cancer and are not influenced by the use of the aromatase inhibitor anastrozole (59).

A feature of the B cell malignancy, multiple myeloma, is the development of osteolytic bone disease. In studies of mice, myeloma cells have been shown to express RANKL and induce bone resorption (60). OPG treatment of mice with myeloma prevents the development of lytic bone lesions (61). Recently, this has also been tested in human patients with myeloma (60, 62). Serum sRANKL was elevated in patients with newly diagnosed multiple myeloma and was related to disease severity in bone (63). The sRANKL/OPG ratio was also higher in these patients and correlated with markers of bone resorption, osteolytic lesions, and markers of disease activity (63). In accordance with these findings, Seidel *et al.* (64) observed lower serum OPG in patients with multiple myeloma compared with age- and sex-matched controls. This was particularly evident in those patients with osteolytic lesions. Patients with monoclonal gammopathy of undetermined significance have increased serum OPG and lowered sRANKL/OPG ratio (65) compared with patients with multiple myeloma. This observation has implications for the use of serum OPG measurements as a noninvasive tool to discriminate between patients with multiple myeloma and monoclonal gammopathy of undetermined significance.

Paget's disease

Enhanced RANKL expression has been observed in a bone marrow stromal cell line developed from a patient with Paget's disease (66) and osteoclastic precursor cells from Pagetic lesions have increased sensitivity to RANKL (67). One clinical study observed that serum OPG was higher in patients with Paget's disease compared with a control population (68).

Pregnancy

Serum OPG increases markedly during the gestation period in mice (69), indicating a possible role for OPG in the prevention of excessive bone loss during pregnancy. Subsequent studies of human pregnancy showed similar increases in serum OPG through the gestational period with a sharp decline after delivery (42, 70). The relevance of these changes to bone loss and remodeling, however, remains unclear. The source of circulating OPG during pregnancy is also uncertain because the placenta and breast tissue produce large quantities of OPG. It has been suggested that increased OPG in pregnancy may protect cells of the fetal membrane against the proapoptotic effects of TRAIL (71). In mice, RANKL is also important for the development of the lactating mammary gland (72).

Fracture healing

Expression of RANKL and OPG during fracture healing has been examined in a mouse model of tibial fracture. Peaks

of OPG expression were seen at 24 h and 7 d after fracture, coinciding with peak cartilage formation. RANKL expression on the other hand peaked at d 3 and 14 when OPG levels were decreasing (73). A study of fracture healing in humans after traumatic tibial fracture showed a decrease in serum OPG concentration with the lowest OPG concentration seen at 8 wk after fracture (74). A negative correlation between serum OPG and the bone formation markers osteocalcin and the N-terminal propeptide of type I collagen was also observed in this study. These data suggest that RANKL/OPG ratios may be implicated in the fracture repair process although they do not necessarily demonstrate cause and effect. It should be noted that the RANK knockout mouse has normal fracture healing (17).

Kidney and liver disease

Patients with renal osteodystrophy or renal failure have increased serum OPG concentration that is decreased by renal transplantation (75–77). Little is known of the mechanisms of OPG clearance, although it is now known that OPG will not pass through a hemodialysis membrane (78). Skeletal resistance to PTH is one of the major abnormalities underlying bone diseases in uremia. These studies suggest that the mechanism for this resistance may be related to increases in skeletal OPG production.

Serum OPG/sRANKL levels have been implicated in liver disease. Serum OPG was higher and serum sRANKL lower in patients with primary biliary cirrhosis compared with healthy controls (79). Interestingly, serum OPG correlated positively with markers of bone turnover in this study.

Vascular disease

Osteoporosis and vascular disease are commonly associated clinically. Animal experiments suggest that OPG and RANKL may be common mediators that affect both bone metabolism and vascular integrity. As well as having severe osteoporosis and a high incidence of fractures, OPG$-/-$ mice have medial calcification of the aorta and renal arteries, a disease model similar to Mocklenberg's sclerosis (80). OPG can inhibit arterial calcification in an experimental mouse model (81). However, OPG administration to adult OPG$-/-$ mice could not reverse calcification but did reverse the observed osteoporosis (82). The role of OPG and RANKL as paracrine regulators of vascular calcification is reinforced by the fact that these factors are produced by vascular endothelial cells (83).

Associations between serum OPG, sRANKL, and vascular calcification have been studied in a clinical setting. It should be noted that these human studies have assessed calcification associated with atherosclerosis, which represents calcification of the intima of the artery, whereas those in mice describe medial calcification. Data from these studies are in contrast to the studies in mice. In patients undergoing hemodialysis, serum OPG was higher in those patients with a high aortic calcification index (84). In patients with coronary artery disease, serum OPG was higher in subjects with significant arterial stenosis than those without (85). Later studies revealed that coronary artery disease in men is associated with high serum OPG and low serum sRANKL measure-

ments (86, 87). These findings are in accordance with an epidemiology study by Browner *et al.* (53) who showed that increased serum OPG was associated with cardiovascular mortality. Interestingly, high serum OPG concentration has been associated with vascular dementia and Alzheimer's disease (88) and with microvascular complications associated with diabetes (89). Increased thickness of the intima media of the carotid artery has also been associated with increased serum OPG (90).

Arthritis

It has been suggested that there may be a role for OPG in the prevention of bony erosions in the rheumatoid joint. Macrophages isolated from rheumatoid joints have been found to express RANKL, RANK, and OPG (91, 92). When these cells are cultured, they are capable of differentiating into bone-resorbing osteoclasts, and their differentiation is blocked by OPG (93). Higher serum OPG levels have been described in adults with seropositive rheumatoid arthritis and also in children with juvenile idiopathic arthritis (94, 95). These measurements did not, however, correlate with the markers of disease activity, erythrocyte sedimentation rate, and C reactive protein.

Response of Serum OPG and sRANKL to Therapy

Therapies that modulate bone turnover most likely influence OPG and RANKL production. Serum OPG and sRANKL may thus have potential use as markers of therapeutic response. Several studies have evaluated the *in vitro* effects of different drugs on OPG and RANKL expression. These effects have also been examined in clinical models.

In vitro, 17β-estradiol increases the production of OPG in human and mouse osteoblast-like cells (29, 29), whereas testosterone appears to inhibit OPG production (96). The effects of estradiol and testosterone therapy on serum OPG are less clear. Using a longitudinal study design, Khosla *et al.* (97) rendered elderly men acutely hypogonadal using a GnRH agonist and an aromatase inhibitor and selectively replaced either estradiol or testosterone or both in different groups. They found that serum OPG increased after selective estradiol replacement but decreased with testosterone replacement (97). In contrast to these findings, women prescribed a GnRH agonist for the treatment of endometriosis showed an increase in serum OPG (98). Serum OPG and sRANKL decreased, after 1 yr of treatment with a preparation of 17β-estradiol combined with norethisterone acetate in a study of 90 early postmenopausal women (99). The use of oral contraceptives has also been associated with higher serum OPG, but the same study reported no differences in serum sRANKL measurements (100). Reasons for these discrepancies may relate to the differences in type and duration of treatment as well as the populations studied.

In vitro, bisphosphonates influence production of OPG. Viereck *et al.* (101) have shown that the bisphosphonates pamidronate and zoledronate increase the expression of OPG mRNA in primary human osteoblasts. One clinical study has demonstrated a decrease in OPG in response to bisphosphonate therapy (tiludronate) in patients with Paget's disease, but in the same study serum OPG was found

to be unresponsive to bisphosphonate therapy in patients with osteoporosis (68). Serum OPG did not respond to 2 yr of etidronate therapy in patients with rheumatoid arthritis, despite observations of a significant decrease in the bone turnover markers (102).

Glucocorticoids have been shown to influence OPG and RANKL production *in vitro* (28). Hofbauer *et al.* demonstrated a decrease in OPG and an increase in RANKL production by osteoblast-like cells in response to dexamethasone (28). This finding may indicate a possible mechanism for glucocorticoid-induced osteoporosis (103). Studies *in vivo* have shown that glucocorticoids suppress serum OPG in patients with renal disease and after cardiac transplantation (76, 104, 105).

Continuous PTH infusion increases RANKL and decreases OPG expression by osteoblasts *in vitro*, whereas intermittent PTH infusion does not alter the RANKL/OPG ratio (26, 106). These observations may help to explain the anabolic effect of intermittent PTH therapy. The effect of intermittent PTH therapy on serum OPG has been investigated in women with glucocorticoid-induced osteoporosis (107). A rapid significant increase in serum sRANKL was seen within 1 month of commencing therapy, whereas serum OPG was only slightly suppressed 6 months after therapy. Any effect of increased endogenous PTH on OPG and sRANKL is less certain. In a small study of 20 patients with hyperparathyroidism, no significant changes in serum OPG were observed after parathyroidectomy (108). However, a later study by the same research group did show that the ratio of RANKL/OPG mRNA expression in transiliac bone biopsies was significantly decreased after parathyroidectomy (109).

Discussion

There can be little doubt of the importance of the contribution the discovery of the OPG/RANKL/RANK system has made to our understanding of bone biology. Not surprisingly, since the development of assays to measure OPG, and later sRANKL, in serum there has been considerable interest in the use of these cytokines as indicators of metabolic bone disease. Overall, there is a lack of consistency in the outcome of studies of circulating OPG and sRANKL, particularly in the field of osteoporosis. There may be several reasons for this.

Serum OPG and sRANKL may not reflect the activity of these cytokines in the bone microenvironment. A proportion of circulating OPG and sRANKL is likely to originate from nonskeletal sources. Furthermore, the source of circulating OPG and sRANKL in different disease states is unknown. Calculation of the ratio of sRANKL to OPG may be a more relevant marker than that of OPG alone. However, serum measurements of sRANKL have limitations. Circulating sRANKL concentrations in normal individuals are typically less than 1 pmol/liter. The early assays for sRANKL were not sensitive enough to detect concentrations at this level. Subsequently, the sensitivity of these assays has been improved.

Although RANKL is expressed in a soluble form (sRANKL), the majority of RANKL is cell bound and thus not detectable in the circulation. Cell surface production of RANKL has been assessed *in vivo* in humans using flow cytometry (110). In this study of pre- and postmenopausal women, cell surface RANKL production by bone marrow cells was higher in untreated postmenopausal women than estrogen-treated women and premenopausal women. This represents an alternative approach to assessing RANKL production *in vivo*. However, it would be impractical to use this method in large epidemiological studies.

At present, it is unclear as to which factors may influence the preanalytical variability of OPG and sRANKL measurements. It is possible that factors such as time of sampling, storage of samples, and methods of collection could influence study outcome. The stability of serum sRANKL has been questioned; one report proposes that samples collected in EDTA may be more stable (36), whereas another advocates the use of serum samples (41). These sources of variability, once identified, are controllable. Other factors, such as age, sex, and comorbidity are not controllable but may be overcome by the use of multivariate analyses.

Other reasons for discrepancies between studies may relate to the assays themselves. The specificity of current methods for detecting serum OPG is uncertain. Different assays have been used in different studies. The assays have also evolved over time such that comparisons cannot be made across studies even though the same assay has been used. OPG circulates in different forms, as a monomer, as a dimer, and as a RANKL/OPG conjugate. The OPG dimer has been described as the active form (38). In the circulation, the monomer is the most abundant form and may be a degradation product of the dimer (38). Commercial assays claim to detect all forms of OPG, but it is unclear as to whether this is the most appropriate measure to make. One research group has used an assay designed to detect the homodimer, which may be a more relevant measurement (40).

There are few convincing data that demonstrate a response in OPG and sRANKL to antiresorptive therapy. The reasons for this may be related to the concomitant changes in bone turnover after therapy. A potential change in the cellular response of serum OPG or sRANKL to antiresorptive therapies may be masked by a reduction in the number of active osteoblasts as bone turnover decreases. In studies where a change in OPG has been observed, these measurements have often been made within a few days of starting treatment before changes in bone turnover have become established (97). Many of the studies of the response of serum OPG to treatment have been made retrospectively and were not designed to allow for measures to be made within days of treatment.

The method of clearance of OPG from the circulation is uncertain. The rate of OPG clearance may be an important consideration when interpreting serum OPG measurements. The data from the study of OPG in renal disease suggest that renal insufficiency is related to increased serum OPG (75, 76). OPG does not appear to be cleared directly by the kidneys (78), although this does not preclude any effect of impaired renal function on OPG clearance. Thus the observed relationship between age and OPG may be explained in part by reduced renal clearance, which is commonly observed in the elderly.

In conclusion, the clinical utility of serum OPG and sRANKL measurements as markers of disease activity re-

quires further investigation. It should be remembered that it is the ratio of OPG/RANKL that determines the net effect on osteoclast activity (Fig. 1A) and that measuring each molecule in isolation has its limitations. In the case of serum OPG, attention should be paid to the development of assays that specifically detect the active form of OPG (the homodimer). The usefulness of circulating sRANKL measurements remains uncertain because the major proportion of RANKL in bone is membrane bound. In both cases, rigorous testing of assays should be carried out and the sources of preanalytical variability identified.

Acknowledgments

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