In the 1990s, the discovery of receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG) was a significant breakthrough that improved our understanding of bone remodelling. Until this discovery, the presence of receptors on osteoblasts for most of the hormones, cytokines and growth factors that regulate osteoclast activity was a paradox.\(^1\) RANKL was found to be the osteoblast-derived factor, which is essential for osteoclast formation, function and survival. RANKL is expressed by bone-forming osteoblasts, bone marrow stromal cells and T and B lymphocytes that activate its receptor RANK, which is expressed on osteoblasts. After RANKL-induced RANK stimulation, several key regulating transcription factors, cytokines and enzymes are induced to promote the differentiation, proliferation, nucleation, activation and survival of osteoclasts. The result is a profound resorption of bone. OPG is a decoy receptor that binds to RANKL, which prevents RANKL/RANK from binding and activating osteoclastogenesis.

Although RANKL and RANK alone are not sufficient for bone resorption, the ratio of RANKL/OPG may be the ultimate determinant of bone resorption. The over-expression of soluble RANKL in transgenic mice results in a skeletal phenotype with many similarities to post-menopausal osteoporosis, including low bone mineral density (BMD), increased porosity, increased bone resorption and skeletal fragility.\(^2\) Each of these skeletal changes is also present in OPG knockout mice.\(^3\) Oestrogen deficiency increases RANKL, which leads to increased osteoclast recruitment and activation of osteoblasts and decreased osteoblast apoptosis. Oestrogen suppresses RANKL production by osteoblastic cells and increases OPG production by osteoblastic cells.\(^4\)

With the availability of serum assays to measure OPG and RANKL, there has been considerable interest in the use of these measures as indicators of risk of osteoporosis and fracture. Candidate biomarkers of disease have the potential to identify individuals early in the disease process and facilitate early interventions to prevent further progression. Are serum measures of OPG and RANKL ready for prime time?

In the current issue of this journal, Jorgensen et al. report prospective results from the Tromso Study, a well-characterized population-based cohort study of 2740 male subjects and 2857 female subjects, followed up for ~15 years.\(^5\) Male subjects with the highest level of OPG had almost a 3-fold increased risk of hip fracture compared with male subjects with the lowest level of OPG. Results were similar in female subjects not on post-menopausal hormone therapy, where female subjects with the highest level of OPG had a 60% higher risk of hip fracture. These associations were independent of key confounding variables including age, BMD and body mass index and are consistent with previous reports from the cohort showing positive associations between OPG and bone loss\(^6\) and height loss.\(^7\) These results are important, given that hip fractures are the major consequence of osteoporosis and have major impacts on disability, institutionalization and death.

But, are these results consistent with the biology of bone remodelling? As noted by the authors, one would have expected that high levels of OPG, given its role as a decoy receptor for RANKL, would be protective of fracture. The previous literature on the association between serum OPG and RANKL to bone turnover, BMD and fracture has been inconsistent.\(^8\) Part of this inconsistency may reflect different assays, and Rogers and Eastell have called for rigorous testing of assays and identification of the sources of measurement variability.\(^9\)

The authors speculate that high serum OPG may reflect an attempt to counterbalance the development of osteoporosis. This is consistent with the hypothesis that, with increased bone resorption, OPG synthesis may increase as a homeostatic mechanism to reduce bone loss.\(^9\) The authors speculate that, because the association was independent of areal BMD, other measures of bone strength and micro-architecture may be in the causal pathway. However, a recent study evaluated cytokine production in bone and bone marrow of
patients with an osteoporotic fracture. The fracture patients had substantially greater amounts of RANKL and OPG and their ratio in the bone marrow, in comparison with patients with osteoarthritis, who showed little difference in these measures in bone. Bone marrow produced greater amounts of RANKL compared with bone, whereas bone produced greater amounts of osteoblast inhibitors, such as sclerostin. These results suggest that an important mediator of the OPG–hip fracture association may be the amount of bone marrow fat (BMF). Mesenchymal stem cells, where osteoblasts originate, are also precursors of adipocytes. Differentiation of mesenchymal stem cells into either adipocytes or pre-osteoblasts is regulated by complex processes involving many growth and transcription factors. This differentiation is thought to favour adipogenesis with ageing because of physiological declines in growth factor secretion, oxygen tension and blood supply within the bone marrow. This complex relationship provides an underlying mechanism explaining the higher level of BMF observed in male and female subjects with osteoporosis. Future studies should include measures of BMF to test whether higher BMF accounts for the greater production of these cytokines.

Finally, these results highlight the need to more fully understand the RANKL/RANK/OPG cascade. What we observe in vitro may not translate to what we observe in human studies, and we need to know why. Many factors regulate OPG and RANKL expression in vitro including 1,25-dihydroxyvitamin D, sex steroid hormones, immunosuppressant drugs, glucocorticoids, other cytokines including interleukin-6, interleukin-1ß and bone morphogenetic proteins. Inclusion of a single biomarker that is part of a complex metabolic pathway may over-simplify these biological associations.

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