Non-osteoporotic post-menopausal women do not have elevated concentrations of autoantibodies against osteoprotegerin

Isabelle Piec1, Sophie Paddock1, Christopher J. Washbourne1, Jonathan C.Y. Tang1, Julie Greeves1, Sarah Jackson2, Stuart Ralston1, Philip Riches1, Helen McDonald1 and William D. Fraser1

Introduction: autoantibodies for OPG in the RANK-RANKL-OPG signalling pathway

The RANK/RANKL/OPG signalling pathway is essential for osteoclastogenesis. Osteoprotegerin (OPG) is a decoy receptor for RANKL. By binding to RANKL, OPG blocks RANKL-RANK interaction, inhibiting the differentiation of the osteoclast precursor into a mature osteoclast and thereby protecting the skeleton from excessive bone resorption.

- Auto-antibodies against Osteoprotegerin (α-OPGAb), by capturing OPG, enable sustained interaction of RANKL with RANK and over-activation of osteoclasts.
- Such antibodies were identified in 2009, in a man with coeliac disease associated with severe osteoporosis1 and later in 2013, in patients presenting with rheumatoid arthritis, systemic lupus erythematosus, spondyloarthropathies and osteoporosis2.
- These findings suggest a role for α-OPGAb as primary cause of high bone turnover.

We developed an enzyme linked immunosorbent assay (ELISA) for detection and quantification of α-OPGAb in patient serum samples3 showing α-OPGAb to be present in 14% of an apparently healthy young adult population. Bone resorption is increased in the elderly, particularly in women who may demonstrate increased α-OPGAb.

We aimed to define a reference range for OPG autoantibodies in non-osteoporotic post-menopausal women.

Method: α-OPGAb assay on serum samples

- Samples from non-osteoporotic 60-65yr-old post-menopausal women (ANSAVID study, n=134)
- Serum samples from healthy volunteers following and in accordance with the Ministry of Defence Research Ethics Committee (MODREC-163) (18-26yrs, n=51).

- ELISA:
  - Plates (Maxisorp, ThermoFisher Scientific) were coated with 0.5μg/mL recombinant OPG (Novoprotein)
  - Samples/standards (rabbit OPGAb, Abnova) and controls (50μL) were incubated for 3hrs at RT
  - A two-step detection was used: goat polyclonal biotin conjugated anti-human OPG antibody (ThermoFisher Scientific) and Streptavidin conjugated horseradish peroxidase (Jackson ImmunoResearch).
  - TMB (Sigma Aldrich) was used as substrate and signal was measure using a Multiskan software linked to a plate reader (ThermoFisher Scientific). Circulating antibody concentration is calculated against a 4-Parameter Logistic equation (Typical obtained using a polyclonal r=0.9916).

Results: Distribution of α-OPGAb

- Skewed distribution of α-OPGAb in both populations
- Adult population would be considered positive with a titer above the cut-off limit (95%) of 191ng/mL calculated using the geometric mean of log10 dataset
- The reference ranges obtained were 134-191ng/mL and 131-184ng/mL for control and post-menopausal women, respectively.

Conclusions

We established that the population of normal post-menopausal women who do not have osteoporosis also do not have elevated concentrations of α-OPGAb when compared to a younger female population (18-26 yrs). This suggest that α-OPGAb is not positively associated with increasing age suggesting that the increased production of α-OPGAb is mainly related to pathologic conditions which can result in significant bone resorption.

Comparison of osteoprotic patient samples to non-osteoporotic post-menopausal women would be interesting to determine whether α-OPGAb can be used to detect patients at high risk of bone resorption and identify appropriate treatment for this particular subgroup of patients.

We are designing a humanized antibody against human OPG in order to eliminate false positive.

References: