# ANTI-INFLAMMATORY ACTION OF DENOSUMAB IN VIVO AND IN VITRO

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## OBJECTIVES

RANKL is a member of tumor necrosis factors superfamily and by binding with its receptor RANK promotes expression of pro-inflammatory cytokines. Recent studies shown that RANKL is implicated in insulin-resistance and inflammatory diseases development.[1-2]
Micro- inflammation is characteristic of chronic Kidney disease (CKD).
We studied RANKL and other inflammatory markers levels in CKD patients before and after administration of Denosumab, a drug that blocks the activation of this complex system.

## METHODS

The study was conducted on 40 patients with IV-V stage of CKD (20 males and 20 females mean age 68.9 14) and a control group (HS). We measured serum levels of erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), interleukin-17 (IL-17), interleukin-1beta (IL-1β), interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-$\alpha$), RANKL, Osteoprotegerin (OPG) and plasmatic and urinary Neutrophil gelatinase-associated lipocalin (NGAL) before and after 6 and 12 months of treatment with denosumab.
*Human peripheral blood mononuclear cells (PBMC)* were isolated and stimulated with phytohaemagglutinin (PHA). In the culture medium we measured NGAL and pro-inflammatory cytokines before and after denosumab .

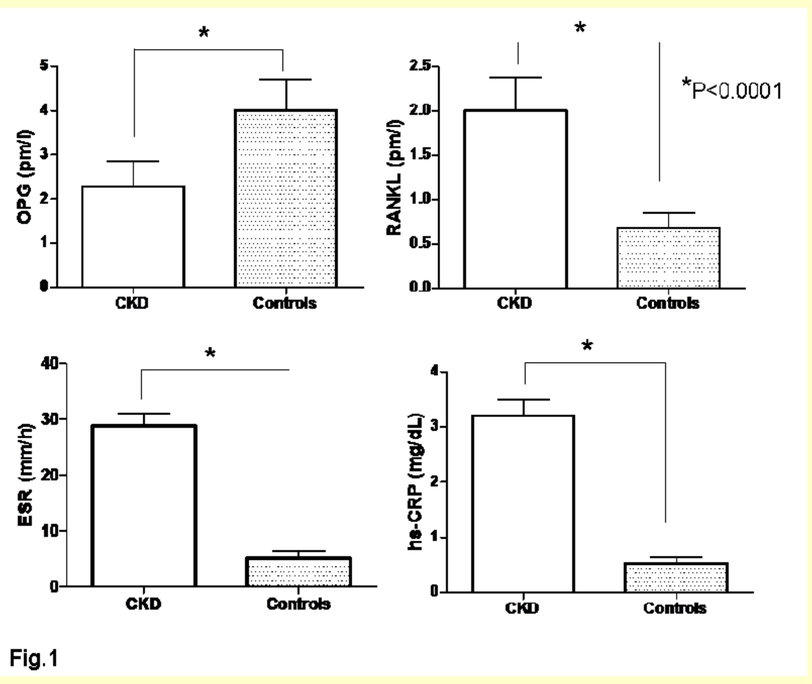

Fig.1

## RESULTS

Patients with CKD have low levels of OPG and RANKL but high levels of inflammatory markers (figure 1). NGAL and cytokines are significantly higher in these patients compared to controls in plasma and in the supernatant of PBMC stimulated with PHA ($p < 0.0001$). After 6 months from denosumab administration NGAL and cytokines levels were significantly reduced both *in vivo* and *in vitro* ( $p < 0.005$). The levels are further reduced after 1 year of treatment ($p < 0.002$ *in vivo*; $p<0.001$ *in vitro*) (Fig.2,3).
At multivariate analysis, RANKL was found to be directly correlated with creatinine, NGAL, IL- 6, IL-17, NF- γ, IL- 1β, TNF- α and CRP (Table).

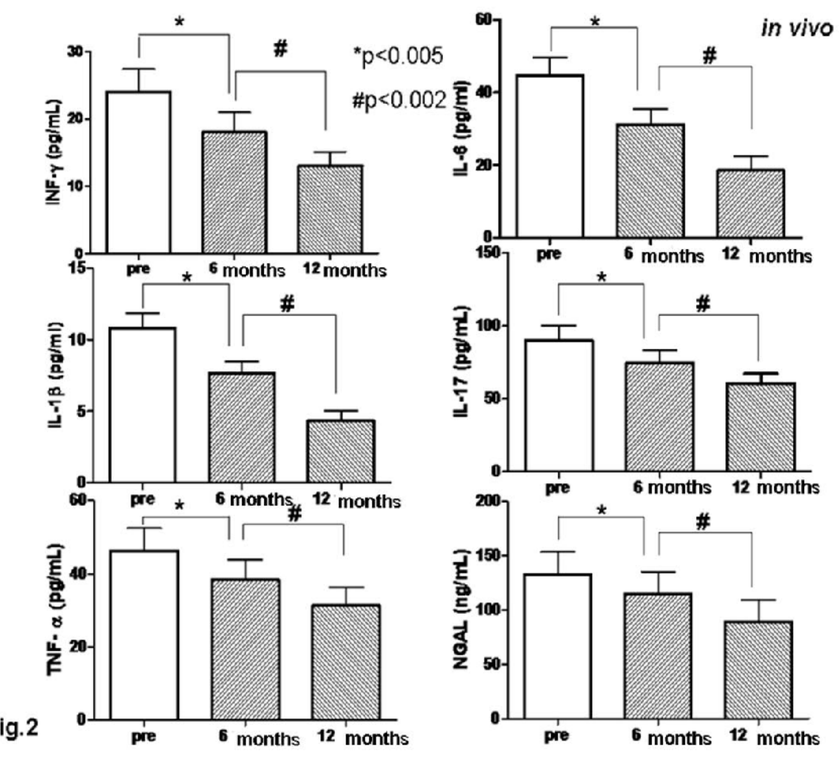

Fig.2

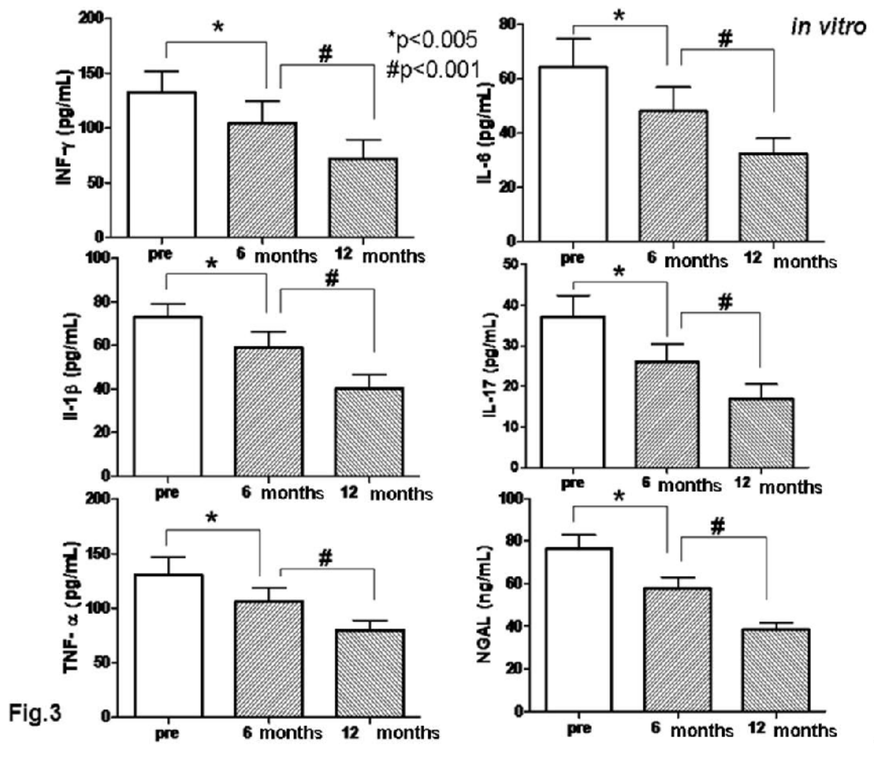

Fig.3

**UNIVARIATE AND MULTIVARIATE CORRELATIONS *in vivo***

| Variable | Partial R | β | P-value |
|---|---|---|---|
| ESR | 0.34 (P= 0.02) | 0.32 | 0.001 |
| hs-CRP | 0.81 (P<0.0001) | 0.81 | <0.0001 |
| IL-6 | 0.90 (P<0.0001) | 0.78 | <0.0001 |
| IL-17 | 0.66 (P<0.0001) | 0.32 | 0.006 |
| IFN-γ | 0.90 (P<0.0001) | 0.75 | < 0.0001 |
| TNF-α | 0.48 (P< 0.0001) | 0.72 | < 0.0001 |
| NGAL | 0.74 (P =0.001) | 0.58 | 0.0001 |
| Il-1β | 0.51 (P =0.0007) | 0.23 | 0.13 |

**UNIVARIATE AND MULTIVARIATE CORRELATIONS *in vitro***

| Variable | Partial R | β | P-value |
|---|---|---|---|
| ESR | 0.35 (P= 0.02) | 0.64 | 0.4 |
| hs-CRP | 0.36 ( P=0.002) | 0.54 | 0.3 |
| IL-6 | 0.94 (P<0.0001) | 0.78 | <0.0001 |
| IL-17 | 0.92 (P<0.0001) | 0.41 | 0.07 |
| TNF-α | 0.88 (P< 0.0001) | 0.43 | 0.0002 |
| Il-1β | 0.78 (P<0.0001) | 0.08 | 0.02 |
| NGAL | 0.48 (P =0.001) | 0.33 | 0.001 |

Table

## CONCLUSIONS

Our study showed an involvement of the RANKL/RANK system in the regulation of immune and inflammatory processes in CKD patients. Denosumab could be used to anti-inflammatory aims in these patients

## References

**1.** Kiechl S, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, Moschen AR, Muscogiuri G, Sorice GP, Kireva T, Summerer M, Wirtz S, Luther J, Mielenz D,Billmeier U, Egger G, Mayr A, Oberhollenzer F, Kronenberg F, Orthofer M, Penninger JM, Meigs JB, Bonora E, Tilg H, Willeit J, Schett G.: Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus.
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**2.** Moschen AR, Kaser A, Enrich B, Ludwiczek O, Gabriel M, Obrist P, Wolf AM, Tilg H.: The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut. 2005 Apr;54(4):479-87.


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