Inflammation may induce an osteoclastogenic phenotype in peripheral mononuclear cells (PBMC) of chronic kidney disease (CKD) patients

Gigante Margherita[1], Cafiero Cesira[1], Brunetti Giacoma[2], Grano Maria[2] , Colucci Silvia[2] , Simone Simona[1], Ranieri Elena[3], Pertosa B.Giovanni[1],Gesualdo Loreto[1].

[1]Department of Emergency and Organ Transplantation, Univ. of Bari, Italy; [2]Department of Basic Medical Sciences, Neurosciences and Sense Organs, Section of Human Anatomy, University of Bari, Italy; [3]Department of Medical and Surgical Sciences, University of Foggia, Italy.

Background: An altered bone remodeling is observed in subjects with progressive loss in renal function, particularly in patients with CKD requiring dialysis (stage V), and in the majority of patients with CKD stages III–V [1]. In pathological condition, immune cells and inflammatory cytokines belonging to tumor necrosis factor superfamily, (LIGHT and RANKL), play a key role in osteoclastogenesis [2]. **Aim:** The aim of our study were to evaluate the osteoclastogenic potential of PBMCs from CKD and HD pts and characterize pro-osteoclastogenic cytokines and immune cell subsets.

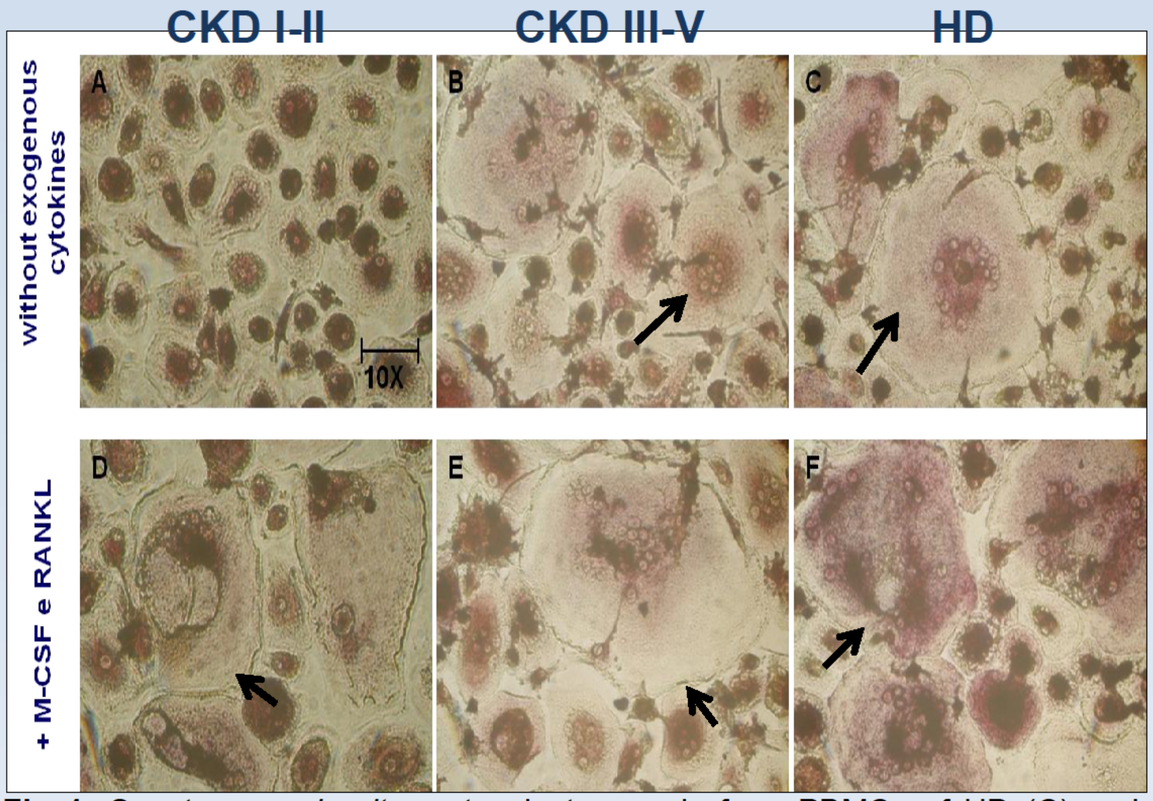

Fig 1: Spontaneous *in vitro* osteoclastogenesis from PBMCs of HD (C) and CKD III-V (B) pts compared to PBMC of CKD I-II pts, where we have not mature osteoclast (A). Induction of osteoclastogeneis from PBMC in HD (F),CKD III-V(E) and CKDI-II (D) with MCSF and RANKL.

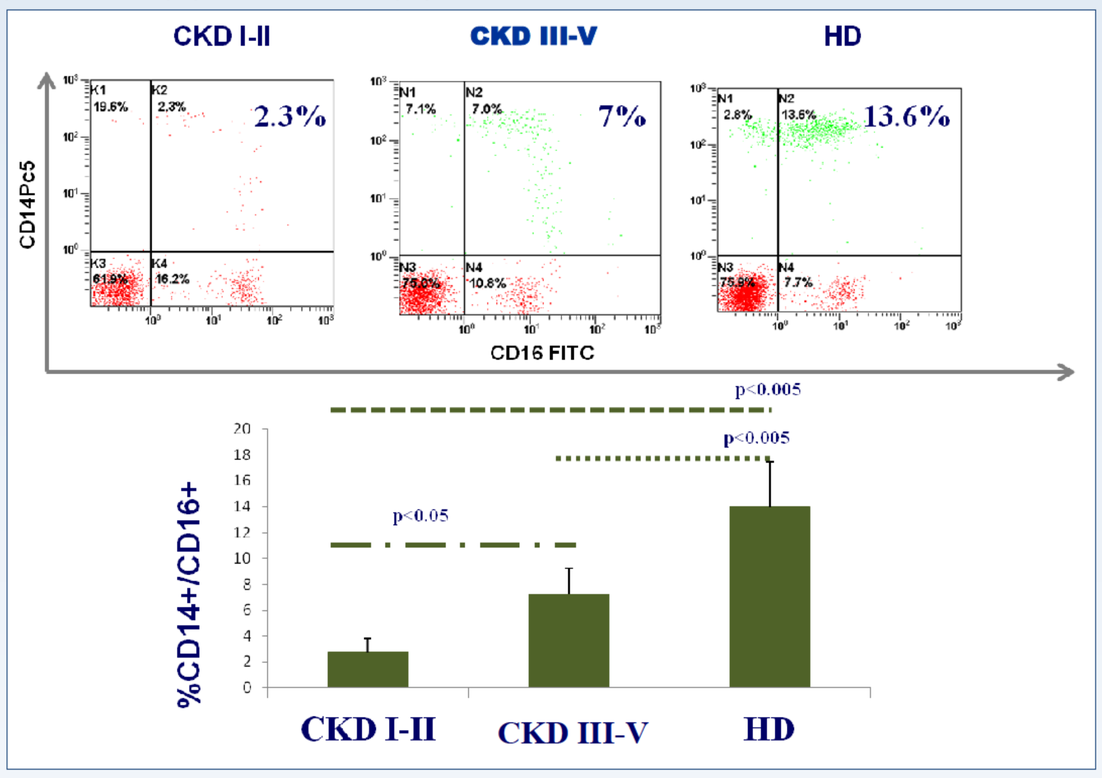

Fig3: Significant increase of $CD14^+CD16^+$ in peripheral blood of HD and CKD pts (stage III-V) compared to controls ($p<0.005$).

Methods: The OCs were obtained from freshly unfractionated and T cell-depleted PBMCs of pts, cells were cultured for 21-25 days with and without exogenous cytokines (MCSF and RANKL). Mature OCs were identified as tartrate resistant acid phosphatase positive ($TRAP^+$) multinucleated cells containing 3 or more nuclei. The presence of circulating inflammatory monocytes ($CD14^+/CD16^+$), LIGHT and RANKL expression in the immune cell subsets were evaluated by flow cytometry. We also evaluated serum levels of *CTX* (carboxy-terminal collagen type I), marker of bone resorption.

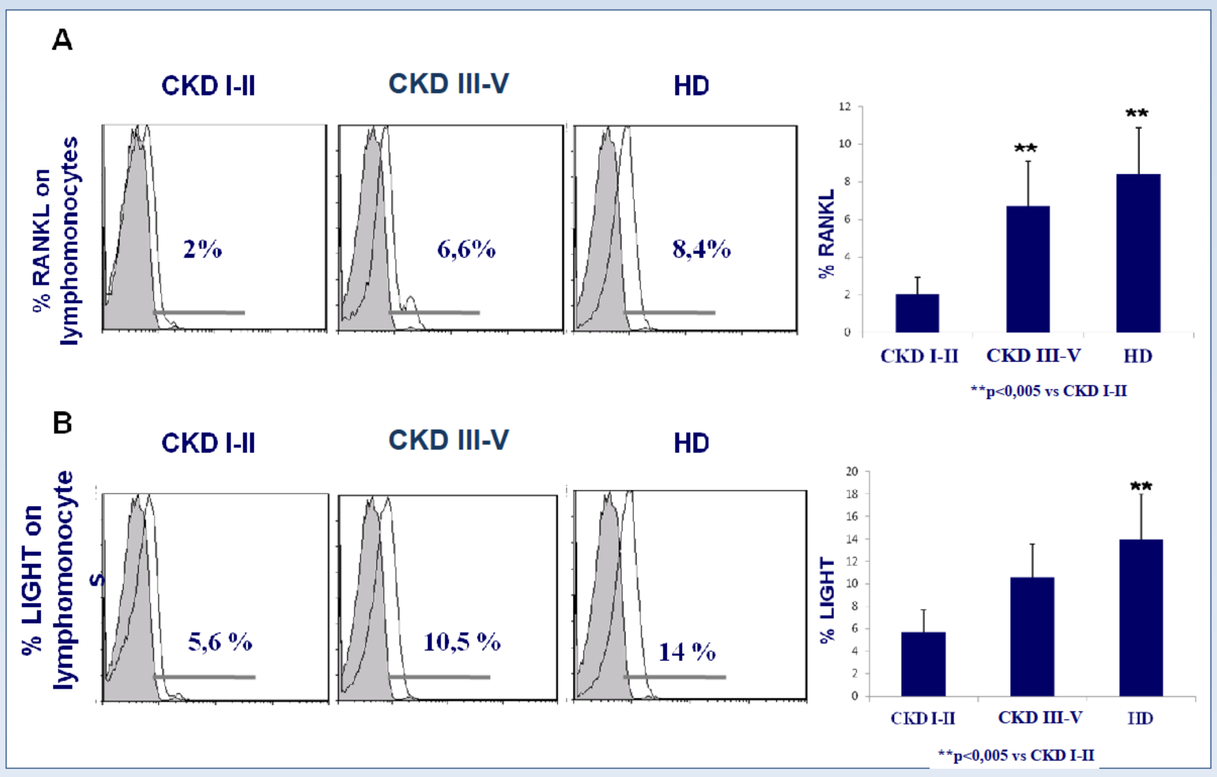

Fig2 Significant increase of RANKL(A) and LIGHT (B) expression on lynfomonocytes of HD and CKD pts (stage III-V) compared to control group.

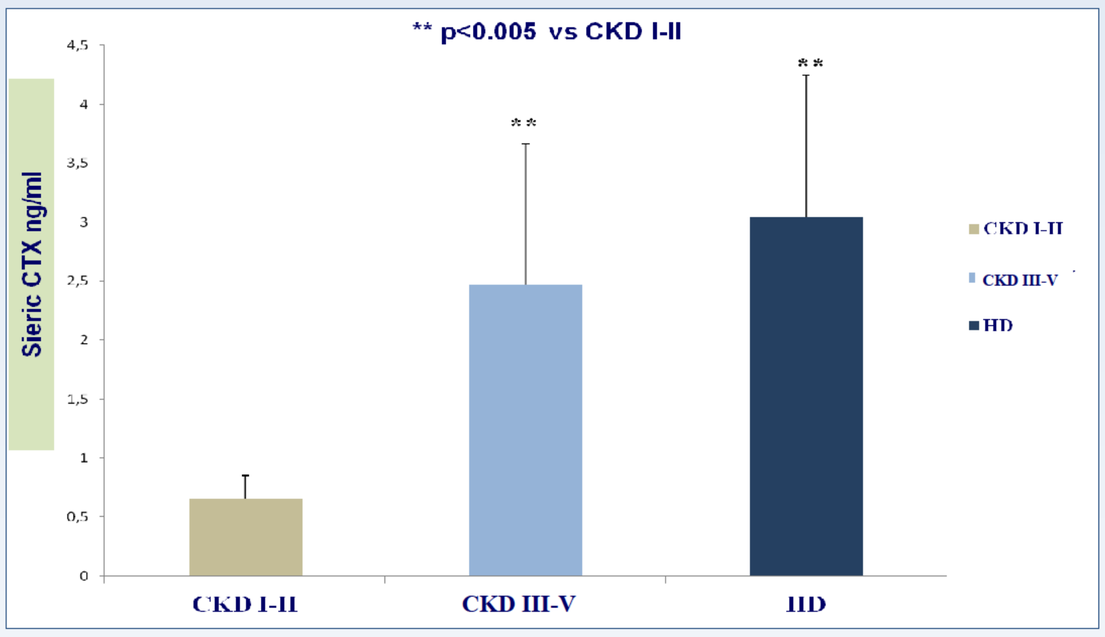

Fig4: Increase serum levels of CTX in HD and CKD pts (stage III-V) compared to control group ($p<0.005$).

Results: PBMCs from HD and CKD pts (stage III-V) showed a spontaneous osteoclastogenesis *in vitro* (80±7) when compared to CKD pts (stage I-II, control group) (10±4, $p<0.001$). Conversely, exogenous cytokines were essential to trigger and sustain osteoclastogenesis in CKD pts (stage I-II) **(Fig1).** The spontaneous osteoclastogenesis correlated with a significant increase of both LIGHT and RANKL expression on lymphomonocytes (**Fig 2)** as well as $CD14^+CD16^+$ monocytes **(Fig3)** in HD and CKD pts (stage III-V) compared to controls ($p<0.005$). The increase of these inflammatory cytokines and $CD14^+CD16^+$ monocytes correlates with the increase of *CTX* serum levels in HD and CKD pts (stage III-V) when compared to controls ($p<0.005$) **(Fig 4).**

Conclusion: uremic PBMC show an increased osteoclastogenic potential that may play a role in the mineral bone disorders observed in CKD and HD patients. Modulation of LIGHT and RANKL expression may represents a new potential therapeutic target in the setting of these patients.

References

1)Mori G, D'Amelio P, Faccio R, et al. *The Interplay between the Bone and the Immune System.* Clin Dev Immunol. 2013;2013:720504.
2) Hemingway F, Kashima TG, Knowles HJ, et al. *Investigation of osteoclastogenic signalling of the RANKL substitute LIGHT*. Exp Mol Pathol. 2013 94(2):380-5

DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014