Impact of chronic kidney disease on the platelet phenotype and the plasma proteomic profile of CAD patients

P. Canzano[1], M. Brambilla[1], M. Brioschi[1], L. Piacentini[1], L. Rossetti[1], G.C. Marenzi[1], C. Banfi[1], G. Teruzzi[1], E. Bono[1], D. Trabattoni[1], G.I. Colombo[1], M. De Metrio[1], A. Bartorelli[1], E. Tremoli[1,2], M. Camera[1,2]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy
[2]Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Italy

BACKGROUND– Chronic kidney disease (CKD) is the most important independent predictor of adverse prognosis of coronary artery disease (CAD): CKD patients are at high risk of cardiovascular and cerebrovascular diseases and they are more likely to die of CAD than to develop terminal renal failure.
Blood platelet activation and platelet interaction with circulating cells increase the risk of thrombosis and, consequently, of cardiovascular events in CKD patients.
Some years ago, we showed that patients with non-ST elevation myocardial infarction (NSTEMI) have a higher number of TF-positive platelets (pTF) and platelet-monocyte aggregates than stable CAD patients or healthy subjects, providing additional insight into the prothrombotic potential of CAD platelet. We also showed that alterations in the platelet transcriptome may account for the platelet phenotype in NSTEMI patients. Moreover, data from the literature indicate the presence of differentially expressed proteins, involved in the pathophysiology of atherothombotic disease, in plasma from NSTEMI compared to stable CAD.
AIMS– Based on the key role played by platelets in the pathogenesis and progression of CAD and on the pivotal role of pTF in the thrombotic complications, the aims of this study were to compare pTF expression, platelet-specific gene expression pattern and plasma proteome profile of NSTEMI and stable angina (SA) patients with or without CKD; in fact, there are no studies focused on the pTF contribution to the higher thrombogenicity in CAD patients with CKD, neither studies on changes that may occur in platelet transcriptome, neither on plasma protein changes that occur in these patients.
METHODS- 58 NSTEMI patients with CKD and 164 without CKD, and 34 SA patients with CKD and 161 without CKD were enrolled. pTF was evaluated by flow cytometry, platelet transcriptome by Illumina BeadChips and plasma protein changes by 2-dimensional electrophoresis (2-DE) and confirmed by ELISA analysis.
RESULTS- The percentage of pTF was significantly lower in NSTEMI patients with CKD compared to subjects without CKD (3.65±1.17 and 4.69±0.8, respectively). A similar trend, but not statistically significant, was observed in SA patients with CKD compared to those without CKD. In order to clarify if the different percentage of pTF in the 2 groups of patients is the result of a global lower number of pTF in patients with CKD or it is due to a different platelet activation state, intracytoplasmic staining for this antigen was performed: patients with CKD have a significant lower intracytoplasmic TF expression compared to patients without CKD (19.49±4.04 and 27.53±5.16, respectively).
Differential expression analysis of platelet transcriptome of NSTEMI patients showed that 28 genes were at least 1.5-fold increased and 16 genes decreased in patients with CKD compared to those without CKD ($p<0.01$). The expression levels of 68 genes showed a positive correlation, whereas those of 19 genes a negative correlation ($p<0.01$) with the severity of CKD.
Comparison of 2-DE maps revealed significant differences among the 2 groups in the expression of 8 plasma proteins; in particular, down-regulation of fetuin-A, a plasma protein important for the regulation of calcium, and up-regulation of alpha-1-microglobulin (A1M) and retinol binding protein 4 (RBP4), 2 proteins used as indicator for some renal disease and metabolic imbalance, were observed in CKD NSTEMI patients. Up-regulation of A1M and RBP4 in these patients was confirmed by ELISA analysis.
CONCLUSIONS- The lower amount of pTF found in NSTEMI patients with CKD may indicate an ongoing platelet activation with TF consumption. Gene expression profiling showed that at least 2 molecular pathways are affected by CKD: extracellular matrix-receptor interaction are up-regulated, and translational elongation genes are down-regulated. Finally, plasma proteins, identified by 2-DE, may have relevance as candidate biomarkers of clinical prognosis.