Platelets in the Uremic Milieu – The link between protein carbamylation and platelet dysfunctions in end stage renal disease

An estimated 850 million people worldwide is affected by chronic kidney disease (CKD) and survival of around 10 million depends on dialysis due to kidney failure. These patients undergo dialysis, often for several years, that leads to a wide range of complications and a dramatically reduce quality of life. Despite continuous improvement, dialysis can only replace circa 10% of physiological kidney function, leaving patients with a chronic overload of toxic metabolites. Uremic bleeding has been a well recognised complication of renal insufficiency with up to 50% of CKD patients affected by hemostatic dysfunctions leading to potentially life-threatening conditions such as gastrointestinal and intracranial bleeding.

Uremia results from the retention of waste products that, under physiological conditions, are cleared by the kidneys. Accumulated, these compounds exert various devastating effects on almost all organ systems of the body. Urea is quantitatively the most abundant retained solute in the body, where it is in equilibrium with the reactive decomposition product cyanate that can react with lysine residues and N-terminal amino groups of proteins and peptides, resulting in the formation of carbamyllysine (homocitrulline; HCit). This irreversible modification can occur at multiple sites within a single protein, altering protein charge, structure, and function. Cyanate concentrations are around 3 fold higher in end stage renal disease (ESRD), patients than in healthy individuals, resulting in excessive carbamylation. Carbamylated proteins in ESRD became important as biomarkers due to their strong association with cardiovascular and overall mortality.

Although carbamylation of plasma proteins was extensively studied and received much attention in the past due to the discovery of its association to mortality in hemodialysis (HD) patients, surprisingly little is known about the effects of uremia on circulating cells. To date, most of the studies investigating carbamylation-induced alterations in cell function were performed using erythrocytes and renal cells, while other cell types remained overlooked. Platelets primary role in circulation is to help maintain primary hemostasis and blood flow. In order to accomplish this goal platelets are equipped with specific receptors including the GpIb/V/IX complex binding to von Willebrand Factor (vWF) as well as the fibrinogen receptor GpIIb/IIIa playing a pivotal role in aggregation and adhesion. Based on our preliminary results we hypothesise that platelet exposure to circulating urea-derived cyanate leads to carbamylation-triggered structural alterations of GpIIb/IIIa, the major platelet membrane protein, whose interactions with vWF and fibrinogen are of critical importance for normal platelet adhesion and aggregation. Within the project we will also investigate enzymatic processing of vWF, to characterise the consequences of carbamylation for binding of vWF to collagen and the platelet receptors GPIb and GPIIb/IIIa. Finally, we plan to study how cyanate exposure modulates platelet activation, shape change and production of the anti-coagulant nitric oxide (NO) that is central in generating an antithrombotic environment by inhibiting platelet adhesion to the intact vessel wall. Carbamylation of platelet receptors may therefore represent a mechanistic link between uremia and the defective primary hemostasis described in HD patients and determining the exact mechanisms of that process is crucial for better management of bleeding episodes.

Therefore, the overall objectives of the project are as follows:

- Characterise the carbamylation pattern of GpIIb/IIIa and other platelet receptors *in vitro* and in clinical samples from CKD patients and determine this projects on their functional integrity.
- Characterise the carbamylation pattern of vWF *in vitro* and in clinical samples from CKD patients and impact of that modification on the proteolytic cleavage of modified vWF by ADAMTS13 as well as vWF multimer distribution in patient plasma
- Examine the impact of vWF carbamylation on interactions with collagen, factor VIII and platelets *in vitro* and establish correlation between vWF carbamylation and vWF-protein and vWF-cell interactions in CKD
- Investigate the protective effect of free amino acids on carbamylation induced hemostasis imbalance as the basis of the future preventive therapy

The ultimate goal of the proposed project is the identification of platelet carbamylation as a new biomarker associated with uremic bleeding in chronic kidney disease and unraveling of underlying molecular mechanisms. That in turn will pave the way for the design of novel treatment strategies targeted at improving primary hemostasis in chronic kidney disease.