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PLATELET MICROPARTICLES AND HEMOSTATIC DISTURBANCES IN RENAL INSUFFICIENCY AND CARDIOVASCULAR DISEASE

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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“We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is a proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium a benefit for humanity.”

Marie Skłodowska-Curie 1867-1934, Nobel Prize in Physics 1903, Nobel Prize in Chemistry 1911.

“When bubbles settle on the surface of the urine, they indicate disease of the kidneys, and that the complaint will be protracted.” -- **Hippocrates** 400 BC.

ABSTRACT

Background: Chronic kidney disease (CKD) affect up to 10-15% of the population worldwide and is a growing global health problem. Diabetes, hypertension and renal diseases, such as glomerulonephritis, are the major cause of CKD in high- and middle-income countries. CKD is a strong independent risk factor for cardiovascular disease (CVD) and CKD patients do worse after a cardiovascular event. This is only partially explained by traditional risk factors. Several non-traditional risk factors for CVD in CKD are becoming recognised, such as inflammation, oxidative stress, calcium-phosphate metabolism, endothelial dysfunction, malnutrition, anemia, and most recently gut microbiota, all in interplay with accumulating uremic toxins as renal function decline.

Hemostatic disturbances including platelet dysfunction are well known in CKD, which is considered a prothrombotic state. However, the paradoxical simultaneous increased risk of bleeding is less understood. Microparticles (MPs) are small sized vesicles (100-1000 nm), which are shed into the circulation from various cells including platelets and endothelial cells due to different stimuli or apoptosis. MPs carry the same surface proteins as their parent cell, and MPs are both markers of disease and can induce inter-cellular cross talk. Platelet MPs (PMPs) are the most abundant MPs in the circulation, and the major significance is their procoagulant activity. MP concentrations are known to correlate with both CKD and CVD.

Vitamin D deficiency and endothelial dysfunction is evident early in CKD and are associated with increased risk of CVD. Normal endothelial function is essential for maintained hemostasis. Vitamin D is involved in calcium-phosphate balance and upregulates production of nitric oxide, essential in endothelial function. Endothelial microparticles (EMPs) may have a role in assessing endothelial dysfunction.

The overall objective of this PhD project was to elucidate some of the complex mechanism causing CKD patients to suffer such high risk for CVD, with focus on hemostatic disturbances, and platelet and endothelial microparticles.

Methods and results:

In **study I**, hemostasis was studied using a global assay in patients with severe CKD, patients on hemodialysis, and healthy controls The results demonstrated a prothrombotic state and an impaired fibrinolysis in severe CKD, as well as a tighter fibrin network assessed with scanning electron microscopy, despite normal concentrations of endogenous fibrinolysis inhibitors.

In **study II**, platelet and endothelial microparticles were investigated in acute coronary syndrome (ACS) patients with and without CKD, using flow cytometry. PMPs and EMPs concentrations were elevated in severe CKD

Study III, a substudy of the SOLID randomized placebo-controlled trial, where paricalcitol treatment in CKD patients demonstrated beneficial effects on endothelial function and reduced proinflammatory cytokines. In our study, paricalcitol treatment reduced concentrations of intercellular adhesion molecule-1 (ICAM-1) positive MPs. Concentrations of cell-activation induced EMPs and PMPs were maintained in the paricalcitol group.

Study IV investigated if biomarkers related to hemostasis, endothelial function and vascular regulation correlated with renal function in a large cohort of ACS patients, and determined

their association with outcome in the subgroups with preserved and with reduced renal function. Biomarkers were quantified by proteomic methods. The biomarkers in ACS patients with reduced renal function indicated a prothrombotic state, with altered endothelial function and vascular regulation, and several of these were associated with outcome in this group.

Conclusions:

We confirm that CKD is associated with a prothrombotic state, and at the same time an impaired fibrinolysis potentially due to a tighter fibrin network.

We show that in ACS patients with CKD, the MP profile indicate higher platelet activation and endothelial dysfunction compared with non-CKD patients, despite concurrent dual antiplatelet and statin treatment.

We show that in CKD patients, paricalcitol treatment reduce concentrations of ICAM positive MPs, indicating a less proatherosclerotic endothelium.

We found that in a large cohort of ACS patients with reduced renal function, the biomarker profile indicates a prothrombotic state. The biomarkers associated with outcome differ between patients with CKD and non-CKD. This indicate that in the field of biomarkers, it might not be sufficient to adjust for renal function. Rather, when renal failure is established, it could be regarded as a separate disease state with its own distinct pathophysiology.

LIST OF SCIENTIFIC PAPERS

I. **Mörtberg J**, Blombäck M, Wallén H, He S, Jacobson SH, Spaak J. Increased fibrin formation and impaired fibrinolytic capacity in severe chronic kidney disease. *Blood Coagul Fibrinolysis*; 2016, 27(4):401-7.

II. **Mörtberg J**, Lundwall K, Mobarrez F, Wallén H, Jacobson SH, Spaak J. Increased concentrations of platelet- and endothelial-derived microparticles in patients with myocardial infarction and reduced renal function *BMC Nephrology*; 2019, 20:71.

III. Lundwall K, **Mörtberg J**, Mobarrez F, Jacobson SH, Jörneskog G, Spaak J. Changes in microparticle profiles by vitamin D receptor activation in chronic kidney disease – a randomized trial. *Revised manuscript, BMC Nephrology.*

IV. **Mörtberg J**, Salzinger B, Lundwall K, Edfors R, Jacobson SH, Wallén H, Jernberg T, Lindahl B, Baron T, Erlinge D, Andell P, James S, Eggers KM, Hjort M, Kahan T, Lundman P, Tornvall P, Rezeli M, Marko-Varga G, Spaak J. Prognostic importance of haemostatic, vascular and endothelial disturbances in acute coronary syndrome patients with impaired renal function. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

ACE	angiotensin-converting-enzyme inhibitor
ADP	adenosin diphosphate
AKI	acute kidney injury
AP	angina pectoris
AV- fistula/graft	arteriovenous fistula/graft
ARB	angiotensin II receptor blockers
ASA	acetylsalicylic acid
CAD	coronary artery disease
CAPD	continous ambulatory peritoneal dialysis
CD	cluster of differentiation
CKD 1-5	chronic kidney disease stage 1-5
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration formula
Cp	coagulation activation profile
CRS	cardiorenal syndrome
CVD	cardiovascular disease
DM	diabetes mellitus
eGFR	estimated glomerular filtration rate
EMP	endothelial derived microparticles
ESRD	end stage renal disease
EV	extracellular vesicles
Fibrin OD-sum	fibrin optical density sum
FMD	flow mediated vasodilation
Fp	fibrinolysis activation profile
HD	hemodialysis
HDF	hemodiafiltration
HDL	high-density lipoprotein
HF	heart failure
HRPR	high residual platelet reactivity
HT	hypertension
ICAM-1	intercellular adhesion molecule-1
LDL	low-density lipoprotein
MDRD	Modification of Diet in Renal Disease (MDRD) Study
MI	myocardial infarction

MP	microparticles
N-STEMI	non-ST elevation myocardial infarctio
NO	nitric oxide
PAR-1	protease activated receptor-1
PCI	percutaneous coronary intervention
PECAM	platelet adhesion cell adhesion molecule
PD	peritoneal dialysis
PMP	platelet derived microparticles
PSGL-1	P-selectin glycoprotein ligand-1
RAAS	renin-angiotensin-aldosterone system
RCT	randomized controlled trial
RRT	renal replacement therapy
STEMI	ST-elevation myocardial infarction
suPAR	soluble urokinase plasminogen activator receptor
TAFI	thrombin activatable fibrinolysis inhibitor
TxA2	tromboxan A2
tPA	tissue plasmin activator
uPA	urokinase plasminogen activator
VCAM	vascular cell adhesion molecule
vWf	von Willebrand factor

1 INTRODUCTION

1.1 CHRONIC KIDNEY DISEASE

In 1836 Dr Richard Bright at Guy´s Hospital, London, England, wrote *Cases and observations illustrative of renal disease* (1). Dr Bright had noticed several patients suffering from a disease he described as "mottling". This disease often came after an episode of scarlatina, or in patients with episodes of hematuria in younger age. The patient became swollen, dropsy[1] and discovered their urine was tinged with blood. The doctor noticed the urine contained albumin by heating the urine in a teaspoon over a light of fire and noticed the amount of precipitate; the albumin (2). The pulse was "full and hard" (1).

Dr Bright noticed that in several cases, as years passed, the patients' health inexorably declined. They suffered increasingly fatigue, headache, vomiting, their healthy color disappeared and the examination of the blood showed presence of urea. These symptoms and signs came and went, the swelling likewise, and after a period of time the patient's status deteriorated and they eventually fell into coma and died. As Dr Bright wrote, "the painful history of this disease is closed" (1), and he was convinced this disease was somehow related to the albuminuria.

Today we know that Dr Bright accurately described glomerulonephritis, acute or chronic, leading to chronic kidney disease (CKD) and end stage renal disease.

CKD is a globally growing health problem, affecting 10-17% of the general population. Diabetes mellitus and hypertension are the major risk factors for CKD as well as various types of "Bright's disease", i.e. glomerulonephritis (3). The prevalence has been rising for decades, but the increase seems to be slowing down since the middle of 2000. The explanation of this is complex, where an increased life expectancy contribute to an age-

[1] Dropsy: old-fashioned term for oedema. Swedish: Vattusot

related increase in prevalence, while better treatment of hypertension, diabetes and changes in life-style contribute to a decrease (4).

CKD has climbed on the list of causes of global death, from 27:th place in 1990, to 18:th place 2010, and in 2016 CKD was ranked 11:th, according to the Global burden of disease study (5). The increase is particularly seen in low- and middle-income countries.

Chronic kidney disease is one of the strongest risk factors for cardiovascular disease (CVD), and in fact, most CKD patients die from cardiovascular disease and not from end-stage renal disease (6).

1.2 CLASSIFICATION OF CHRONIC KIDNEY DISEASE

The Kidney Disease Global Outcomes (KDIGO) define CKD as reduced glomerular filtration rate and evidence of kidney damage (albuminuria) persistent for 3 months. In CKD 1-2 there has to be evidence of kidney damage to fulfil the criteria for CKD (7). Based on this there are five stages of CKD (8-10).

Table 1. CKD classification according to international guidelines.

Chronic kidney disease CKD stages			eGFR ml/min/1.73 m^2	Frequency %	
1		Normal renal function with signs of kidney damage*	>90	4.0	
2		Mildly impaired renal function*	60-89	3.2	
3a	T**	Mildly to moderately impaired renal function	45-59	6.2	4.7
3b	T**	Moderately to severely impaired renal function	30-44		1.5
4		Severely impaired renal function	15-29	0.4	
5	D**	Kidney failure (End stage renal disease)	<15	0.1	

Chronic kidney disease (CKD) defined as abnormalities of kidney structure or function, present for >3 months.
* Albuminuria present
**Add suffix T if patient is kidney transplanted, D if patient is on dialysis. Frequency % in the population
eGFR estimated glomerular filtration.

1.3 ESTIMATION OF RENAL FUNCTION

The estimated glomerular filtration rate (eGFR) can be calculated from a number of equations based on creatinine concentrations. The Cockcroft-Gault formula estimates creatinine clearance without correction for body surface area, and thus calculate the absolute value of filtration (11). The MDRD equation from The Modification of Diet in Renal Disease (MDRD) Study 1999 (12), revised 2006 provided an improved equation and related eGFR to body surface area 1.73/min/min, but was less reliable in older patients, i.e.in m^2 over 70 years of age (13). The CKD-EPI Chronic Kidney Disease Epidemiology Collaboration formula was developed and implemented after 2014 and provides more reliable eGFR estimates in all adults (14). Both the MDRD and CKD-EPI formulas include age, gender and race in the equation calculated from creatinine and there is consensus they are both superior to the Cockcroft-Gault to estimate renal function. In Sweden, the Revised Lund-Malmö GFR estimating equation is calculated from both creatinine and cystatin C and is considered superior in a Swedish population (15).

1.4 CARDIOVASCULAR DISEASE IN CHRONIC KIDNEY DISASE

Dr Bright noticed in the autopsies of several patients, a remarkable left ventricular hypertrophy, without any signs of valvular disease. He stated “The two most ready solutions (for this hypertrophy) appear to be either that the altered quality of the blood affords irregular and unwanted stimulus to the organ immediately; or that it so affects the minute and capillary circulation as to render greater action necessary to force the blood through the distant subdivisions of the vascular system”(2). Bright discovered that renal disease could cause cardiovascular disease, but he did not know the mechanisms behind it.

Today we consider already mild to moderate CKD to be an independent predictor of all-cause mortality and cardiovascular mortality in the general population (16). In the 2016 “European guidelines on cardiovascular disease prevention in clinical practice”, the risk prediction score for a 10-year risk of fatal CVD, classify CKD at an eGFR below 60 ml/min/1.73 m^2 as high-risk, and eGFR below 30 ml/min/1.73 m^2 as very high-risk, in the same risk group as patients with a previous myocardial infarction or stroke (Figure 1)(17).

The increase in premature CVD and the poor outcome after a CVD event in CKD patients is caused by a complex interplay between several pathophysiological mechanisms. Platelet

dysfunction and hemostatic disturbances both contributes to the very last step in the pathophysiology in CVD: the formation of a thrombus and is therefore of particular interest in CKD.

Figure 1. Estimated 10-year risk of future cardiovascular death based on risk factors and co-morbidities (17) *Used by permission from European Heart Journal.*

Very high-risk	Subjects with any of the following: • Documented CVD, clinical or unequivocal on imaging. Documented clinical CVD includes previous AMI, ACS, coronary revascularization and other arterial revascularization procedures, stroke and TIA, aortic aneurysm and PAD. Unequivocally documented CVD on imaging includes significant plaque on coronary angiography or carotid ultrasound. It does NOT include some increase in continuous imaging parameters such as intima–media thickness of the carotid artery. • DM with target organ damage such as proteinuria or with a major risk factor such as smoking or marked hypercholesterolaemia or marked hypertension. • Severe CKD (GFR <30 mL/min/1.73 m2). • A calculated SCORE ≥10%.
High-risk	Subjects with: • Markedly elevated single risk factors, in particular cholesterol >8 mmol/L (>310 mg/dL) (e.g. in familial hypercholesterolaemia) or BP ≥180/110 mmHg. • Most other people with DM (with the exception of young people with type 1 DM and without major risk factors that may be at low or moderate risk). • Moderate CKD (GFR 30–59 mL/min/1.73 m^2). • A calculated SCORE ≥5% and <10%.
Moderate-risk	SCORE is ≥1% and <5% at 10 years. Many middle-aged subjects belong to this category.
Low-risk	SCORE <1%.

1.4.1 Traditional and non-traditional risk factors

This greatly increased risk of CVD in CKD patients is only partially explained by high prevalence of the traditional Framingham risk factors such as smoking, dyslipidemia, hypertension, advanced age, diabetes and obesity (18). Patients with CKD have the traditional risk factors for CVD to a great extent, partly because the impaired renal function itself can be a manifestation of the atherosclerotic burden. However, non-traditional risk factors such as endothelial dysfunction, oxidative stress, inflammation, malnutrition, anemia, hyperhomocysteinemia, abnormal calcium-phosphate metabolism and hemostatic

disturbances, all have been shown to contribute to the increased risk of CVD in patients with CKD (19). These metabolic and inflammatory alterations, contributing to the accelerated vascular disease are induced by, or amplified by, retention of a range of organic compounds termed uremic toxins, which normally are metabolized or excreted by the kidney (20). Thus, the traditional risk factors are of relatively less importance in more advanced renal failure (19).

1.4.2 Uremic toxins

Impaired renal function leads to retention of a range of organic products, uremic toxins, e.g. tryptophan-derived uremic toxins (TDUT) such as indoxyl sulfate, indole-3-acetic acid (IAA), kynurenine (KYN), anthranilic acid (AA), and p-cresyl sulfate, trimethylamine-N-oxide (TMAO), all normally metabolized or excreted by the kidney. Over 90 different compounds have been identified, and they are often classified according to their solubility and size: small molecules with a molecular weight (MW) below 500 Dalton, middle molecular weight compounds (MW between 500 and 12,000 Dalton) and high molecular weight compounds (> 12,000 Dalton). Smaller, water-soluble compounds are easily removed by highly permeable hemodialysis filters while protein-bound molecules are difficult to remove (20).

Another way to classify uremic toxins is based on their origin. Uremic toxins can be produced by endogenous metabolism, which is the majority of the uremic toxins, or by microbial metabolism from protein degradation, such as phenolic and indolic short-chain fatty acids, ammonia, amines, and thiols, which all are potentially toxic, or they can be ingested, e.g. oxalate or advanced glycation end-products (20). Uremic toxins contribute in various ways to both progression of CKD and to the pathophysiology of CVD by proinflammatory, profibrotic mechanisms, such as in vitro demonstrated inhibition of NO production, stimulation of vascular smooth muscle cell proliferation in the vessel wall, hereby, all of this contributing to development of atherosclerosis (21, 22).

1.4.3 Gut microbiota, a putative risk factor for CVD

Gut microbiota is becoming recognized as a risk factor for CVD and atherosclerosis (23, 24). In CKD patients, uremic toxins accumulate and affect the gut microbiota composition and metabolism, which further contribute to production of more uremic toxins, as previously discussed. Several of these toxins are associated with altered hemostasis, atherosclerosis and increased platelet reactivity (25, 26). These toxins impair endothelial function (27), and may cause vascular and renal toxicity (22), and are associated with outcome in CKD patients (21, 28). Heianza et al concluded in a meta-analysis from 2017 in Journal of American Heart Association that metabolites from gut microbiota in humans were independently associated with risk of CVD and all-cause mortality (29). It was also demonstrated that gut microbiota composition in humans related to blood pressure (30). Another study in humans published in Nature 2013, showed that obese persons had less diversity of bacteria composition compared to non-obese individuals, and an inflammatory phenotype (31). Gut microbiota also affects the immune system by influencing the differentiation of T-cell-subsets, and a "healthy" microbiota may shift these towards less pathogenic circulating T cells (Foxp3+ Tregs), suppressing immune processes involved in atherosclerosis (32). Mice models raised in a sterile environment demonstrate unaffected initial platelet adhesion, but significant less platelet aggregation, reduced thrombosis growth, and lower concentration of von Willebrand factor (vWf) compared with mice whit normal gut flora (33). A novel study from 2018 demonstrated that transplantation of gut microbiota from humans to mice, can alter the generation of specific uremic toxins, depending on the gut microbiota composite transplanted, altering platelet in vitro aggregation and reactivity, as well as in vitro thrombus formation (34). Recent data also supports that Vitamin D can affect the gut microbiota both in mice and human (35), and also that loss of muscle mass, and the concept of physical frailty in elderly persons might have a relation to the gut microbiota composition (36).

In short, gut microbiota is altered in CKD patients (25, 26), it seems to have a role in early mechanisms in atherosclerosis and CVD (22, 27, 29), it is involved in immune system modulations (34), it seems to affect hemostasis, and transplantation of gut flora has the ability to alter hemostasis (34). It is tempting to speculate that gut flora might contribute to CKD patients increased risk of CVD, but this is far beyond the scope of this thesis.

1.5 THE CARDIORENAL SYNDROME

Kidney disease and cardiovascular disease, both acute and chronic, interact in a complex manner. Atherosclerosis can cause both renal impairment and ischemic heart disease. Impaired heart function may cause decreased kidney perfusion (37).

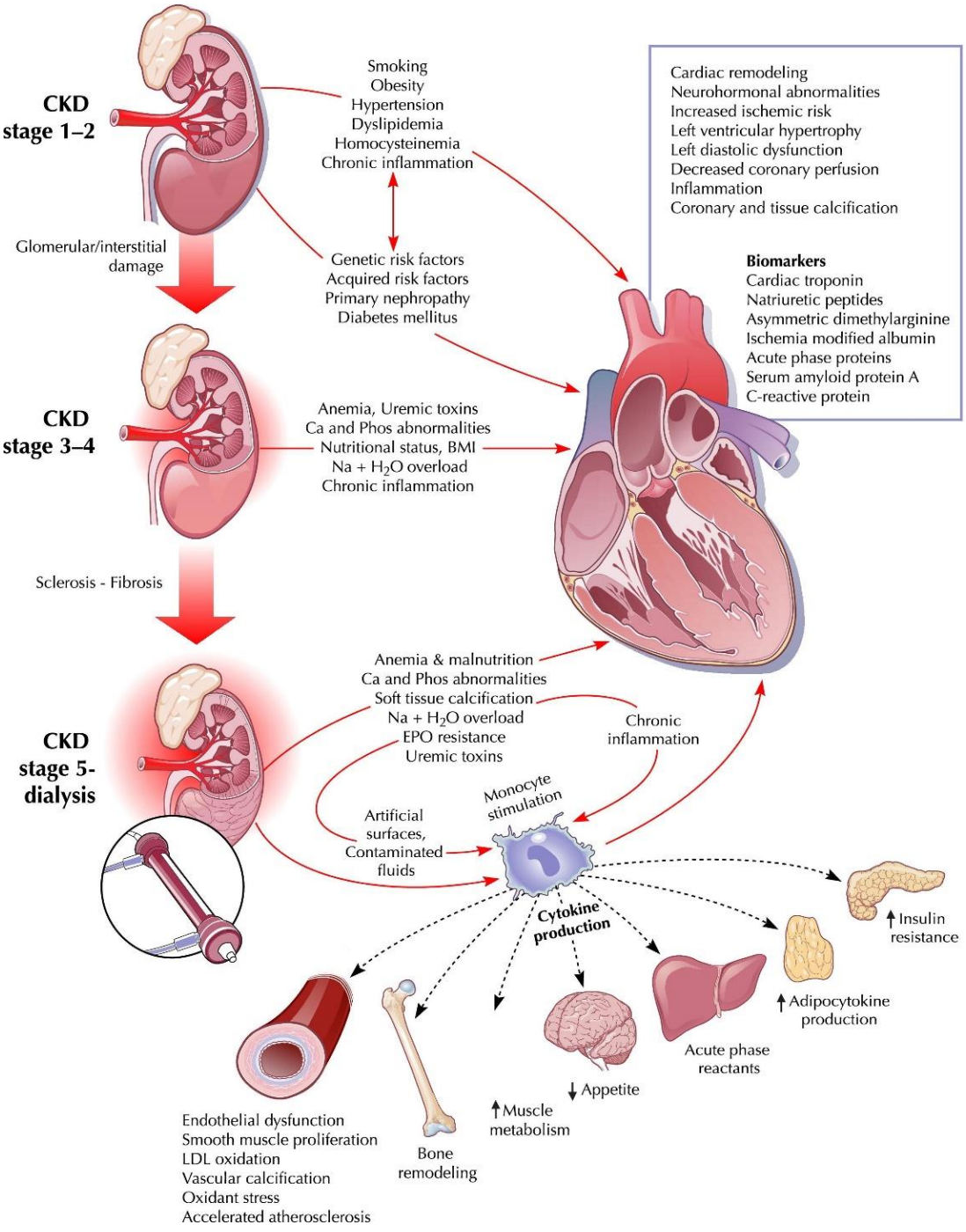

Figure 2. The type 4 cardiorenal syndrome, “chronic renocardiac syndrome”.
Reprinted from Cardiorenal syndrome by Claudio Ronco et al. 2008 (37). Journal of the American College of Cardiology, with permission from Elsevier.

Decreasing renal function accelerates atherosclerosis by several mechanisms, and in severe renal failure, the accumulation of uremic toxins and volume overload more directly impair heart function (38).

The cross-talk between the heart and the kidneys has such a dignity that it can really be considered a clinical syndrome. In 2008, Dr Claudio Ronco published one of the first comprehensive classification of the cardio renal syndrome (CRS) (37).

The syndrome embraces several of the disturbances listed above and both traditional and non-traditional risk factors. These all contribute to premature vascular ageing and early CVD in these patients. The initial classification has recently been more clearly defined and used to stratify outcomes in clinical trials (38). An integrated view of this syndrome will guide clinicians in designing future clinical trials, and hopefully towards multidisciplinary teamwork of cardiologists, nephrologists and nurses, to provide better care in this patient group (39).

Table 2. Cardiorenal syndrome, classification, modified from American Heart Association (38).

Classification	**Description**	**Description**	**Clinical example**
Type 1 CRS	Acute CRS	HR resulting in AKI	ACE, acute HF
Type 2 CRS	Chronic CRS	Chronic HF resulting in CKD	Chronic HF
Type 3 CRS	Acute renocardiac syndrome	AKI resulting in acute HF	HF in the setting of AKI from volume overload
Type 4 CRS	Chronic renocardiac syndrome	CKD resulting in chronic HF	CKD associated HF and cardiomyopathy
Type 5 CRS	Secondary CRS	Systemic disease resulting in HF and kidney failure	Liver cirrosis, amyloidosis, sepsis

1.6 HEMODIALYSIS TREATMENT

The Scottish chemist Thomas Graham already in 1850 made the first scientific contribution to dialysis by his discovery of diffusion and osmosis (40, 41). In the early 20th century, there were attempts to perform hemodialysis on humans without success on survival. It took until 1945, when Dr Willem Kolff from the Netherlands managed to treat a patient with acute renal failure to full recovery (42, 43). The hemodialysis was further developed and refined in the 40´s and 50´s and Dr Nils Alwall Sweden is generally recognized as the inventor of the first practical dialysis machine (44).

Currently over 2 million people in the world, mostly in developed countries, receive renal replacement treatment (RRT), as dialysis or kidney transplant, to stay alive (45).

Dialysis treatment is performed either as peritoneal dialysis or as hemodialysis. In peritoneal dialysis, the dialysis fluid is introduced and maintained in the abdomen through a permanent catheter. Uremic toxins and excessive fluid diffuse from the blood, over the peritoneal membrane into the dialysis fluid, which is exchanged 3-4 times daily by the patient. In hemodialysis, the blood passes through an external dialyzer with a semi-permeable membrane where toxins and fluid can be removed. Each session last 3-4 hours and is repeated preferably at least 4 times weekly, at home during either the day or night, if the patients have the ability to learn the procedure, otherwise as in-centre treatment. The optimal hemodialysis regimen is an ongoing debate: frequent shorter five times weekly, or daily dialysis, or overnight longer dialysis sessions. However, it clearly seems that the still commonly prescribed regimen of three times weekly is the least favorable (46).

Maintenance hemodialysis treatment is challenging from several aspects; the vascular access, the bio incompatibility of the materials in the dialyzers, endotoxins in dialysis fluid, micro-air embolism, ultrafiltration rate, length of each dialysis session, inter dialytic weight gain and many more (46). Adding a convective component by using hemodiafiltration to mimic the filtration in the glomeruli for better removal of so called “middle sized molecules” is suggested to prolong survival on hemodialysis, but data is still incongruent (46, 47). Several factors not directly related to the dialysis procedure, such as team-based care with a person-centered approach, patient education, access to physiotherapy, dieticians, social workers and other support services are also of great importance for the patients to endure this demanding life-supporting chronic treatment (48).

Hemodialysis patients are in a constant pro-inflammatory state, due to the past chronic renal failure exposure, the inability of the dialysis to completely replace a normal kidney, and to

the dialysis procedure per se(49) . This contributes to the concept of protein-energy wasting (PEW) state, described as a progressive loss of muscle mass and energy reserves. PEW in hemodialysis patients is associated with a further increased risk of CVD (50). Hemodialysis treatment is also closely associated with both bleeding, anemia and with arterial, venous and vascular access thrombosis (51).

1.7 ENDOTHELIAL DYSFUNCTION AND VASCULAR CALCIFICATION

The endothelium consists of a single cell layer of endothelial cells that line the inside of the blood vessels and is a vital organ system that closely and intricately interacts with platelets, the coagulation and immune systems. It consists of 700 gram tissue with a surface-size of a soccer field (6000 m^2) (52). The healthy endothelium expresses a balanced production of substances involved in vascular tone, platelet activation, coagulation and immune responses. Nitric oxide (NO) is an important vasoactive substance produced by endothelial cells, essential for maintaining vascular homeostasis (53). The endothelium produces multiple substances, platelet adhesion molecules such as P-selectin, leucocyte recruitment molecules E-selectin, intercellular adhesion molecule (ICAM), vascular cell adhesion molecule 1 (VCAM), vWf, profibrinolytic factors such as tissue plasminogen activator (tPA), urokinase plasminogen activator inhibitor (uPAR),thrombomodulin and fibrinolytic inhibitors such as plasminogen activator inhibitor 1,(PAI-1) (53-55).

The endothelial dysfunction in the early atherosclerosis process is characterized by impaired endothelium mediated vasodilatation, and a shift towards “activated” endothelial cells expressing substances and surface receptors that interact and activate inflammatory cells, causing chronic vasoconstriction and a procoagulant state (56, 57). As endothelial dysfunction and atherosclerosis advance, this result in a functional stiffening of the arteries which contributes both to accelerated CVD and to the progression of renal dysfunction (56).

Vascular calcification affects the intima or media in the vessel wall in two separate and often parallel processes: atherosclerosis with plaque development, and arteriosclerosis with vascular stiffening and calcifications (58). The atherosclerotic process with the inflammatory plaque in its more advanced stages also causes vascular calcification.

Atherosclerosis starts early, often already in adolescence (59), but is accelerated by hypercholesterolemia, hypertension, smoking, diabetes, i.e. all the established Framingham

risk factors (60). In more advanced CKD, the dominant form of vascular calcification is the media sclerosis, also known as Mönckebergs sclerosis (58). The calcification involves primarily the intima media layers of the vessels and is related to the duration of dialysis, presence of hyperphosphatemia and the calcium-phosphate balance. Media sclerosis is no longer considered a passive biochemical process, rather it involves smooth muscle cells, whom under influence of the uremic milieu transform to express osteoblasts markers, and they to some extent become bone cells (58).

1.8 VITAMIN D AND CHRONIC KIDNEY DISEASE

Vitamin D deficiency is common in CKD patients due to loss of the available enzyme 1,25-hydroxylase, which activates 25-OH-vitamin D to 1,25-dihydroxyvitamin D. Lack of active vitamin D causes hypocalcemia, by decreased absorption of calcium from the gut, and decreased reabsorption of calcium filtered in the kidney. Low calcium concentrations stimulate release of parathyroid hormone (PTH), and vitamin D is widely used in CKD to treat secondary hyperparathyroidism and prevent CKD mineral bone disorder (MBD) (61, 62).

Short-term treatment studies with active vitamin D have shown beneficial effects with reduction of proteinuria, reduction of inflammatory cytokines, and preserved macrovascular function assessed by flow-mediated dilatation (FMD) (63-65). FMD measure the capacity of the brachial artery to dilate, after ischemia induced by a pneumatic tourniquet. The vasodilation is measured at certain time points, and the relative change of the diameter of the artery is determined. The largest randomized controlled trials using vitamin D in CKD were the PRIMO trial (66) and the OPERA trial (67), both failed to demonstrate beneficial effects on left ventricular mass index. However, both PRIMO and OPERA studied whether vitamin D treatment could regress left ventricular mass in patients with more advanced CKD. It is plausible to argue that potential effects of vitamin D treatment or supplementation would be of more benefit earlier in the disease development, and be related to endothelial and vascular function, not myocardial structure.

In summary, there are indications of beneficial effects of Vitamin D treatment on endothelial function, but there is lack of large RCTs to demonstrate outcome as hard endpoints such as progression of kidney disease or mortality. Studies on the effects of vitamin D treatment in CKD on microparticles have not been previously been performed.

1.9 PLATELET FUNCTION

Platelet activation is considered to be the initial step in hemostasis and can be induced by endothelial injury exposing subendothelial collagen, fibronectin, tissue factor(TF) and vWf, or soluble platelet activators which, interact with various receptors on the platelet (68). When platelets are activated they release platelet activating substances from dense and alpha granules including ADP, serotonin (69) and P-selectin, CD40ligand (CD40L) (70) and they also synthesize and release thromboxane A_2, a potent vasoconstrictor and platelet activating compound (70). This leads to modifications of the receptor GPIIb/IIIa, towards a high affinity state stimulating platelet aggregation. GPIIb/IIIa is regarded as the most important receptor for platelet aggregation (68). The platelets also change shape from a discoid form to a form with pseudopodias leading to increased membrane surface that adhere to the endothelium. Thromboxane A_2 mediates an important enforcement of platelet aggregation by binding to the receptor on the surface on the platelets (71) . The action of thromboxane is limited due to the short half-life (68). There are multiple signaling pathways all reinforcing platelet aggregation by positive feedback mechanisms. All of this together with a simultaneous release of microparticles expressing phosphatidylserine, possibly also carrying TF, trigger the blood coagulation process (68). GP Ib-IX-V has high affinity for thrombin (72), linking the first wave of hemostasis, which is the phase were platelets adhere and aggregate, with the second wave of hemostasis where the coagulation cascade is activated. TF further triggers platelet activation by forming complexes with active FVII, causing activation of FX and ultimately generating thrombin, which is a very potent platelet agonist, generated locally on the surface of activated platelets. Thrombin-induced platelet activation is elicited through the protease-activated receptor-1 (PAR-1) as described below.

ADP, released by activated platelets, interacts with $P2Y_{12}$ receptors on surrounding platelets leading to further platelet aggregation (68). P-selectin, an adhesion molecule expressed on a variety of cells but mainly activated platelets and endothelial cells, is involved in cellular interactions causing proinflammatory and prothrombotic effects (73). The main receptor for P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1), expressed on leucocytes and endothelial cells. P-selectin is shedded from the cell surface and soluble P-selectin is considered a marker for platelet activation (70).

The CD40-CD40L system connects the platelet, the coagulation and the immune systems(74). CD40L is a transmembrane protein, expressed mainly on activated platelets, and platelets are also the main source of the soluble form sCD40L. The receptor CD40 is found

on a variety of cells, platelets, immune cells such as T cells, neutrophils, and endothelial cells. In platelets CD40L mediate the stability of the thrombus(70). Interaction of CD40L with the receptor CD40 expressed on endothelial cells, both resting and activated, promote expression of adhesion molecules such as E-selectin, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)(75).

The protease-activated receptors are a class of receptors, where the PAR-1 is most important type of PAR receptor on human platelets (76). Notably, PAR-1 is a thrombin specific receptor that can activate platelets at lower concentrations than required to fully activate the coagulation cascade. Platelet PAR-1 activation results in platelet activation with release of other platelet activating factors like thromboxane A_2 and ADP (76).

There is new and interesting knowledge on the role of micro RNAs in platelet signaling. Platelets do not have a nucleus with DNA, but still carry genetic information as microRNA from their parent megakaryocyte. MicroRNAs are small non-coding strands of RNA that can regulate gene expression when transferred to other cells. Hundreds of microRNAs are identified in platelets and their role in CVD and as biomarkers for platelet function are being investigated (77).

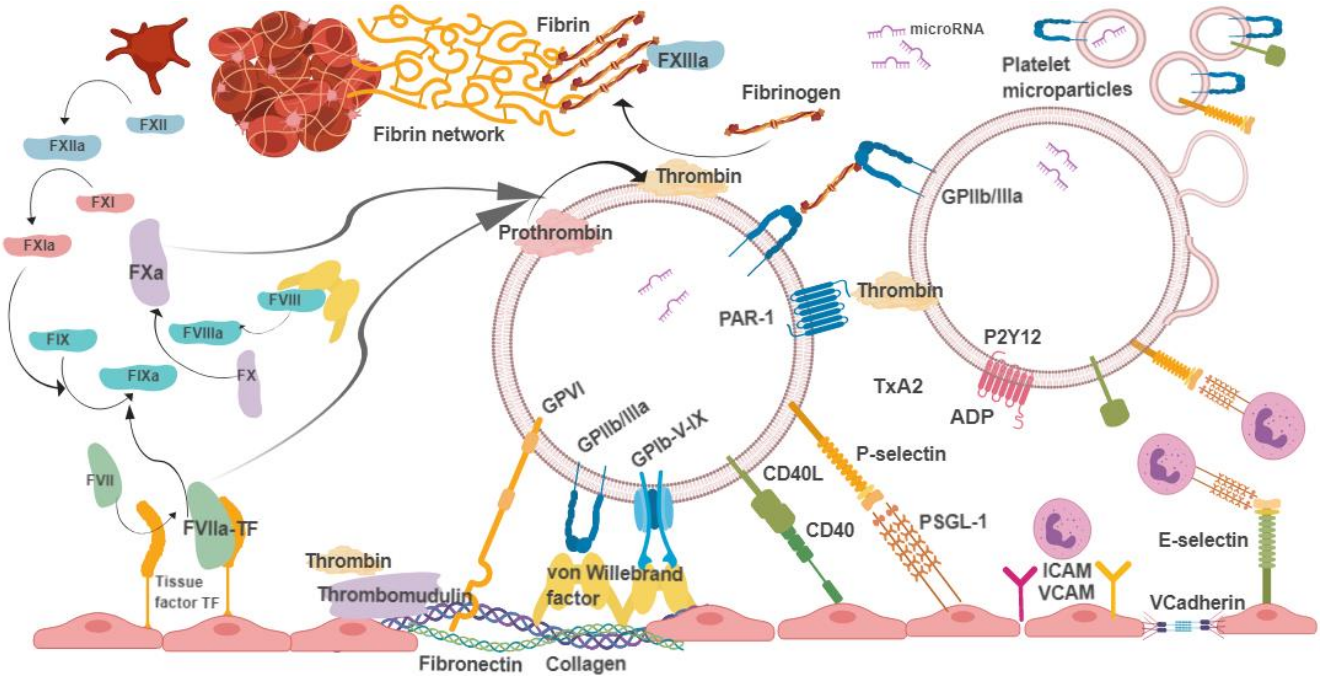

Figure 3. Overview of receptors involved in platelet function. Large circles are platelets. Microparticles budding from the platelet. Josefin Mörtberg/Biorender.com.

1.10 HEMOSTATIC DISTURBANCES IN CKD

Hemostasis is the delicate state that can stop local bleeding without clotting the entire circulation. It is a complex system with multiple parallel processes involving the vascular endothelium and subendothelium, platelets, the coagulation cascade finally generating thrombin and insoluble cross-linked fibrin, and the fibrinolytic system.

There is a range of hemostatic disturbances reported in CKD, see Table 3. Based on clinical experience and previous research, CKD is considered a prothrombotic (78) and hypofibrinolytic state (79). The paradox that CKD patients have a high risk of thrombosis while at the same time suffer an increased risk of bleeding is not well understood, but underpin the importance of a well-balanced hemostatic system.

Table 3. Hemostatic disturbance described in CKD.

Hemostasis factors grouped according to function		
Pro-coagulation	Fibrinogen (FI)	increased (80-85)
	TF (FIII, former thromboplastin)	increased (78, 80-82, 86)
	Factor VIIa	increased (78, 80, 82, 83),
	Factor X	decreased (78)
	Von Willebrand factor	increased (81-83, 86, 87)
	Factor XIIa	increased (80, 82)
	Factor VIII	increased (81, 83, 85, 88, 89)
	Factor V	no difference (83)
	APTT (activated partial thromboplastin time) (prekallikrein, FXII, FXI, FIX, FVIII, FX, FV, prothrombin, fibrinogen)	no difference (83)
	PT prothrombin time/PK/INR(international normalized ratio) (extrinsic pathway F II-prothrombin, FVII, FX)	no difference(83)
Weakens coagulation	Antithrombin activity	reduced (78, 80) no difference (87)
	AT III (antithrombin III)	no difference (83)
	TF pathway inhibitor (TFPI)	increased (81)
	Protein C	CKD 5 decreased (83)
	Active Protein C	increased (80)
	Protein S	no difference (83)
Anticoagulant properties	Soluble thrombomodulin	increased (81, 84, 86, 88, 90)

Inhibit fibrinolysis	TAFI	no difference (87)
	PAI-1, plasminogen activator inhibitor	increased (82, 84, 85) no difference (81, 86) increased in CKD + CVD (91)
	PAI-1 antigen	decreased (87)
	PAP plasmin-antiplasmin complex	increased (81)
Fibrinolysis activators	tPA	reduced (80) increased in CKD+CVD (91)
	tPA ag/ tissue plasmin activator ag	increased (84)
	plasminogen	
Markers of fibrin turnover	D-dimer	increased (80, 81, 83, 84)
Global methods		
Fibrin network structure	Fibrin network	denser (87) denser in CKD + CVD (91)
	Fibrin clot	denser in CKD + CVD (92, 93)
Global methods	Fibrinolysis potential	decreased (87)
	Euglobulin clot lysis time/ fibrinolytic activity	impaired fibrinolysis (79)
	Thromboelastography, fibrinolytic kinetics, clot lysis time	impaired fibrinolysis (94)
	Thromboelastography, coagulation kinetic analysis	increased coagulation (83, 94)
	Occlusion time	reduced (95)
	Lysis time	impaired (95)
	APTT(activated partial thromboplastin time) (prekallikrein, FXII, FXI, FIX, FVIII, FX, FV, FII-prothrombin, fibrinogen)	no difference (83)
	PT prothrombin time/PK/INR(international normalized ratio) (extrinsic pathway FII-prothrombin,FVII, FX)	no difference (83)
Markers of fibrin turnover	D-dimer	increased (80, 81, 83, 84)
	Thrombin-anti thrombin complex	increased (80, 84)
	Prothrombin fragment 1+2	increased (78)

1.10.1 Coagulation

The first step in the coagulation cascade is initiated either by the extrinsic or the intrinsic pathway (96). Activation by the extrinsic pathway is triggered when FVII encounters TF, forming a complex that further activates FIX and FX. The intrinsic or contact activation pathway is initiated when prekallikrein and high-molecular-weight kininogen binds to a surface, activating FXII (Hageman factor) to FXIIa which in turn activate FXI and further activate the cascade (96).

The intrinsic pathway is likely not as essential for hemostasis as the extrinsic system, as patients with deficiencies in the intrinsic pathway (e.g. FXII deficiency) do not have severe bleeding disorders (97). The last step in the coagulation cascade, independent of the activation pathway, is the conversion of plasma fibrinogen to an insoluble fibrin clot.

CKD patients suffer an increased risk of both arterial thrombosis as well as deep venous thrombosis and pulmonary embolism (98, 99). In hemodialysis patients the most common thrombotic event is thrombosis in their life supporting dialysis access; the arteriovenous (AV-) fistula or graft (51).

There are some similarities, but also some differences between a venous and an arterial thrombosis. Arterial thrombosis occurs in the system with high blood flow and is more dependent on direct platelet aggregation after vessel damage and subendothelial exposure. Venous thrombosis occurs in the low flow system, and are more dependent on the coagulation system. The concept of Virchow's triad: change in blood flow such as stasis or turbulence, endothelial damage or dysfunction, and a hypercoagulable state, is important in all thrombosis mechanisms (57). Venous thromboses are loosely bond to the vessel wall and contain entrapped red blood cells, which make them red or dark in color. In contrast, the arterial thrombus is mainly built up by platelets, fibrin, white blood cells, and are whiter in color and usually more firmly attached to the injured vessel wall or plaque. Interestingly, CKD patients with acute coronary syndrome (ACS) have denser fibrin clots assessed by permeation assays, and denser clot structure has been related to mortality in ESRD (91, 93).

1.10.2 Fibrinolysis

The central step in fibrinolysis is the conversion of the inactive circulating proenzyme plasminogen to plasmin. Insoluble fibrin within the hemostatic clot or thrombus is degraded by plasmin into fibrin degradation products. There are two major plasminogen activators: tissue-type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA), of which tPA is considered the most important and its activity profoundly increased in the presence of fibrin (100). The most important endogenous inhibitor of tPA and uPA is likely the plasminogen activator inhibitor (PAI-1) (80).
Thrombin activatable fibrinolysis inhibitor (TAFI) is another important inhibitor of fibrinolysis (101). TAFI is activated by thrombin in the presence of thrombomodulin, and contributes to fibrin clot stability (100). Other proteins with inhibitory effect on fibrinolysis are of importance for the stability of the fibrin clot, including alpha2-antiplasmin and complement C3 (100). Alpha-2-antiplasmin forms a complex with plasmin hereby inactivating it. In addition, FXIII crosslinks fibrin polymers to a more stable fibrin network(100).

With respect to CKD, studies have indicated a hypofibrinolytic state in patients suffering impaired renal function, which has been associated with poor cardiovascular outcome (95).

1.10.3 Bleeding in CKD

The knowledge that CKD patients have an increased risk of bleeding goes back to 1764 where Giambattista Morgagni in his book “Opera Omnia, Epistola Anatomico-Medica XLI”, Venice, Italy, described a renal patient with bleeding complications, “Sermo est de Urinae Suppressione” (102).

This bleeding tendency with prolonged bleeding time is well known also for clinicians today taking care of CKD patients. It includes both mild bleedings such as nose bleeds, prolonged bleeding after vein puncture as well as life threatening bleedings from the gastrointestinal tract and an increased risk of intracranial hemorrhage (103). The early reports in the 1950´s focused mainly on platelet dysfunction in CKD patients(104).

The platelet dysfunction observed in advanced CKD patients includes intrinsic platelet abnormalities such as defect intracellular granule content, altered platelet-vessel wall interactions and defect platelet aggregation caused by an impaired function of GPIIb/IIIa (82), as well as abnormal NO production (103). Many of these disturbances seems to be

caused by the uremic milieu itself, since some of them are reduced by dialysis (103), but despite that the bleeding tendency remains in dialysis patients (105).

It has been proposed that elevated parathyroid hormone could contribute to the bleeding tendency (106). However, a study from 2016 demonstrated that parathyroid hormone did not affect platelet aggregation, in non-CKD patients with primary hyperparathyroidism compared with control (107), indicating that this does not contribute to the bleeding tendency.

Normal endothelial function is dependent on the continuous release of NO as described above in chapter 1.7. NO inhibits platelet aggregation, and it has previously been shown that uremic plasma increase NO formation in vitro (108) which could contribute to a bleeding tendency.

Altered blood rheology due to anemia and lower hematocrit makes platelets flow in the center of the vessel, reducing platelet-vessel wall interaction, and hereby contributing to increased risk of bleeding (109). Correction of anemia with either erythrocyte stimulating agents, or blood transfusion improves symptoms and signs of prolonged bleeding (110).

When evaluating bleeding in CKD patients, there are usually no disturbances noticed in routine laboratory markers such as platelet count, prothrombin time (PT), partial thromboplastin time (APTT)(82). Test of bleeding time is usually prolonged, due to the platelet deficiency (82).

Microparticles might have a role in the increased bleeding tendency in CKD since one study has reported PMPs to correlate with bleeding in non CKD patients (111), were simultaneously the PMPs were increased. In CKD, most studies report of elevated concentrations of PMPs (Table 4) and in general PMPs are considered procoagulative (112-114), so it is difficult to interpret that finding.

The bleeding tendency in uremia is complex, multifactorial, and difficult to understand in the context of the simultaneously reports of CKD as a prothrombotic state (82, 85). Further studies are needed, and evaluation of the role of PMPs in the bleeding tendency in CKD would be of great interest.

1.11 MICROPARTICLES

1.11.1 Microparticle discovery and classification

In 1946, Dr Erwin Chargaff and Dr Randolph West reported that human platelet free plasma, created by extended centrifugation, promoted coagulation, indicating the presence of an additional clotting factor beside the known platelets (115). Twenty-one years later, in 1967, Dr Peter Wolf reported in *The nature and Significance of Platelet Products in Human Plasma*, that coagulant material originating from platelets, identified by electron microscopy, was this clotting factor. He called the material "platelet dust" (116).

Today we know it as extracellular vesicles (EV), a term describing cell-derived membranous structures, surrounded by a phospholipid bilayer, without a cell nucleus and thus without ability to replicate. There is still not a clear definition of EV, and the classification is evolving, depending on size, markers on their surface or mechanisms of their release (117). A classification that is common today is into either apoptotic bodies/vesicles, exosomes with endosome origin or microparticles/microvesicles (118). In earlier research, the most common term for all EV was "exosomes", important to know when studying the field, but today this term most often refers only to the smallest EV (119). In this Thesis the term microparticles is used.

Microparticles (MPs) are small sized vesicles (100-1000 nm), circulating in plasma under normal conditions. They are shed from various cell types such as platelets, endothelial cells, and leucocytes by different biological stimuli, shear stress, or during apoptosis. MPs carry bioactive surface proteins originating from the parent cell, and they were initially considered mainly to be biomarkers of various disease, but later studies have shown that they have important biological effects and induce various biological responses (120).
The most common method to study MPs is flow cytometry, and this technique is described in the methods part 3.2.2. Data on MPs are usually reported as counts, and monoclonal antibodies against surface proteins are used to identify their cellular origin, for instance platelet (GPIIb/IIIa; CD 41/61) or endothelial origin (platelet adhesion cell adhesion molecule [PECAM]; CD31), and activation state (e.g. P-selectin expression).

Since many surface proteins are expressed on different cell types, MP specificity is often expressed as MPs positive for one marker and negative for another. For instance, CD31+/CD41- MPs, are MPs positive for PECAM, negative for GPIIb and thus considered to be an EMP.

1.11.2 Platelet microparticles

Platelet derived MPs (PMPs) are the most abundant MPs in the circulation. They express several surface proteins and cytokines normally present on platelets, such as GPIb (CD42b), GPIX (CD42a), GPIIb/IIIa (CD41/61). The also express activation markers like P-selectin (CD62P), and CD40L (CD154), all involved in recruitment of inflammatory cells especially leukocytes and monocytes, inducing adhesiveness to endothelial cells and influencing hemostasis (120, 121).

Table 4. Studies of microparticles in CKD.

Authors	Population	Marker	Results
Mörtberg et al 2019 (122)	N=49; All ACS patients non-CKD, CKD 3, CKD 4-5	CD62P+PMP	↑ in CKD 4-5
		CD40l+PMP	↑ in all CKD
		CD62E+EMP	↑ in all CKD
Almquist et al 2016 (123)	N=39; Statin +/-Ezetimibe vs placebo All CKD 3-4; +/- DM	CD62P+PMP	Baseline: ↑ in CKD Posttreat:↓ in CKD,↓nonCKD
		CD40l+PMP	Baseline: ↑ in CKD, Posttreat: ↓ in all
		CD62E+EMP	Baseline: ↑ in CKD Posttreat:↔ CKD,↑ non-CKD
		CD144+EMP	Baseline: ↑ in CKD Posttreat: ↑ in all
Chen 2015 (124)	N=88, CKD+AP, CKD+CAD, controls	CD31+CD42b+PMP	↔ CKD
		CD31+CD42b-EMP	↔ CKD
Burton 2013(114)	N=57; HD, PD, controls	CD42b+PMP	↑ in CKD
		CD144+EMP	↑ in CKD
Trappenburg 2012 (125)	N=37; CKD 4, PD, HD vs controls	CD41+PMP	↑ in CKD
		CD62P+PMP	↑ in CKD 4, HD,↔PD
		CD144+EMP	↑ in CKD 4, HD, ↔PD
Amabile 2012 (126)	N=81; HD. Follow up median 50 month	CD41+CD31-PMP	predicted all cause and CVD mortality
		CD31+CD41-EMP	did not predict outcome

Dursun 2009 (127)	N=88; HD, PD, CKD 4 vs controls	CD144+EMP	↑ in all CKD
Boulanger 2007 (128)	N=34; HD correlation with shear stress in vivo	CD41+CD31+PMP	no correlation
		CD41-31+EMP	Correlation
		CD144+ EMP	Correlation
Faure 2006 (129)	N=110; HD, CKD 4-5 vs controls (before HD session)	CD41+PMP	↑ in CKD, ↔ HD
		CD144+EMP	↑ in CKD, ↑HD
		CD146+EMP	↑ in CKD,↑ in HD
Amabile 2005 (130)	N=76; HD, controls (after HD session)	CD41+CD31+PMPs	↑ in CKD not corr with FMD
		CD31+CD41-EMPs	↑ in CKD corr with FMD
		CD144+EMPs	↑ in CKD corr with FMD
Ando 2002 (131)	N=118, CKD 4-5, HD, PD, controls (no DM)	CD42+PMP	↑ in CKD

One major functional significance of PMPs is associated with their procoagulant activity; PMPs can express phospholipids (the negatively charged phosphatidylserine (PS), on the outer surface, offering a negatively charged surface that promotes coagulation and fibrin formation to a much greater extent than activated platelets (112). PMPs can also carry TF, further contributing to their procoagulant properties (113), although the origin of TF may not be from platelets but rather other cells.

The role of PS-negative MPs is not yet clear (132), but recent studies indicate that PS-negative MPs are more common than previously reported (133). PMPs seem to have a direct effect on platelet aggregation and hemostasis (134), and in vitro they have the ability to make the fibrin clot more dense (135).

Circulating PMPs are increased in numbers in various diseases such as ACS, diabetes mellitus, hypertension, asymptomatic arteriosclerotic lesion, and in CKD (118, 136). Platelet activation measured by expression of soluble P-selectin seems to be associated with the concentrations of PMPs in patients with ACS (137).

In a study of non-ST elevation myocardial infarction (N-STEMI) patients without CKD, elevated concentrations of CD61 and CD62P+PMPs were found, as compared to healthy controls (138), and another study demonstrated CD62P+PMP could predict outcome(139). However, in yet another study, concentrations of CD41+PMPs were not significantly different in patients with STEMI without CKD, in comparison to healthy individuals, and their concentrations were not associated to outcomes (140). These conflicting results

demonstrates the need for further studies to evaluate the role of PMPs as potential biomarkers in MI. High concentrations of PMPs have been found to associate with the severity of myocardial infarction, in patients without CKD (141). Patients with ongoing clopidogrel treatment and high remaining platelet reactivity (HRPR), in in-vitro tests, have higher concentrations of PMPs compared with patients without HRPR, indicating a putative role for PMPs in evaluating treatment effect (142). Patients with NSTEMI treated with aspirin or statins did not differ in concentrations of PMPs, compared with patients without treatment (138). In CKD, the studies mostly report of elevated concentrations of MP (Table 4). The reason why is not clear but the uremia per se seems to contribute, since an in vitro study demonstrated that the uremic toxins p-cresol and indoxyl sulphate could increase CD146+EMPs from endothelial cells (129).

1.11.3 Endothelial microparticles

Endothelial microparticles are shed from activated or damaged endothelial cells. Several cell surface antigens are used to identify EMPs. In vitro studies demonstrate that MPs promote monocyte recruitment and adhesion to the endothelium and have the ability to impair endothelial function by decreasing NO production and by this mechanism, alter vascular tone (143). The markers chosen to identify EMPs are important when studying their relation to diseases. There are several endothelial cell markers such as the mandatory markers CD31 (platelet endothelial cell adhesion molecule- PECAM-1), an adhesion protein involved in leucocyte migration, and CD144 (VE-cadherin) an endothelial cell specific molecule located in the intercellular junction important for vascular permeability and the extravasation of leucocytes, also involved in cell proliferation and apoptosis (143). CD31 and CD144 are considered markers of apoptosis induced EMPs, but there is still a lack of knowledge in which marker is best for identifying the different EMPs in various diseases (143).

The inducible endothelial marker E-selectin (CD62E) is upregulated by cytokines in inflammation (52). It is an adhesion protein involved in recruitment and adhesion of platelets and leucocytes to the endothelial cell. Other inducible markers of endothelial activation are vascular cell adhesion molecules (VCAM-1; CD 106) and ICAM-1 (CD 54), both involved in leukocyte recruitment in the inflammation process in atherosclerosis (52, 144).

Concentrations of CD31 and CD144 positive EMPs are known to increase in impaired vascular function assessed with flow mediated vasodilation and pulse wave velocity (143). EMPs can be considered a surrogate marker for endothelial dysfunction (126). EMP

concentrations are increased in a variety of diseases such as diabetes mellitus, heart failure, severe hypertension, ACS and CKD (130, 136), and increased concentrations are associated with worse outcome (145).

1.12 ANTIPLATELET THERAPIES

1.12.1 Acetylsalicylic acid

The enzyme cyclooxygenase (COX) is needed for the conversion of arachidonic acid into thromboxane A2 and prostaglandins such as prostacyclin (146). Acetylsalicylic acid (ASA) inhibits cyclooxygenase 1 (COX-1) irreversibly, hereby diminishing the production of these substances (147), inhibiting platelet aggregation moderately. The importance of ASA in reducing cardiovascular event as secondary prevention was established first in 1987, in a large randomized, double blind, placebo controlled trial *The Physicians' Health Study* that was terminated in advance by the Data Monitoring Board, due to the marked risk reduction for recurrent myocardial infarction, noticed in the aspirin arm vs. placebo (148). ASA treatment is presently a cornerstone in secondary prevention together with $P2Y_{12}$inhibitors in cardiovascular disease (149).

1.12.2 $P2Y_{12}$ inhibitors

Blocking of the platelet ADP receptor results in a strong inhibition of platelet aggregation (150). ADP released from the platelets granulae at activation, strongly activates platelets both by autocrine and paracrine mechanisms, through the ADP receptor (151). This class of antiplatelet agents, the $P2Y_{12}$ inhibitors, clopidogrel, ticagrelor and prasugrel, are commonly used as secondary prevention following a cardiovascular event.

Genetic variability, diabetes and probably also CKD is associated to high residual platelet reactivity, the concept where some patients do not fully respond to clopidogrel as $P2Y_{12}$ rec blockage. Impaired renal function did not explain HRPR in a European study but in a large Chinese study HRPR was associated with renal function (152, 153). Recent studies support that newer $P2Y_{12}$ ticagrelor is more potent than previously most commonly used clopidogrel (154).

1.12.3 GP IIb/IIIa receptor blockage

GP IIb/IIIa receptor blockage inhibits binding of fibrinogen to the receptor and inhibits platelet aggregation. These types of drugs are administered intravenously, and are used predominantly when performing high-risk percutaneous coronary interventions (PCIs)(155, 156). Orally administered GP IIb/IIIa receptor blockage were showed to be associated with increased risk of death but also bleeding when compared to aspirin (157, 158). In that study, the protocol was aspirin vs GP IIb/IIIa blockage, not adding it on top of aspirin.

1.12.4 PAR-1 antagonists

Locally generated thrombin acts on the protease activated receptor-1 (PAR-1), and hereby potently stimulates platelet activation. A potent antiplatelet drug class PAR-1 antagonist have also been developed (159). A large study of the PAR-1 receptor antagonist vorapaxar, added on to standard treatment as secondary prevention in stable patients with history of CVD, demonstrated a decreased risk of events, but unacceptable high severe bleeding complications, particularly intracranial hemorrhage (160, 161).

1.12.5 Dipyridamole

Dipyridamole is a phosphodiesterase inhibitor, where the main antiplatelet mechanism of action likely is through inhibiting adenosine uptake in red blood cells, platelets and endothelial cells, hereby increasing extracellular concentrations of ADP, which reduce platelet aggregation (162). Dipyridamole also increases endothelial NO signals, and stimulate release of prostaglandins from the vessel wall (162). Dipyridamole was used to achieve coronary vasodilation in the 1960: s. However, the phenomena coronary steel, in high dose treatment, in which dilated resistance vessels directed blood away from the ischemic myocardium made this treatment a failure. Since then the role has primarily been for secondary prevention after stroke, now generally replaced by $P2Y_{12}$ inhibitors added to aspirin (163). Recent studies of dipyridamole indicate other potentially beneficial anti-inflammatory and antioxidant properties, including a possible future role in preventing proteinuria and preserving endothelial function, as well as a possible improvement in survival for CKD patients (162, 164).

2 AIMS OF THE PROJECT

The overall objective of this PhD project was to elucidate some of the complex mechanisms causing CKD patients to suffer such high risk for cardiovascular events, by investing the role of hemostatic disturbances, platelet and endothelial microparticles in CKD and CVD, and evaluate if selected markers are associated with outcome in CKD patients.

The specific objectives were:

Study I

To study whether the prothrombotic state in severe CKD is characterized by an increased fibrin formation, an impaired fibrinolysis or both, and to investigate the fibrin network structure.

Study II

To investigate whether patients with ACS and impaired renal function have further elevated concentrations of platelet- and endothelial microparticles, indicating more activated platelets and endothelial dysfunction, as compared to patients with preserved renal function.

Study III

To assess if paricalcitol treatment have beneficial effects on endothelium assessed by concentrations of endothelial microparticles expressing proatherosclerotic markers (ICAM-1 and VCAM-1), as well as cell specific MPs, in a substudy of a randomized-controlled trial in CKD patients.

Study IV

To determine if concentrations of biomarkers related to hemostasis, endothelial function and vascular regulation correlate with renal function in ACS patients, and to assess if these biomarkers are of distinct prognostic importance in the subgroup of ACS patients with renal dysfunction.

3 MATHERIALS AND METHODS

3.1 STUDY DESIGN AND POPULATION

3.1.1 Study I

In the cross-sectional study I we assessed hemostasis using a global assay, in patients with CKD 4, patients on hemodialysis, and healthy controls.

Patients with CKD were recruited from the outpatient clinic and the hemodialysis unit at the Division of Nephrology, Danderyd University Hospital, Stockholm, Sweden. Exclusion criteria were ongoing treatment with vitamin K antagonists or active malignancy, and in the control group also past or present cardiovascular disease, active malignancy, chronic kidney disease, rheumatic disease, diabetes mellitus, and ongoing medication with any anticoagulants. Hypertension, treatment for hyperlipidemia and ongoing aspirin treatment were accepted. The control group was recruited from the Swedish national SPAR registry including all persons registered as residents in Sweden. The control group were selected to match age and sex with the CKD 4 and hemodialysis patients. The kidney function was determined by calculating the estimated glomerular filtration rate, eGFR, using the MDRD formula as ml/min/1.73m^2, in the following abbreviated to ml/min.

The aim was to assess hemostasis with a global method, which determine the amount of fibrin formed over time, fibrin optical density sum, and fibrin degradation, to determine if CKD was related to increased fibrin formation or degradation. Scanning electron microscopy also visualized the fibrin network structure in a randomly selected patient from each group.

The study was approved by the Regional Ethics committee North (Lokala forskningsetikkomitten Nord), Stockholm, Sweden, (Dnr 98-341).

3.1.2 Study II and IV

Study II and IV used the patient population in the TOTAL-AMI (tailoring of treatment in all-comers with acute myocardial infarction) project, and the Swedish Web-System for

Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies (SWEDEHEART) biobank, were consecutive patients with an ACS were included between 2008 and 2015. All Swedish hospitals (n=72) are participating in the SWEDHEART national registry, and over 90 % of patients that are hospitalized for ACS and 100 % of all undergoing coronary interventions are included. Three Swedish regions, Lund-Malmö, Stockholm and Uppsala have biobanks linked to the SWEDEHEART registry (165).

In study II, we used patient samples from patients included in Stockholm from start 2008 until 2015, in a descriptive study of platelet and endothelial microparticle-concentrations in patients with various renal function. Flow cytometry was used to measure microparticle-concentrations in frozen/thawed plasma. Forty-seven patients from the biobank were selected for analysis, to represent the three groups; normal renal function, moderate impaired renal function (CKD 3), and severely impaired renal function (CKD 5), but not on dialysis. All patients were on dual anti-platelet treatment with aspirin and a $P2Y_{12.}$

We hypothesized that patients with ACS and CKD have elevated concentrations of platelet- and endothelial microparticles, as compared to patients with preserved renal function, indicating more activated platelets and endothelial dysfunction.

Study IV was a prospective cohort study of the first 1 394 consecutive included patients in the biobank. We excluded patients on dialysis treatment. Proteomic methods were used to quantify 179 proteins, classified in advance by the TOTAL-AMI study group according to their main known biological function.

In this study it was prespecified to study biomarkers classified as being involved in hemostasis or endothelial and vascular regulation, see Paper IV, Table 4S (supplement).

We hypothesized that some of the selected biomarkers would be of distinct prognostic importance in the subgroup of ACS patients suffering renal dysfunction, but not in patients with preserved renal function, indicating an importance of separate disease mechanisms in renal dysfunction.

The TOTAL-AMI study was approved by the Ethics committee in Stockholm (Dnr: 2017/759-31) and conducted according to the Declaration of Helsinki. The project and the proteomic analyses were supported by the Swedish Foundation for Strategic Research (grant no. KF10-0024).

3.1.3 Study III

Study III was a substudy of the SOLID trial (166). This was a double blind randomized placebo controlled trial with CKD 3-4 patients, without diabetes mellitus. GFR was estimated using the MDRD formula. Patients were randomized to either placebo, 1 µg, or 2 µg paricalcitol daily for 12 weeks of treatment. The patients were recruited from the Department of Nephrology at Danderyd University Hospital, Stockholm, Sweden. The SOLID study showed that vitamin D treatment preserved macrovascular endothelial function assessed by FMD, and that it reduced proinflammatory cytokines (64, 167).

In the present substudy, we hypothesized that the maintained endothelial function and reduced inflammatory activation would be reflected in reduced concentrations of subtypes of microparticles, measured by flow cytometry.

All patients provided written informed consent and the study protocol was approved by the regional Ethics Committee of Stockholm, Sweden. The trial was registered on clinicaltrials.gov (SOLID study; NCT01204528).

3.2 METHODS

3.2.1 Global hemostasis

The assay Fibrin OD sum evaluates the global hemostasis by measuring the summation of fibrin optical density using a spectrophotometer. The readings from the spectrophotometer, expressed as arbitrary units, reflects the capacity of fibrin formation in a plasma sample, under the influence of both coagulation and fibrinolysis, thus independent of the endothelium and platelets. By analyzing different parts of the spectrophotometer-signal over time, the Coagulation activation profile (Cp), Fibrinolysis activation profile (Fp) and the amount of fibrin formed over time, as an indicator of overall hemostatic dynamics, termed Fibrin optical density sum (Fibrin OD-sum) can be calculated.

The procedures of the global assay are described in detail elsewhere (168). In brief, in each well of a microtitre-plate, an rt-PA solution is mixed with the plasma sample and a coagulation triggering solution is added. A spectrophotometer at the wavelength of 405 nm and 24°C, records the optical density (OD) every 30 sec for 300 min creating a fibrin-time. The curve reflects the rates of fibrin formation and degradation (fibrinolysis) over time. The

software analyses the data along the curve and identify the time to the start of fibrin, the maximum rate of formation, the time to maximum rate of fibrin formation, time to detectable fibrin degradation, the maximum rate of fibrin degradation, and the time to the maximum rate of degradation.

The parameters obtained from analysis of ΔODs were put into two formulas that define Cp and Fp:

$$C_P = (t_1)^{-1} * \frac{h_1}{(t_2 - t_1)} \qquad F_P = t_1 * \frac{|h_2|}{(t_4 - t_3)}$$

The Fibrin OD-sum was determined as the sum of all the absorbance values obtained from the 601 readings, using Microsoft Office Excel, where the "blank" value had been subtracted from each OD value.

3.2.2 Microparticles

Antecubital venous blood was sampled in test tubes containing 1/10 0.129 M sodium citrate. Samples were centrifuged within 20 min at 2 000 g for 20 min in room temperature (RT), and platelet poor plasma (PPP) collected and frozen in aliquots at -80 °C until analysis.

PPP was thawed and centrifuged again at 2 000 g for 20 min at RT. The supernatant was then centrifuged again at 13 000 g for 2 min. Twenty µl of the supernatant was incubated for 20 min in dark with lactadherin, and the antibodies.

Lactadherin was used as a probe to detect phospholipid phosphatidylserine (PS)(113).

MPs were defined as particles < 1.0 µm in size positive to lactadherin, and platelet microparticles PMPs were detected with antibodies against cytokines or proteins exposed on the platelet surface, such as integrin glycoprotein GP IIb (CD41), platelet activation markers P-selectin (CD62P) and CD40L (CD154).

Endothelial microparticles EMPs were detected by using antibodies against E-selectin (CD62E), intercellular adhesion molecule (ICAM-1, CD54) or vascular cell adhesion molecule (VCAM-1, CD106). Leucocyte derived MPs were detected with an antibody directed against the pan-leukocyte antigen CD45.

Flow cytometry counts, sorts and detect cells or particles, suspended in a liquid, by letting them pass through a flow chamber, one by one, while laser beams pass them. The size of the cells or particles is determined by detecting the light passing, in line with the laser beam: the

forward scattering. The light hits the antibodies labelled to the cells or microparticles, and the side scattering of the light is detected, each cell with specific fluorochromes enabling the flow cytometer to detect several properties of the cell simultaneously.

The study II, and III used a Beckman Coulter Gallios flow cytometer. The MP-gate was determined using Megamix beads (0.5 μm, 0.9 μm and 3.0 μm, BioCytex, Marseille, FR).

The MP counts are presented as MPs per μl plasma.

3.2.3 Proteomics

3.2.3.1 Multiple reaction monitoring assay

The targeted proteomic analysis by the Mass spectrometry method Multiple reaction monitoring (MRM) assay is an analytical technique to determine and quantify proteins. It sorts and analyses the peptides/proteins studied according to the mass-to-charge ratio and can perform parallel analysis of a large number of proteins in one sample, previously described in detail (169, 170).

A mass spectrometer works in several steps. First, the sample is vaporized and ionized by an ion source. The ions are then accelerated. The acceleration and tendency for an ion to move straight forward is proportional to its mass. The ions are deflected by electromagnets and the force needed to deflect the ion is proportional to its charge. The path the ion takes is proportional to its mass-charge ratio. The detected ion sequences are then matched to known peptide-sequences, and in turn matched to known proteins (1-3 peptides per protein). In this study, 87 proteins were quantified. A triple quadrupole mass spectrometer (TSQ Vantage) was used for the MRM assay, with two acceleration steps analyzing the mass-to-charge ratio. The Nano spray Flex Ion source, and an EASY n-LC II pump were used. The raw data were handled by software Skyline v3.5 (macCoss Lab Software, Seattle, WA, US).

3.2.3.2 Proximity extension Assay

Proseek Multiplex OLink CVD-1 chip is based on the technique Proximity Extension Assay (PEA), previously described in detail (171, 172). This technique enables analysis of multiple biomarkers simultaneously with high specificity. 92 selected proteins all related to cardiovascular disease were included in this commercially available analysis kit. Briefly, a pair of antibodies targeted to a specific biomarker, each carry a short oligonucleotide/ DNA

molecule with weak affinity to each other, PEAprobes. When the antibodies attach to the target biomarker, the DNA molecules become in proximity of each other, a DNA polymerase extend the oligonucleotides so that a new DNA sequence is formed, specific for every biomarker. The DNA sequence can be amplified and quantified by quantitative real time PCR, the numbers of PCR templates reflecting the concentration of the biomarker in the sample.

3.2.3.3 Statistical methods

The two proteomic methods used in this study quantified 179 biomarkers. A priori there were 36 proteins selected for analysis in this hypothesis-testing study, all considered either involved in prothrombotic pathways, anti-thrombotic or profibrinolytic pathways, or related to endothelial function or vascular regulation, and listed in Paper IV, Table 4S (supplement).

First, it was assessed which biomarkers that correlated with renal function. Second, the patients were stratified into two groups, eGFR $\geq$ 60 and eGFR < 60 ml/min/1.73 m^2, and associations with outcome were estimated using Cox regressions analysis.

Sample size in studies with multiple markers cannot be based on a traditional power calculation. We considered this a high-risk group for recurrent events; they were followed for a median of 3 years, so the number of patients included in the study was considered large enough to answer the research question in the pre-specified subgroups.

4 RESULTS, DISCUSSION AND METHODOLOGICAL CONSIDERATIONS

4.1 STUDY I

4.1.1 Results and discussion

In study I, we showed that the hemostasis in patients with CKD was shifted towards a pro-coagulative state, due to both an increased fibrin formation and an impaired fibrinolytic capacity, despite no change in the concentrations of the fibrinolytic inhibitors PAI-1 and TAFI. We included 49 patients in the study, 13 controls, 21 patients with CKD 4 and 15 patients on hemodialysis, see Table 5 and 6.

Table 5. Markers of hemostasis.

	Controls (C; $n=13$)		CKD 4 ($n=21$)			Haemodialysis ($n=15$)			
	Median	IQR	Median	IQR	*P* vs. control	Median	IQR	*P* vs. control	*P* vs. CKD 4
Fibrin OD-sum (a.u.)	115.1	82.4–233.8	292.9	169.4–433.6	**0.0119**	311.7	277.9–435.2	**0.0013**	0.500
F_p (a.u.)	0.58	0.44–0.71	0.47	0.29–0.66	0.288	0.24	0.18–0.55	**0.030**	0.102
C_p (a.u.)	16.84	11.40–19.56	22.83	16.88–28.72	0.164	17.06	14.12–27.17	0.164	0.164
Fibrinogen (g/l)	3.27	2.84–4.05	4.54	3.35–6.46	**0.030**	4.36	3.64–5.58	**0.016**	0.810
Albumin (g/l)	35.40	33.20–36.10	29.90	29.10–32.30	**0.001**	27.8	25.90–29.50	**0.000**	**0.007**
Von Willebrand factor (KIU/l)	1.39	1.09–1.65	1.74	1.44–2.11	0.117	2.19	1.58–2.67	**0.010**	0.401
PAI-1 antigen (μg/l)	17.7	10.9–26.9	16.5	11.0–24.0	0.613	12.5	8.6–17.6	**0.034**	0.166
TAFI-activity (% of normal)	125.0	107.9–143.6	128.9	120.2–146.9	0.477	131.8	122.2–155.8	0.188	0.758
Antithrombin (IU/ml)	0.99	0.98–1.06	1.02	0.91–1.07	0.183	0.89	0.85–1.01	0.183	0.183
CRP (mg/l)	1.40	1.30–2.10	2.40	1.50–4.00	0.177	5.30	1.1–13.60	0.177	0.177

CKD 4, chronic kidney disease stage 4; CRP, C-reactive protein; F_p, fibrinolysis activation profile; IQR, interquartile range; TAFI, thrombin-activatable fibrinolysis inhibitor. Bold font indicates significant *P* values.

The global assay Fibrin Od sum reflects the capacity of plasma to form fibrin, under the influence of both coagulation and fibrinolysis. The fibrinolysis activation profile (Fp) reflects the fibrinolytic capacity.

We found an impaired fibrinolysis (Fp) in patients with severe renal impairment. At the same time, we observed low or normal fibrinolytic inhibitors in plasma, i.e. PAI-1 and TAFI, indicating that mechanisms other that those involving PAI-1 and TAFI are involved.

The fibrin network demonstrated by SEM showed a denser network in patients with severely impaired renal function, and fibrinogen concentrations were higher in both patient groups. High fibrinogen have been shown to be associated with dense fibrin clots (91). The

architecture of the fibrin network and fibrin clot is of importance for the ability to degrade the fibrin (173), partly because the involved enzyme plasmin is quite large and needs to enter the clot to lyse it. Thus, the denser fibrin network per se hinder plasmin and this might be a partial explanation to the impaired fibrinolysis observed.

It has previously been shown that impaired fibrinolysis assessed by this global method is associated with worse outcomes in young patients with myocardial infarction (174), and it has been shown in CKD patients that other global methods (Global Thrombosis Test, and Fibrin Clot structure) can predict major adverse cardiovascular events (93, 95).

Patient characteristics in this study are demonstrated in Table 6. Patients were well matched regarding age and gender.

Table 6. Study population characteristics.

	Controls (C)	CKD 4	Haemodialysis
Number of individuals	13	21	15
Mean age (years)	69	65	69
Female/male	7/6	9/12	7/8
Mean eGFR ml/min per 1.73 m^2 (range)	(n.a.)	17 (6–44)	–
Mean duration of haemodialysis (month)	–	–	15 (1–48)
Diabetes mellitus	0%	38%	13%
Hypertension	8%	90%	80%
Previous CABG-surgery	0%	10%	7%
Previous myocardial infarction	0%	24%	33%
Angina pectoris	0%	33%	13%
Previous venous thromboembolism	0%	0%	13%
Hyperlipidemia	8%	62%	47%
Peripheral artery disease	8%	14%	13%
Previous stroke/TIA	0%	14%	13%
ASA/clopidogrel	15%	38%	20%
Statins	8%	52%	13%
ACE/ARB	8%	29%	33%
ESA (EPO)	0%	38%	100%
Kidney disease aetiology			
Atherosclerotic kidney disease	0%	19%	13%
Polycystic kidney disease	0%	10%	7%
Others	0%	33%	27%
Diabetes mellitus	0%	38%	13%
Glomerulonephritis	0%	0%	40%

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; CABG, coronary artery bypass graft; CKD 4, chronic kidney disease stage 4; eGFR, estimated glomerular filtration rate; EPO, erythropoetin; ESA, erythropoiesis-stimulating agent; TIA, transient ischaemic attack.

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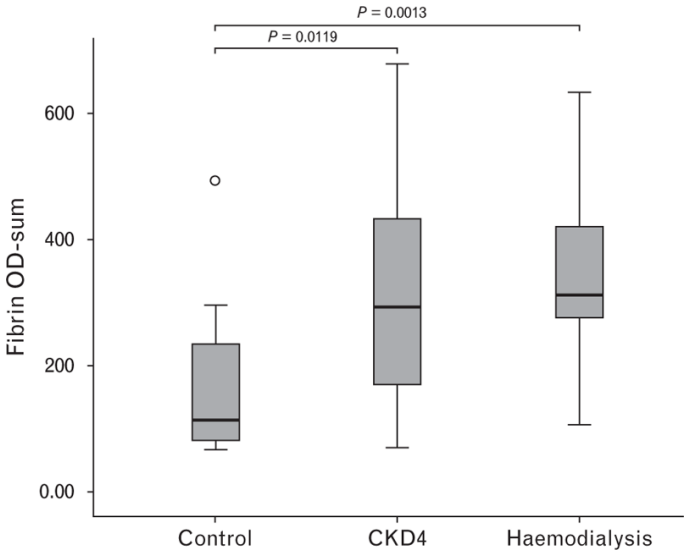
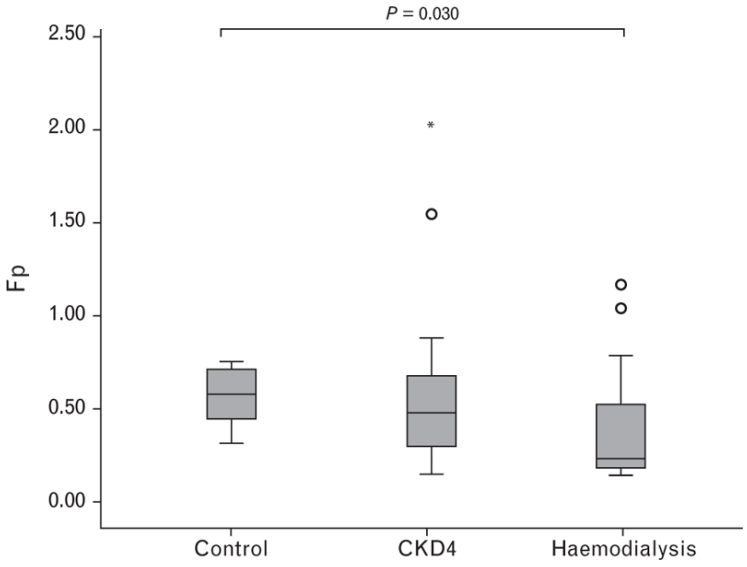
Diabetes mellitus which is an important factor that can influence hemostasis, was however more common in the CKD 4 group; 38% vs. 13 % in the hemodialysis group, and none in the control group. There were too few cases with respectively without diabetes mellitus in each group to be able to perform a formal regression analysis. Instead, we tested between-group differences by Kruskal–Wallis test and when only including the diabetes mellitus patients, we found the same result for fibrin OD sum between the three groups. When testing the whole cohort with Mann-Whitney U test, for differences between diabetes mellitus group and no diabetes mellitus, there were no differences regarding Fibrin ODsum, Cp or Fp, but fibrinogen was significantly higher in the diabetes groups. It cannot be completely excluded that the comorbidities affect our findings, but it seems that diabetes mellitus does not have an effect.

Figure 4. Box plots of the global assay fibrin OD-sum.



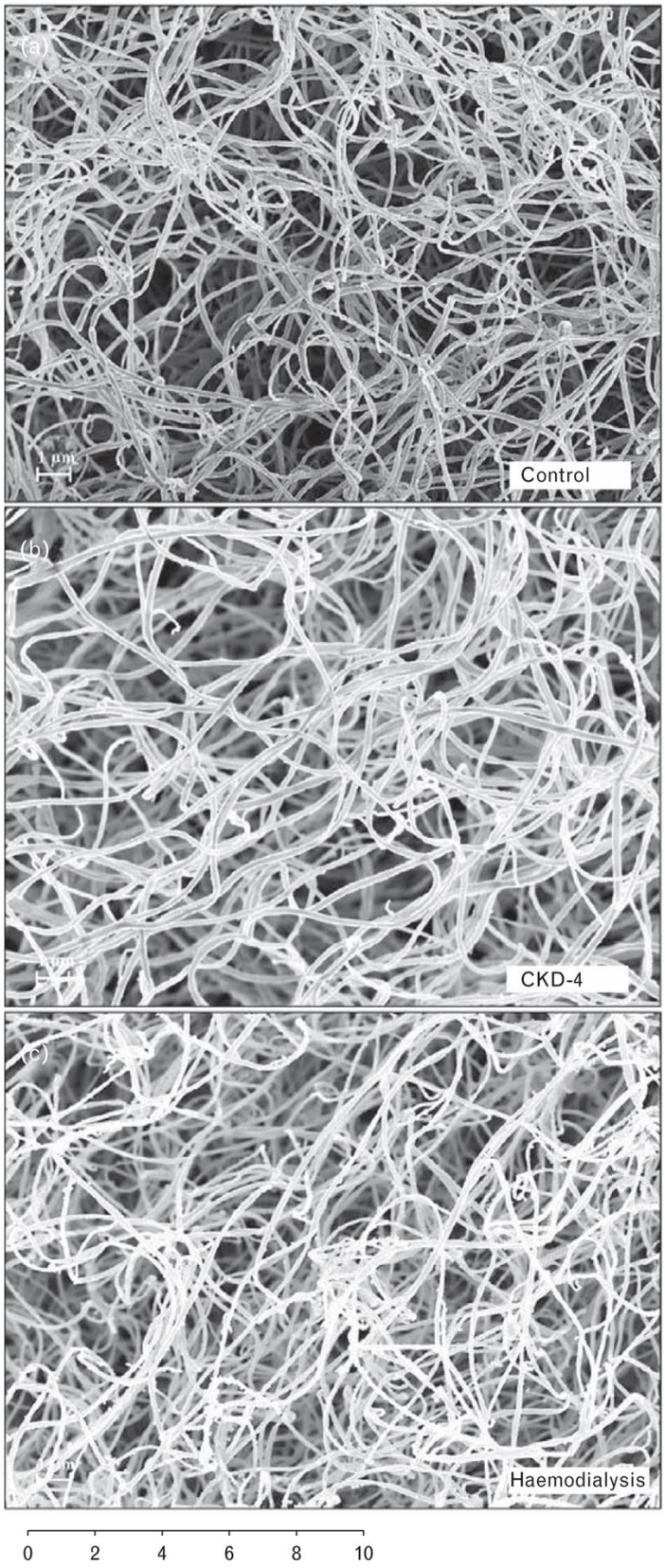
Fibrin OD-sum in arbitrary units that measures the summation of fibrin optical density reflecting the capacity of fibrin formation under the influence of both coagulation and fibrinolysis. Both CKD groups show increased fibrin formation. Values for the thick bar shown in the boxes are median, the box include the lower and upper quartile. Whiskers show 90 % and outliers are represented by (*).

Figure 5. Box plots of fibrinolysis activation profile (Fp).



Fibrinolysis activation profile (Fp) reflecting the fibrinolytic capacity in arbitrary units. The hemodialysis group showed an impaired fibrinolysis. Fp in control group vs CKD 4 non-significant. Values for the thick bar shown are median, the box include the lower and upper quartile. Whiskers show 90% and outliers are represented by (*).

Figure 6. Scanning electron microscopy of the fibrin network from a control subject (a), a CKD stage 4 patient (b), and a hemodialysis patient (c). The tighter fibrin network is illustrated in both kidney disease groups



4.1.2 Limitations and methodological considerations

There are several limitations in this study. The study is rather small, and that always adds a risk that simply chance affected the result. The comorbidity differed between the three groups, and comorbidity may affect the result. Between group differences are possible to adjust statistically for in larger samples, but in smaller studies it is difficult. Hemodialysis is also a potential confounder when studying hemostasis. The patients are treated with iv heparin or low molecular weight heparin right before or during the hemodialysis session, but the effect of these drugs administered iv only stays up to 4-6 hour. To minimize this potential effect, the samples for the study were taken right before a dialysis session after the 2-3 day long interdialytic period.

The method of studying the overall hemostasis with a functional assay, to evaluate coagulation and fibrinolysis, has obvious advantages compared with studying single parameters, but it does not include influential effects of platelets and dysfunctional vascular endothelial cells that may contribute to the pathology.

4.2 STUDY II

4.2.1 Results and discussion

In study II, we showed that among patients with ACS, patients with impaired renal function had increased concentrations of PMPs, and also of PMPs expressing platelet activation markers CD40L (CD154) and P-selectin (CD62P). CKD patients also had elevated concentration of EMPs expressing markers of endothelial activation E-selectin (CD62E), Table 5. These results indicate higher platelet activation in CKD patients, despite concurrent dual antiplatelet treatment, and further impaired endothelial function despite statin treatment.

There were 47 patients included in the study, all with ACS, 19 with preserved renal function, 15 with CKD 3, and 13 with CKD 4-5.

Table 7. MP concentrations.

	Normal kidney function (**H)** n=19	**CKD 3** N=15	**CKD 4-5** N=13	p-value[a]
PMP(CD41) count/µL	424 (328-534)	600 (401-888)	1576 (666-2351)	0.001[a] (CKD3 vs CKD 4-5, **p=0.022**)[b] (CKD3 vs H, p=0.186)[b] (CKD4-5 vs H**, p=0.002**)[b]
P-selectin positive PMP (CD 62P) count/µL	106 (79-158)	147 (111-174)	253 (227-461)	<0.001[a] (CKD3 vs CKD 4-5, **p<0.000**)[b] (CKD3 vs H, p=0.106)[b] (CKD4-5 vs H**, p<0.000**)[b]
CD40L positive PMP (CD154) count/µL	101 (71-134)	142 (125-187)	210 (174-237)	<0.001[a] (CKD3 vs CKD 4-5, **p<0.003**)[b] (CKD3 vs H, **p=0.006**)[b] (CKD4-5 vs H, **p<0.000**)[b]
E-selectin positive MP (CD 62E) count/µL	83 (53-140)	197 (120-245)	245 (189-308)	<0.001[a] (CKD3 vs CKD 4-5, p<0.118)[b] (CKD3 vs H, **p=0.002**)[b] (CKD4-5 vs H, **p<0.000**)[b]

Values expressed as median and interquartile range. a = p value for comparison between the three groups. b = p value for comparison between the two groups listed. *Published by permission from BMC Nephrology 2019.*

Comparisons between PMP concentrations between the three groups are demonstrated in Figures 5-7. Most previous studies reports that both ACS patients (138), and CKD patients (136), have increased concentrations of PMPs compared with controls. A recent study demonstrated that CD62P+ PMPs predicted increased risk of a new cardiovascular event(139). However, there are conflicting data, as a study from 2014 showed that CD41 PMPs were not elevated in ACS patients compared with controls, and could not predict outcome (140). In study I, the concentration of MPs expressing the platelet activation marker P-selectin were elevated in patients with severe CKD, but not in moderate CKD. In a previous study, it was found that platelet reactivity was altered only in patients with moderate renal impairment, not in mild renal impairment, and the authors discussed a possible threshold where renal impairment starts inducing platelet activation (175).

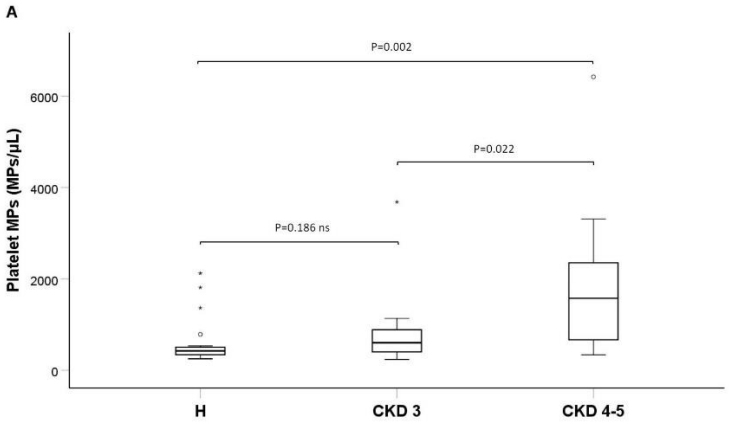


Figure 7. Boxplot of PMPs (CD41) in the tree groups.

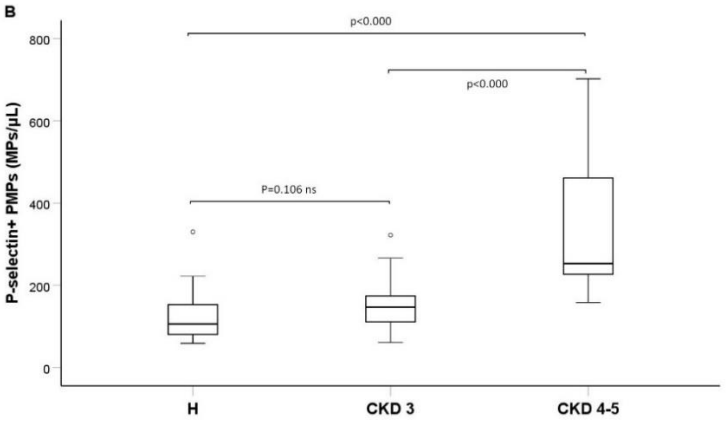


Figure 8. Boxplot of P-selectin (CD62P) positive PMPs.

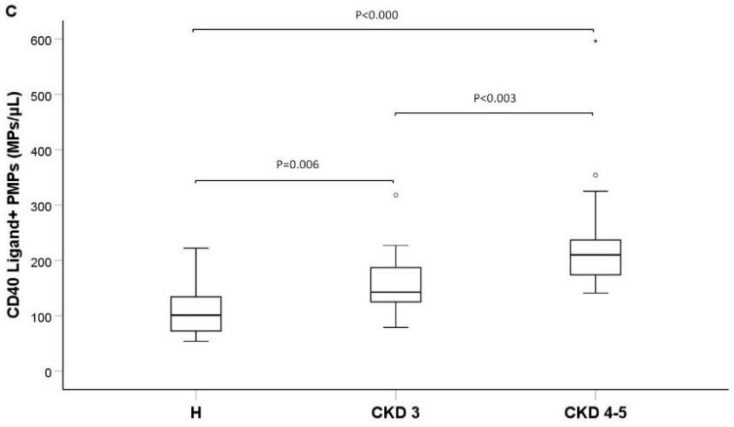


Figure 9. Boxplot of CD40L (CD154) positive PMPs.

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EMPs expressing the endothelial activation marker E-selectin CD62E were elevated in both CKD groups, with increasing concentrations as the renal function declined. EMPs have been

shown to be elevated in CKD (136), and that CD31+ EMPs is associated with outcome in ESRD patients (126). The results from study II indicates a more pronounced endothelial dysfunction in CKD patients. Further studies are needed to provide information on whether EMPs are a valuable surrogate marker in ACS patients, and if so, which endothelial antigen is best to use.

There were more diabetes mellitus patients in the CKD groups. We performed a regression analysis including diabetes mellitus, but found no differences in correlation with MPs and renal function with and without diabetes mellitus.

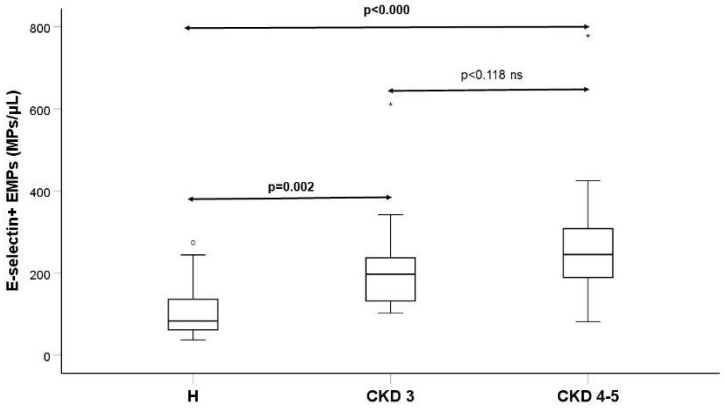

Figure 10. Boxplot of E-selectin positive EMPs count/µL in the tree groups.
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4.2.2 Limitations and methodological considerations

Major limitations are the small number of patients included, with differences between the groups regarding co-morbidity, which we tried to assess by performing a regression analysis. To determine renal function and diagnose patients with CKD is not possible based on just one value of creatinine and without data regarding albuminuria, since the diagnose CKD require at least 3 months stable renal function loss. This is the same problem in many studies where renal function is studied in an acute setting. In the present study, we adjudicated all patient's electronic medical records and could determine that the eGFR level at admission reflected a true chronic state. Another limitation is that samples were collected 1-3 days after admission due to the ACS, and that might affect the concentrations of the MPs. It would have been desirable to collect the samples the same day for every patient included in the study. A methodological limitation may be that the freezing procedure of the sample could produce cell membrane fragments disturbing the analysis. A study of the flow cytometry method of

detecting MPs has been performed in our laboratory, and showed low presence of cell fragments after the same sample handling that was used in our study (176).

4.3 STUDY III

4.3.1 Results and discussion

Study III was a substudy of the SOLID trial (166), were 2 µg daily paricalcitol treatment demonstrated preserved macrovascular function, assessed by FMD, after 12 weeks treatment, in patients with CKD stages 3-4 without diabetes mellitus.

The aim of this study was to assess if paricalcitol treatment affected concentrations of endothelial microparticles expressing the proatherosclerotic markers ICAM-1 and VCAM-1.

There were 36 patients included in the study, divided into three treatment groups, 1 µg, 2 µg or placebo. The groups had similar eGFR and stage of CKD and baseline measurements of vascular function (FMD and pulse wave velocity) were well matched (Table 8, Figure 11) (64). Age was statistically different at baseline, the patients in the placebo group were slightly older. There were no statistical differences regarding medication and previous CVD, however there was a difference in absolute numbers with less previous CVD in the 2 µg group.

Paricalcitol treatment in CKD patients did reduce the concentrations of ICAM-1+ MPs, indicating a less proatherosclerotic endothelium. Furthermore, concentrations of EMPs, PMPs and leukocyte derived MPs (LMPs) induced by cell-activation were maintained, which might be signs of a more preserved and reactive endothelium.

Table 8. Baseline characteristics in the SOLID trial.

Characteristics	Placebo (n=12)	Paricalcitol 1µg (n=12)	Paricalcitol 2 µg (n=12)	p-value
Age (years)	70.8 (10.0)	66.1 (7.9)	59.1 (11.6)	0,02
Sex (% male)	9 (75%)	11 (92%)	8 (67%)	ns
Smokers (current)	1 (8%)	1 (8%)	0	ns
BMI	28.1 (2.4)	26.4 (3.5)	26.8 (2.8)	ns
eGFR (mL/min/1.73 m^2)	41.6 (12.9)	38.9 (13.6)	42.1 (8.0)	ns
CKD duration (years)	10.3 (8.8)	5.8 (6.0)	9.7 (10.5)	ns
Cause of CKD				ns
Hypertension	3 (25%)	4 (33%)	4 (33%)	
Polycystic disease	4 (33%)	2 (17%)	1 (8%)	
Glomerulonephritis	4 (33%)	3 (25%)	4 (33%)	
Other cause	0	2 (17%)	2 (17%)	
CVD at inclusion				ns
Myocardial infarction	3 (25%)	2 (17%)	0	
Atrial Fibrillation	1 (8%)	1 (8%)	0	
Stroke	3 (25%)	0	0	
TIA	1 (8%)	0	0	
Heart failure	0	0	1 (8%)	
Aortic aneurysm	0	1 (8%)	1 (8%)	
Medication				ns
ACE-i/ARB	11 (92%)	9 (75%)	9 (75%)	
β-blockers	6 (50%)	8 (67%)	4 (33%)	
Ca flow-inh	10 (83%)	8 (67%)	4 (33%)	

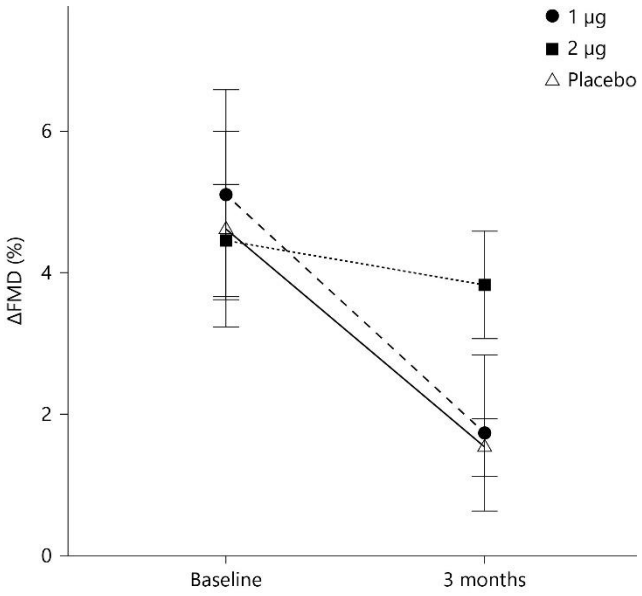

Figure 11. Maximum increase in brachial artery diameter in percentage from baseline by FMD, before and after 3 months treatment with paricalcitol or placebo. *© 2015 S. Karger AG, Basel. Published by permission of American J Nephrology 2015.*

Baseline plasma levels before treatment of 25OH-vitamin D, calcium, phosphate or levels of parathyroid hormone (PTH) did not differ between the groups (Table 9). Data post-treatment showed an expected PTH suppression, but no statistically significant increase in calcium levels or other routine laboratory markers.

Table 9. Clinical characteristics and laboratory markers before and after the intervention.

	BASELINE			POST TREATMENT			
Clinical characteristics	**Placebo (n=12)**	**Paricalcitol 1µg (n=12)**	**Paricalcitol 2µg (n=12)**	**Placebo (n=11)**	**Paricalcitol 1µg (n=12)**	**Paricalcitol 2µg (n=12)**	**p-value**
Systolic BP (mmHg)	134 (12.8)	147(21.1)	130 (14.7)	129 (9.2)	149 (20.3)	128 (16.5)	ns
eGFR (mL/min /1.73 m^2)	41.6 (12.9)	38.9 (13.6)	42.1 (8.0)	40.6 (11.4)	38.3 (12.7)	38.8 (7.3)	ns
Ualb/crea (g/mol)	22.8 (29.0)	60.5 (89.1)	36.3 (44.6)	26.1 (25.3)	57.1 (71.8)	30.9 (39.9)	ns
PTH (pmol/L)	9.3 (3.5)	7.3 (3.6)	7.0 (2.6)	8.7 (3.1)	5.4 (3.1)	2.8 (1.5)	0,006
25-OH-Vit D (nmol/L)	64.6 (27.9)	71.7 (34.2)	69.3 (22.4)	63.6 (22.6)	74.8 (34.7)	72.3 (21.6)	ns
Calcium (mmol/L)	2.26 (0.07)	2.26 (0.08)	2.28 (0.07)	2.26 (0.08)	2.25 (0.12)	2.36 (0.15)	ns
Phosphate (mmol/L)	1.0 (0.2)	1.1 (0.2)	1.1 (0.2)	1.1 (0.2)	1.1 (0.1)	1.23 (0.21)	ns
CRP (mg/L)	3.3 (5.3)	4.3 (7.0)	6.7 (10.2)	2.3 (1.8)	4.8 (4.6)	3.6 (5.3)	ns

BP: blood pressure, eGFR: estimated glomerular filtration rate, Ualb/crea: urine albumin creatinine ratio, PTH: parathyroid hormone. Values are expressed as mean (SD). P-value for interaction of treatment and time.

Table 10. MP concentrations before and after the intervention.

	BASELINE			POST TREATMENT		
MP expression	**Placebo(n=12)**	**Paricalcitol 1µg (n=12)**	**Paricalcitol 2µg (n=12)**	**Placebo (n=12)**	**Paricalcitol 1µg (n=12)**	**Paricalcitol 2µg (n=12)**
ICAM-1+MP (MP/µL)	189.2 (33.9)	205.7 (39.8)	210.8 (31.3)	203.8 (36.4)	193.6 (40.4)	194.7 (35.7)
VCAM-1+MP (MP/µL)	71.6 (21.2)	64.3 (23.8)	77.8 (37.4)	56.7 (18.8)	63.8 (31.0)	59.0 (24.5)
CD62e+MP (MP/µL)	764.4 (176.9)	689.3 (259.9)	575.6 (157.6)	510.6 (234.4)	557.0 (175.8)	499.0 (214.7)
CD45+MP (MP/µL)	692.9 (151.8)	703.4 (210.5)	627.4 (176.2)	599.5 (105.5)	618.9 (108.0)	650.7 (135.7)
CD41+MP (MP/µL)	678.5 (290.3)	703.8 (310.7)	610.3 (287.6)	523.1 (130.2)	553.3 (171.9)	613.0 (156.0)
CD41+62p+MP (MP/µL)	134.8 (56.4)	163.0 (67.3)	141.0 (95.8)	126.8 (72.9)	123.3 (60.5)	158.4 (45.7)
CD41+154+MP (MP/µL)	110.9 (39.8)	115.3 (42.0)	104.6 (41.2)	99.2 (72.9)	84.5 (22.8)	111.3 (47.7)

ICAM-1: intercellular adhesion molecule-1, VCAM-1: vascular adhesion molecule -1, CD62E+: endothelial MPs, CD45+: Leukocyte MPs, CD41+: platelet MPs, CD41+62p+: platelet MPs, $CD41+154^{+}$: platelet MPs. Values are expressed as mean (SD).

Before treatment there were no significant differences regarding the ICAM-1+ and VCAM-1+ MP concentrations in the three groups (Table 10).

The concentrations of ICAM-1+ MPs showed a significant decline in the treatment groups, assessed by repeated measures ANOVA (p=0.04), and increased in the placebo group.

VCAM-1+ MPs did not change significantly during the study period. The concentrations of MPs with origin from activated endothelial cells, platelets and leucocytes remained unchanged in the treatment group of 2 µg and declined in the placebo and 1 µg group. The CD62E+ EMPs decreased in the placebo group.

The SOLID trial demonstrated that three months treatment with 2 µg paricalcitol preserved endothelial function and reduced inflammatory cytokines. Soluble ICAM-1 is known to reflect inflammation and can predict mortality in CKD patients (177). The result of reduced ICAM-1+ MPs goes in line with these previous findings that can be interpreted as a potential protective effect of paricalcitol.

When evaluating the significance of MPs in disease it is important to be aware of the MP subtype. Jimenez et al. demonstrated in vitro that CD31+ EMPs where induced by apoptosis, and CD62E induced by activation (178). There are in vitro studies on endothelial cell lines demonstrating lower concentrations of EMPs after vitamin D treatment, interpreted as a protective effect against apoptosis (179, 180), but there are no in vivo studies. There are several studied indicating that high concentrations of CD31+ and CD144+ EMPs and also CD62E+ EMPs are related to endothelial dysfunction (127, 181-187). A recent study by Sansone et al. on hypertension and EMPs, demonstrated CD144+ EMPs and CD62E+ EMPs more related to mechanical injury and activation, and CD31+ more responsive of chronic changes (188).

There are not many studies on clinical interventions and EMPs. A study on atorvastatin treatment in non-CKD patients demonstrated a rise in CD144+ EMPs following treatment (189, 190), and the authors interpreted that the rise of EMPs were a sign of a more reactive and healthy endothelium. In another study, Almquist et.al. demonstrated that statin treatment vs. placebo caused elevated concentrations of CD144+ EMPs, in CKD patients with diabetes mellitus. In that study, the concentrations of CD62E+ EMPs were higher at baseline in the CKD group, compared to the non-CKD group, all with diabetes. Only the non-CKD groups increased their concentration of CD62E+ EMPs on statin treatment (123). Since several studies have demonstrated beneficial effects of statin treatment, the rise of the CD62E+EMPs may be interpreted as a possible beneficial effect.

The SOLID trial demonstrated protective effects on the endothelium, and reduced levels of proinflammatory cytokines by paricalcitol treatment. This was further demonstrated in this substudy as a decline in ICAM-1+ EMPs. We speculate these findings of MPs to reflect a preserved endothelial cell function. However, in the field of MPs, further research is

necessary to better understand what subpopulations of MPs that are important in which disease, and what a change in concentrations implicates.

4.3.2 Limitations and methodological considerations

There are several limitations in this study. The study is relatively small in number of patients and with a short follow up, which limits the generalizability of the results. The differences in age between the groups despite the randomization is unfortunate, but may happen by chance in small studies, and is not considered to have a major influence on the results in the present study, as the RCT remains the best available methodology for causal inference.

4.4 STUDY IV

4.4.1 Results and discussion

In study IV, 36 biomarkers, all associated with hemostasis, vascular and endothelial function were evaluated in a prospective cohort study of 1 370 ACS patients. First, it was assessed if the biomarkers correlated with renal function. Second, the patients were stratified into two groups, eGFR $\geq$ 60 and eGFR < 60 ml/min/1.73 m^2, and associations with outcome were estimated using Cox regressions analysis.

There were 1 393 patients included, and after excluding patients on dialysis and patients without data on eGFR, 1 370 patients remained for the statistical evaluation. The cause of the impaired renal function was not available. The patients with impaired renal function were older, had more comorbidities, and more on-going medical treatments. Baseline data of the cohort demonstrated in Table 11.

Table 11. Baseline characteristics of study population

	eGFR < 60 (n=234)	eGFR ≥ 60 (n=1 136)	All (n=1 370)
Age, year	74 (9)	63 (10)	65 (11)
Female sex	30 %	22 %	24 %
Current smokers	16 %	29 %	27 %
Hypertension	78 %	45 %	51 %
Claudication	10 %	2 %	4 %
Diabetes	34 %	21 %	23 %
Heart failure admission [a]	24 %	5 %	9 %
Atrial Fibrillation admission [a]	25 %	7 %	10 %
History of IHD	36 %	16 %	19 %
Previous Stroke	15 %	4 %	6 %
Previous CVD	43 %	18 %	22 %
Medications and lab values at admission			
Single platelet treatment	62 %	29 %	34 %
DAPT [b]	10 %	4 %	5 %
Oral anticoagulation [c]	10 %	3 %	4 %
ACEi/ARB	54 %	27 %	32 %
Statin	48 %	27 %	30 %
eGFR mL/min	45.4 (11.9)	87.1 (13.3)	80.0 (20.6)
Haemoglobin g/l	135 (18)	143 (15)	142 (16)
Cholesterol mmol/l	4.6 (1.4)	5.1 (1.2)	5.0 (1.3)
LDL mmol/l	2.8 (1.3)	3.2 (1.1)	3.1 (1.1)
HDL mmol/l	1.1 (0.4)	1.2 (0.4)	1.2 (0.4)
Triglycerides mmol/l	1.6 (1.0)	1.7 (1.0)	1.7 (1.0)
Urate µmol/l	427 (110)	339 (80)	360 (96)
Albumin g/l	32 (5)	35 (5)	34 (5)
Calcium mmol/l	2.2 (0.1)	2.3 (0.5)	2.2 (0.5)
Phosphate mmol/l	1.1 (0.2)	1.0 (0.2)	1.1 (0.2)
Diagnoses and medications at discharge			
STEMI	38 %	50 %	48 %
Coronary angiography	87 %	97 %	95 %
PCI	69 %	83 %	81 %
Heart failure at discharge [d]	46 %	32 %	35 %
Atrial fibrillation discharge [d]	28 %	9 %	12 %
Aspirin	91 %	98 %	96 %
DAPT [b]	76 %	88 %	86 %
Oral anticoagulants [c]	20 %	8 %	10 %
Antithrombotic combination therapy [f]	8 %	6 %	6 %
ACEi/ARB	84 %	83 %	83 %
Statin	92 %	96 %	95 %

[a] known diagnosis before admission for the index ACS, [b] Dual anti platelet treatment (DAPT), [c] Vitamin K antagonist treatment, [d] Diagnosis and medication at discharge from the index ACS, [f] DAPT and Oral anticoagulation

In total 18 of the 36 biomarkers correlated with renal function, after adjusting for eGFR, age, sex, smoking habits, diabetes, previous CVD and heart failure diagnose at discharge. Out of these 18, there were 7 that correlated with eGFR with $r^2 > 0.05$, and were included in the statistical analysis: TF, protease activated receptor (PAR-1), soluble urokinase plasminogen activator receptor (suPAR), thrombomodulin, adrenomedullin, renin, angiotensinogen (Figure 12). vWf, fibrinogen gamma, alfa and beta chains, and coagulation factors IX, XI and factor XIII B-subunit, beta-2-glycoprotein 1, plasma protease C1 inhibitor, vitamin K-dependent protein S, and tetranectin correlated with renal function but with $r^2 < 0.05$.

The analysis of biomarkers in the study indicated an overall prothrombotic state without a rise in fibrinolysis activation, in patients with reduced renal function. TF had a strong correlation with renal function. In previous studies, TF demonstrated a correlation with both CKD and CVD (191-193), and the same was found in the present study where TF could predict the composite outcome of MI, HF, stroke or death in the group of patients with impaired renal function. There were several biomarkers that correlated inversely with renal function: vWf, fibrinogen alpha, beta and gamma chains, coagulation factors IX, XI and factor XIII B-subunit, fibrinogen gamma, alfa and beta chains, factor XI, all indicating a prothrombotic state in CKD.

PAR-1 increased as renal function declined but was not associated with outcome. This finding might indicate a state of more activated platelet, in ACS patients with CKD. On the other hand, we did not find elevated soluble CD40L in the CKD patients, which in in contrast to previous finding (74, 194). This may perhaps, be explained by the intense antiplatelet therapy used in ACS patients.

The fibrinolytic system counteracts the coagulation system and is activated in response to the coagulation process. In the state of an activated coagulation system in ACS patients and further activated among those with CKD, we did however, not find signs of increased fibrinolytic activity. There were no elevations in endogenous plasminogen activator tPA. Tetranectin, that increases fibrinolytic activity, was decreased. Plasminogen was not increased. This was together interpreted as signs of attenuated fibrinolysis.

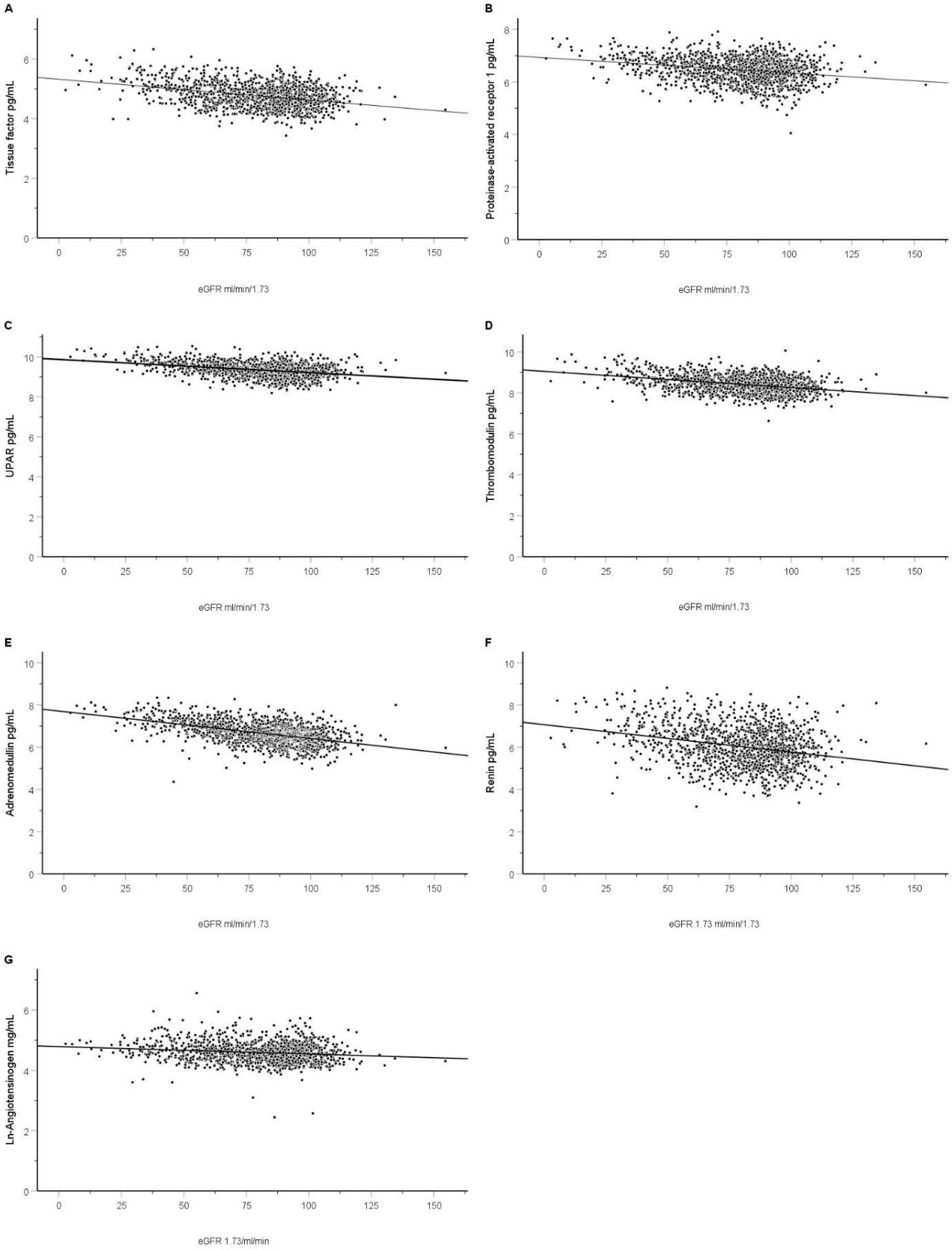

Figure 12. The 7 biomarkers that correlated significantly with eGFR with a coefficient of determination (r^2) > 0.05.

UPAR is expressed by a variety of cells and is an important plasminogen activator when it ligates to urokinase plasminogen activator (uPA). In the soluble circulating form suPAR has less profibrinolytic properties (195), and is involved in a variety of biological processes, including immune modulating and fibrosis (196, 197) and can predict both a decline in renal function and predict incidence of heart failure and cardiovascular death (198, 199). SuPAR correlated with renal function in this present study. Other studies have showed that the uPA/suPAR system correlates with uremic toxins accumulated as renal function decline, but that this correlation only is present in mild, not severe renal impairment (200). Thus, suPAR can be affected of the degree of renal impairment and exert different functions in different stages if CKD. This could be an explanation why suPAR predicted heart failure only in the renal group.

Thrombomodulin has important anticoagulant and vascular protective properties, and soluble thrombomodulin is considered a marker of endothelial dysfunction (201, 202). It has a complex role where increasing concentrations is considered anticoagulative in healthy individuals, and simultaneously is associated with severity of disease in patients with CVD (54). There is conflicting data regarding outcome related to thrombomodulin. There are reports of increased risk of CVD with high concentrations as well as with low (90, 203-205).

In our data set, thrombomodulin increased as renal function declined, and was associated with reduced risk of heart failure in the ACS patients with impaired renal function. The Cox regressions in our analysis were adjusted for age, sex, diabetes mellitus, previous CVD and heart failure. This is a novel finding and warrant further studies.

Adrenomedullin concentrations increased as renal function declined. Adrenomedullin release is regarded as a compensatory response to organ damage, and have positive effects on the endothelium (206). In line with other studies (207), our finding was that adrenomedullin was associated with increased risk of death and heart failure, but only in the group with preserved renal function. In the group with impaired renal function it did not predict any outcome. One interpretation of this may be that when the endothelial dysfunction aggravates as renal function decline, other disturbances become relatively more important, and adrenomedullin loses its predictive value.

Renin and angiotensinogen correlated with renal function as eGFR declined. Renin was associated with bleeding in the normal renal function group, and angiotensinogen with death in the reduced renal function group. The importance of the renin-angiotensin-aldosterone system (RAAS) for cardiovascular outcome is well recognized (208, 209). The substrate of all angiotensin peptides is angiotensinogen, and intra-renal angiotensinogen concentrations have been linked to hypertension and cardiovascular disease (210). However, the RAAS system, and the constituents involved such as renin and angiotensinogen, is affected of treatment with RAAS inhibitors, which was more common in the group with impaired renal function (54 % vs. 27 %). This association may be biased due to differences in medication in the groups.

In summary, the correlations indicated a prothrombotic state, without a simultaneous activation of the fibrinolytic system, in patients with reduced renal function. Our data are in line with our hypothesis that biomarkers associated with hemostasis, vascular and endothelial function could predict outcome among patients with impaired renal function.

Our main conclusion is that the predictive value of biomarkers differed in renal patients, so in the field of biomarkers, it might therefore not be sufficient to adjust for renal function, rather, we speculate that renal failure could be regarded as a separate disease state with its own distinct pathophysiology.

There were additional interesting findings of thrombomodulin as having a potential protective role among CKD patients with ACS, and the increase in suPAR noticed, potentially involved in pathophysiology of heart failure.

Table 12 Cox Models of HRs with 95% confidence intervals of biomarkers for predicting outcome in ACS patients stratified by renal function group.

	eGFR < 60 (n=234)			**eGFR ≥ 60 (n=1136)**		
		HR(95%CI)	p-value		HR(95%CI)	p-value
Death	*n=45*			*n=50*		
First step	TF	3.55 (0.85-14.76)	0.082	TF	2.95 (1.01-8.68)	0.049
	PAR-1*	0.48 (0.15-1.55)	0.221	PAR-1	0.82 (0.35-1.91)	0.648
	suPAR**	1.16 (0.19-7.01)	0.873	suPAR	0.85 (0.21-3.35)	0.813
	Thrombomodulin	0.53 (0.16-1.77)	0.305	Thrombomodulin	0.89 (0.31-2.61)	0.835
	Adrenomedullin	0.99 (0.36-2.74)	0.987	Adrenomedullin	2.47 (1.02-5.93)	0.044
	Renin	1.10 (0.74-1.64)	0.643	Renin	0.95 (0.69-1.32)	0.779
	Angiotensinogen***	2.61 (0.95-7.19)	0.063	Angiotensinogen	2.15 (0.88-5.25)	0.093
Last step	Angiotensinogen	2.91 (1.04-8.13)	0.042	Adrenomedullin	2.50 (1.26-4.97)	0.009
				Angiotensinogen	2.17 (0.93-5.06)	0.072
MI	*n=32*			*n=90*		
First step	TF	2.10 (0.48-9.21)	0.325	TF	1.03 (0.47-2.23)	0.950
	PAR-1	1.28 (0.34-4.79)	0.714	PAR-1	1.06 (0.60-1.87)	0.837
	suPAR	1.47 (0.18-12.07)	0.717	suPAR	2.37 (0.89-6.31)	0.084
	Thrombomodulin	0.27 (0.06-1.21)	0.087	Thrombomodulin	0.75 (0.33-1.69)	0.491
	Adrenomedullin	0.76 (0.27-2.13)	0.602	Adrenomedullin	0.81 (0.45-1.47)	0.484
	Renin	0.80 (0.51-1.26)	0.332	Renin	1.03 (0.80-1.33)	0.823
	Angiotensinogen	0.50 (0.14-1.74)	0.274	Angiotensinogen	0.79 (0.40-1.55)	0.489
Last step				suPAR	2.07(1.09-3.96)	0.027
Ischemic stroke	*n=8*			*n=23*		
First step	TF	****		TF	0.99 (0.20-4.93)	0.991
	PAR-1			PAR-1	1.22 (0.37-4.08)	0.746
	suPAR			suPAR	2.06 (0.28-15)	0.480
	Thrombomodulin			Thrombomodulin	0.38 (0.07-1.97)	0.249
	Adrenomedullin			Adrenomedullin	1.22 (0.35-4.26)	0.759
	Renin			Renin	1.00 (0.61-1.63)	0.998
	Angiotensinogen			Angiotensinogen	3.93 (1.02-15)	0.046
				Angiotensinogen	3.10 (0.85-11)	0.086
Bleeding	*n=7*			*n=18*		
First step	TF	****		TF	3.10 (0.55-18)	0.202
	PAR-1			PAR-1	1.26 (0.32-4.96)	0.746
	suPAR			suPAR	2.05 (0.18-23)	0.559
	Thrombomodulin			Thrombomodulin	1.75 (0.26-12)	0.567
	Adrenomedullin			Adrenomedullin	0.29 (0.08-1.15)	0.078
	Renin			Renin	2.36 (1.30-4.31)	0.005
	Angiotensinogen			Angiotensinogen	0.71 (0.16-3.04)	0.642
Last step				TF	3.61 (0.89-15)	0.073
				Renin	2.09 (1.19-3.66)	0.010

		HR(95%CI)	p-value		HR(95%CI)	p-value
HF	*n=27*			*n=47*		
First step	TF	1.68 (0.35-8.15)	0.520	TF	1.16 (0.38-3.52)	0.796
	PAR-1	0.86 (0.22-3.36)	0.823	PAR-1	0.75 (0.35-1.60)	0.449
	suPAR	7.15 (0.68-75.54)	0.102	suPAR	0.94 (0.23-3.88)	0.929
	Thrombomodulin	0.26 (0.06-1.08)	0.064	Thrombomodulin	0.65 (0.22-1.95)	0.441
	Adrenomedullin	0.98 (0.26-3.77)	0.979	Adrenomedullin	6.72 (2.67-17)	0.000
	Renin	1.03 (0.65-1.63)	0.912	Renin	1.24 (0.87-1.79)	0.235
	Angiotensinogen	1.22 (0.32-4.64)	0.771	Angiotensinogen	0.60 (0.22-1.61)	0.313
Last step	suPAR	10.54 (1.99-55.86)	0.006	Adrenomedullin	5.15(2.67-9.93)	0.000
	Thrombomodulin	0.25 (0.08-0.81)	0.021			
Composite	n=68 [a]			n=165[a]		
First step	TF	1.93 (0.70-5.38)	0.207	TF	1.21 (0.67-2.17)	0.529
	PAR-1	0.85 (0.35-2.06)	0.726	PAR-1	0.94 (0.62-1.44)	0.786
	suPAR	1.38 (0.34-5.55)	0.648	suPAR	1.81 (0.88-3.73)	0.110
	Thrombomodulin	0.43 (0.17-1.10)	0.078	Thrombomodulin	0.56 (0.31-1.02)	0.060
	Adrenomedullin	0.95 (0.46-2.07)	0.952	Adrenomedullin	1.83 (1.16-2.91)	0.010
	Renin	0.95 (0.70-1.29)	0.736	Renin	1.09 (0.90-1.31)	0.383
	Angiotensinogen	1.88 (0.85-4.14)	0.120	Angiotensinogen	1.07 (0.64-1.78)	0.799
Last step	TF	2.53 (1.04-6.16)	0.040	suPAR	2.22 (1.15-4.29)	0.017
	Thrombomodulin	0.47 (0.20-1.09)	0.077	Thrombomodullin	0.58(0.34-0.96)	0.036
				Adrenomedullin	2.00(1.28-3.10)	0.002

Hazard Ratios for the biomarkers n=7 related to eGFR with a coefficient of determination (r^2) above 0.05.
Cox Regression performed by Backword selection procedure. First and last step reported in this table. HRs Adjusted for eGFR, age, sex, smoking habits, diabetes, previous CVD and heart failure diagnose at discharge.
Composite outcome of MI, HF, stroke or death. Myocardial infarction (MI). Heart failure (HF). * Proteinase activated receptor 1 (PAR-1). **Urokinase plasminogen activator surface receptor (suPAR). ***Angiotensinogen: value transformed to natural logarithmic scale. ** Too few events to perform an analysis.**

4.4.2 Limitations and methodological considerations

There are several potential limitations with the present study. First, the study population was relatively younger and healthier compared to an unselected ACS population. There were more STEMI and more PCIs performed, as well as fewer patients with CKD. This is a common observed selection bias in studies like this, and something to consider when interpreting data and applying results on the general ACS population. Second, this was a hypothesis testing study and corrections for multiple testing was not performed. Third, there were too few events of bleeding and stroke in the group with impaired renal function.

Fourth, all markers selected were based on those that were available through the PEA/MRM methods, while several markers of known prognostic importance were absent. Fifth, data on concentrations need to be verified, with more established quantitative methods. Fifth data on some important risk factors were missing, due to lack of reliable data at the time of inclusion, so adjustments for some known risk factors such as blood pressure levels, was not possible. Last, the proteomic methods analyze semi-quantitative concentrations of biomarkers. Consequently, results regarding individual marker concentrations need to be verified, with more established quantitative methods.

5 GENERAL DISCUSSION AND METODOLOGICAL CONSIDERATIONS

The key findings of this study were that:

- CKD is a prothrombotic state, with impaired fibrinolysis, activated platelets and a dense fibrin network.
- Patients with ACS and renal dysfunction have higher concentrations of microparticles indicating platelet activation and endothelial dysfunction.
- Paricalcitol treatment in patients with CKD affect concentrations of endothelial microparticles expressing surface markers that may indicate a protective effect.
- In patients with ACS and renal dysfunction, several biomarkers indicate a prothrombotic state.
- Biomarkers associated with hemostasis, vascular regulation and endothelial function are associated with long-term outcome among patients with impaired renal function.

There are several strengths and limitations with the current study designs and populations investigated. Study I was cross sectional, with a control group. There is always a risk of selection bias both for the patients included as for the controls. In study II, the patients came from a larger cohort, based on a set of criteria, consecutive but not random, which might have induced some bias. Study IV was performed on consecutive patients included in a large cohort, including three centers in Sweden, which adds strengths and external validity to the result. Study III, the randomized trial, has the highest evidence grade, as the randomization is performed in order to eliminate systematic errors. Although in smaller randomized trials, there can be differences between groups in baseline data, as practical issues and cost limit the study size.

The methods used in the studies have several strengths but also limitations, as discussed in the respective chapters. MP analysis with flow cytometry is an extremely sensitive method, but also complicated to perform and to analyze. It is a strength that there is a published study regarding method reproducibility from our laboratory (176). The global hemostasis assays have also been developed and refined in our research group, and used in several publications (168, 174, 211). The proteomic methods are developed within the research groups involved in the TOTAL-AMI (tailoring of treatment in all-comers with acute myocardial infarction) project, and there are several publications using the methods (170, 171).

Current drug treatments to reduce thromboembolic events includes platelet inhibition and anticoagulants, whereas these results indicate that attempts to restore fibrinolysis or normalize fibrin cross-linking may be of clinical importance. There are currently no drugs available to restore fibrinolysis in clinical use. However, a recent study demonstrated that valproic acid could increase concentrations of the endogenous fibrinolysis activator tPA in mice, and through this diminish thrombus formation (212).

PMPs and EMPs concentrations were elevated in patients with CKD, as reported in Study I. Data regarding the role of MPs in ACS and CKD are not consistent. PMPs are even considered more procoagulant than platelets. This is due to their ability to carry TF, where PMPs are the main source of circulating TF (191), and to their ability to bind factor Va, factor VIII and that they can express phosphatidylserine on their surface (112). PMPs also have the ability to enhance thrombus formation and deposition of fibrin on atherosclerotic vessels, and decrease clotting time, as assessed by thromboelastography (134). These effects could explain the poorer outcome associated with increased concentrations of PMPs (139, 140, 213).

Most studies of PMPs procoagulant properties are performed on non-CKD patients. In one study, it was shown that patients with CKD had elevated PMPs compared with controls (125). However, these MPs had less procoagulant capacity when transferred into non-uremic plasma samples (125). Thus, it is not only the concentrations of MPs that are important but also their functional capacity. The platelet dysfunction noticed in patients with CKD probably translate to dysfunctional PMPs. Another study assessed MPs from patients with CKD and compared these with subjects with normal renal function, and noted that when the MPs were removed from the plasma samples, the increased thrombin generation normalized, and suggested that MPs are a major cause of the hypercoagulability noticed in patients with CKD (114). On the other hand, one study showed that increased concentrations of CD42+PMPs correlated to increased risk of bleeding in patients with non-STEMI and normal renal function (111).

In the same study, the MP concentrations correlated inversely with concentrations of the fibrinolysis activators tPA and uPA, i.e. higher MPs related to less profibrinolytic markers (111). Taken together, these reports regarding PMP concentrations are difficult to interpret. It appears that they have different effects depending on the clinical context. The findings of PMPs correlating to bleeding in patients with normal renal function, would certainly be of value to investigate further for a putative role in CKD-associated bleeding.

Proteomic approaches have several limitations. The methods used to quantify the protein concentrations cannot directly be translated to concentrations by conventional methods, and

confirmatory analyses are often required. The large number of proteins analyzed also makes the statistical analysis difficult, with risk for mass-significance. The current approach to test a pre-specified hypothesis reduces some of these issues. Our main conclusion is that the predictive value of biomarkers differed in renal patients, which appears safe to conclude despite these limitations.

It appears that CKD have several unique pathophysiological characteristics, and may even be considered a separate disease state once developed (214). The similar concept of "multiple states" is commonly used in clinical practice, for instance primary vs. secondary prevention, i.e. the states before or after a CV event.

One example of how three chronic diseases may develop and co-exist, has recently been used by Siriwardhana et al. to explain the progression from CKD, CVD and diabetes mellitus to the endpoint of death (Fig 13)(215).

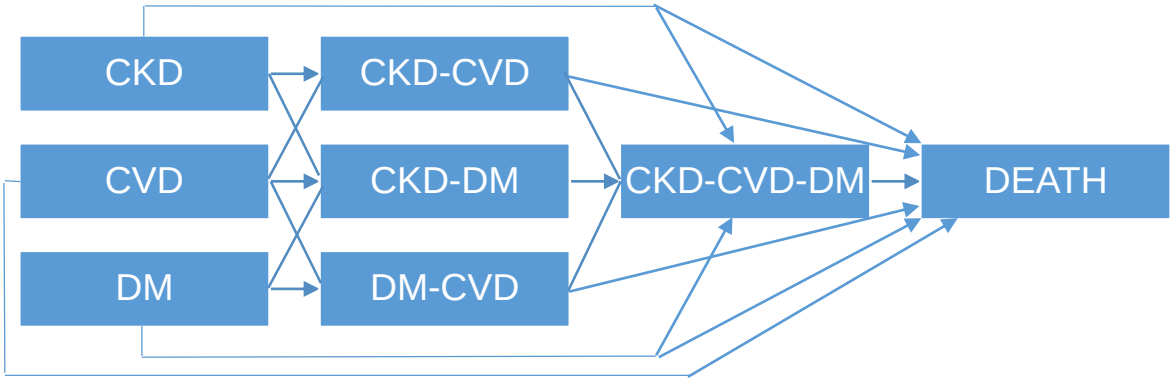

Figure 13. Illustration of distinct chronic disease states and outcomes. Based on a thee chronic condition multistate model by Siriwardhana et al.

Thus, it might not be sufficient to adjust for renal function, rather we argue that renal failure, once developed, could be regarded as a separate disease state with its own distinct pathophysiology. Certainly, the etiology of the CKD is of great importance, but once developed, the "non-Framingham" factors induced by CKD would come into play.

6 CONCLUSIONS

Patients with CKD have hemostatic disturbances demonstrating a procoagulant state, in part due to an impaired fibrinolytic capacity and an increased fibrin formation, as well as a tighter fibrin network, despite normal concentrations of endogenous fibrinolysis inhibitors

Patients with ACS and CKD have increased concentrations of PMPs and EMPs indicating more activated platelets and endothelial dysfunction, compared to patients without CKD, despite concurrent dual antiplatelet and statin treatment.

Paricalcitol treatment in CKD patients reduces the concentrations of ICAM-1 positive EMPs, indicating a less proatherosclerotic endothelium. Concentrations of cell-activation induced EMPs, PMPs, and LMPs were maintained, which may be signs of a more preserved and reactive endothelium. EMPs might be useful to evaluate endothelial activation, function, or damage, but further studies are needed to understand the complex physiology.

Several biomarkers related to hemostasis, endothelial and vascular function correlate with renal function in ACS patients, indicating a procoagulative state, increased platelet activation and increased endothelial activation. Some of these were associated with long-term outcome, but only in patients with reduced renal function. Thus, it might not be sufficient to only adjust for renal function when using biomarkers to predict outcome. Rather, once developed, renal failure might be considered a separate disease state with its own distinct pathophysiology

7 FUTURE PERSPECTIVE

The pathophysiology of how CKD interact with hemostasis, platelets, endothelium and calcium-phosphate metabolism in CVD is indeed complex. The spectrum of mechanisms involved concerns all levels, from microRNA to cells, to vessels and organs, causing the clinical phenotype with CKD and evident CVD. This PhD project have demonstrated some of these mechanisms, hopefully contributing to a better understanding of these.

There have been many large randomized trials performed on CVD prevention with for instance lipid lowering and antithrombotic treatments, involving thousands of patients. These trials were not designed to study patients with CKD, and often excluded more advanced CKD. Thus, regarding antiplatelet therapy, the current recommendations are largely based on result extrapolated after subgroups analyses (216). Regarding vitamin D treatment, there have

been no randomized trials with CVD as endpoint in patients with CKD. Since CKD is common, and carry a poor prognosis, there is obviously a need for sufficiently powered randomized trials in patients with CKD using hard, cardiovascular endpoints. Trials are needed to demonstrate safety and efficacy of new antiplatelet therapies, and of vitamin D treatment. Until then, contributions to increase the knowledge on specific disease mechanisms and surrogate markers in patients with CKD, are valuable. Studies on microparticles represent a promising field, both for risk prediction and for evaluating treatment effect.

Our results from the global hemostatic assay indicate that restoring fibrinolysis and normalizing fibrin network structure may be a new field in CKD. Currently there are no treatments known to achieve this in man, but it may be worth to study the effects of currently available drugs, in the right doses, and the right combinations.

The novel field of gut microbiota is most certainly an interesting future perspective, worth to study as a potential risk factor for CKD and CVD.

Microparticles, flow cytometry investigations and proteomics are important high-technology areas that contribute to our increased understanding. However other very important areas in current and future clinical nephrology concerns how we can improve patient education and patient involvement. How can we help patients to adhere to prescribed medication and improve on life style associated risk factors? A better understanding of the pathophysiological mechanisms involved and potential treatment effects achieved, may enable clinicians to better explain the importance of all these medications, and empower the patients. The patients with CKD suffer from their premature myocardial infarctions or strokes, with all their terrible consequences; with the words from Dr Bright, still “a painful history”.

8 SVENSK SAMMANFATTNING

Kronisk njursvikt innebär nedsatt förmåga av njurarna att rena blodet från slaggämnen och hålla vätskebalansen. Njursvikt är vanligt, det är en tyst folksjukdom utan mycket symtom i början, och mellan 10-15% av befolkningen är drabbad. Diabetes (”sockersjuka”), högt blodtryck och primära njursjukdomar s.k. .glomerulonefriter är de vanliga orsakerna till njursvikt i västvärlden. ”Brights disease” är ett äldre namn på glomerulonefrit. Dr Bright, njurmedicinens fader, beskrev redan i mitten av 1800-talet denna grupp av sjukdomar, som ofta ledde till allvarlig njursvikt, svårt långdraget lidande och död. Han noterade tecken på allvarlig hjärt-kärlsjukdom hos patienterna, och bedömde att detta måste ha något samband med själva njursjukdomen.

Numera är det klarlagt att kronisk njursvikt är en stark riskfaktor för hjärt-kärlsjukdom i form av hjärtinfarkt och stroke, och att efter en hjärtkärlhändelse så har patienter med njursvikt sämre prognos. Det finns en rad riskfaktorer som bidrar, såsom rökning, fysisk inaktivitet, övervikt, diabetes, högt blodtryck som benämns traditionella riskfaktorer. Dessutom finns en rad riskfaktorer som är speciellt viktiga för patienter med njursvikt så som inflammation, oxidativ stress och kalk-fosfat rubbningar. Även låggradig inflammation i det innersta lagret av celler, endotelcellerna, som bekläder kärlväggarna s.k. endoteldysfunktion. Rubbningar i tarmflora har kommit upp som en tänkbar riskfaktor på senare år. Vid allvarligare njursvikt ansamlas en rad slaggprodukterna som samverkar med alla dessa riskfaktorer och ökar risken för hjärtkärlsjukdom.

Rubbningar av blodets koagulationssystem är välkända bland njursvikts patienter, som både har en ökad risk för blodproppar och paradoxalt nog samtidigt en ökad risk för blödning. Blodplättar, trombocyter, är centrala i många hjärt-kärlsjukdomar. Deras uppgift är att stoppa den allra tidigaste blödningen, och aktivera koagulationssystemet. Det är blodplättarnas aktivering som skapar blodproppen som ger hjärtinfarkt eller stroke.

Mikropartiklar är små cellstrukturer, med cellmembran, som knoppas av ifrån celler som aktiveras eller håller på att dö. De cirkulerar i blodet, och bär samma ytproteiner och receptorer som sin ursprungscell. Mikropartiklar är både markörer för sjukdom, och överför även aktiverings och signaleringsämnen mellan celler. Mikropartiklar analyseras med flödescytometri, där man låter mikropartiklarna passera i en tunn kanal en efter en, och

belyser dem med en laserstråle. Det ljus som reflekteras mäts, och beroende på ljusstyrkan och våglängden kan man klassificera och räkna antal mikropartiklar. Mikropartiklar från blodplättar är de vanligaste i cirkulationen, och koncentrationen av dessa korrelerar med en rad sjukdomar som njursvikt och hjärtkärlsjukdom. Mikropartiklar från endotelets celler korrelerar också med en rad sjukdomar, och kan ha en roll i endotelets funktion. D vitamin brist är mycket vanligt vid njursvikt, eftersom enzymet som aktiverar D-vitamin finns i njuren. Endoteldysfunktion är också vanligt bland njursviktspatienter. D-vitaminbrist har visats korrelera till endoteldysfunktion.

Proteomic kallas en annan avancerad metod där man kan analysera en stor mängd proteiner samtidigt, flera hundra, i en mycket liten blodvolym.

Det övergripande målet med det här doktorandprojektet var att klarlägga några av de mekanismer som kan orsaka att patienter med njursvikt har ökad risk för hjärt-kärlsjukdom, genom att undersöka koagulationsrubbningar och mikropartiklar, samt undersöka om olika koagulationsmarkörer kan förutsäga utfall i form av framtida hjärt-kärlhändelser.

I den första studien visade vi att njursvikts patienter har en ökad koagulation jämfört med friska kontroller, på grund av en minskad förmåga att lösa upp blodproppen. Vi såg också en tätare organiserad blodproppstruktur på bilder med svepelektronmikroskop.

I den andra studien visade vi att patienter med hjärtinfarkt och samtidig njursvikt har ökade koncentrationer av mikropartiklar än patienter utan njursvikt, vilket talar för en mer uttalad aktivering av blodplättarna och mer endoteldysfunktion.

I den tredje studien gjorde vi en analys av prover insamlade vid en tidigare randomiserad kontrollerad studie, Solid studien. Den studien visade gynnsamma effekter av D-vitamin på endotelet. Vi visade att D-vitamin även minskade koncentrationen av mikropartiklar med markörer för inflammation.

I den fjärde studien undersökte vi ett antal proteiner inblandade i blodkoagulation och endotel, i en stor grupp av patienter med hjärtinfarkt, och tittade på om de var relaterade till njursvikt. Proteiner som kan förutsäga utfall kan kallas biomarkörer. Flera av dessa var relaterade till njurfunktion, och där undersökte vi om dessa kunde förutsäga utfall som död, hjärtinfarkt, stroke eller hjärtsvikt. Det visade sig att om man jämförde grupper med eller utan njursvikt så var det olika proteiner som var associerade med dessa utfall.

Det behövs omfattande framtida forskning för att bättre förstå dessa mekanismer och vad som kan göras åt dem. Både stora randomiserade studier över behandlingar, men även fler studier

för att klargöra vilken roll t.ex. mikropartiklar har i olika sjukdomar, och om man kan använda dessa för att t.ex. styra behandling. Andra viktiga områden är studier hur man ska förbättra det multidisciplinära omhändertagandet och teamarbetet med patienten, för förbättrad överlevnad och ökad livskvalitet.

Patienter med njursvikt drabbas fortfarande ofta av hjärtinfarkt och stroke med hemska kvarstående men. Dr Bright beskrev njursviktspatientens kamp som “a painful history”. Framtida forskning har ett viktigt uppdrag att försöka minska hjärtkärlsjukdom hos dessa patienter.

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10 REFERENCES

1. Bright R. Cases and observations illustrative of renal disease accompanied with the secretion of albuminous urine. Guy's Hospital Trans. 1836;1836;(1:):338–79.

2. Keith NM, Keys TE. Contributions of Richard Bright and his associates to renal disease. AMA archives of internal medicine. 1954;94(1):5-21.

3. Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, et al. Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. Lancet (London, England). 2017;390(10105):1888-917.

4. Murphy D, McCulloch CE, Lin F, Banerjee T, Bragg-Gresham JL, Eberhardt MS, et al. Trends in Prevalence of Chronic Kidney Disease in the United States. Annals of internal medicine. 2016;165(7):473-81.

5. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England). 2017;390(10100):1151-210.

6. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet (London, England). 2017;389(10075):1238-52.

7. Levey AS, Coresh J. Chronic kidney disease. Lancet (London, England). 2012;379(9811):165-80.

8. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international. 2005;67(6):2089-100.

9. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2014;63(5):713-35.

10. CKD K. kdigo.org/wp-content/uploads/2017/02/KDIGO 2012 [

11. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

12. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Annals of internal medicine. 1999;130(6):461-70.

13. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Annals of internal medicine. 2006;145(4):247-54.

14. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009;150(9):604-12.

15. Nyman U, Grubb A, Larsson A, Hansson LO, Flodin M, Nordin G, et al. The revised Lund-Malmo GFR estimating equation outperforms MDRD and CKD-EPI across GFR, age and BMI intervals in a large Swedish population. Clinical chemistry and laboratory medicine. 2014;52(6):815-24.

16. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet (London, England). 2010;375(9731):2073-81.

17. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). European heart journal. 2016;37(29):2315-81.

18. Zoccali C. Cardiovascular risk in uraemic patients-is it fully explained by classical risk factors? Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2000;15(4):454-7.

19. Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimburger O, Massy Z. Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clinical journal of the American Society of Nephrology : CJASN. 2008;3(2):505-21.

20. Moradi H, Sica DA, Kalantar-Zadeh K. Cardiovascular burden associated with uremic toxins in patients with chronic kidney disease. American journal of nephrology. 2013;38(2):136-48.

21. Hung SC, Kuo KL, Wu CC, Tarng DC. Indoxyl Sulfate: A Novel Cardiovascular Risk Factor in Chronic Kidney Disease. Journal of the American Heart Association. 2017;6(2).

22. Evenepoel P, Poesen R, Meijers B. The gut-kidney axis. Pediatric nephrology (Berlin, Germany). 2016.

23. Lippi G, Danese E, Mattiuzzi C, Favaloro EJ. The Intriguing Link between the Intestinal Microbiota and Cardiovascular Disease. Seminars in thrombosis and hemostasis. 2017.

24. Brial F, Le Lay A, Dumas ME, Gauguier D. Implication of gut microbiota metabolites in cardiovascular and metabolic diseases. Cellular and molecular life sciences : CMLS. 2018;75(21):3977-90.

25. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. Cell. 2016;165(1):111-24.

26. Kaminski TW, Pawlak K, Karbowska M, Mysliwiec M, Pawlak D. Indoxyl sulfate - the uremic toxin linking hemostatic system disturbances with the prevalence of cardiovascular disease in patients with chronic kidney disease. BMC nephrology. 2017;18(1):35.

27. Lin CJ, Wu CJ, Wu PC, Pan CF, Wang TJ, Sun FJ, et al. Indoxyl Sulfate Impairs Endothelial Progenitor Cells and Might Contribute to Vascular Dysfunction in

Patients with Chronic Kidney Disease. Kidney & blood pressure research. 2016;41(6):1025-36.

28. Lin CJ, Wu V, Wu PC, Wu CJ. Meta-Analysis of the Associations of p-Cresyl Sulfate (PCS) and Indoxyl Sulfate (IS) with Cardiovascular Events and All-Cause Mortality in Patients with Chronic Renal Failure. PloS one. 2015;10(7):e0132589.

29. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut Microbiota Metabolites and Risk of Major Adverse Cardiovascular Disease Events and Death: A Systematic Review and Meta-Analysis of Prospective Studies. Journal of the American Heart Association. 2017;6(7).

30. Kim S, Goel R, Kumar A, Qi Y, Lobaton G, Hosaka K, et al. Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. Clinical science (London, England : 1979). 2018;132(6):701-18.

31. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541-6.

32. Yamashita T. Intestinal Immunity and Gut Microbiota in Atherogenesis. Journal of atherosclerosis and thrombosis. 2017;24(2):110-9.

33. Jackel S, Kiouptsi K, Lillich M, Hendrikx T, Khandagale A, Kollar B, et al. Gut microbiota regulate hepatic von Willebrand factor synthesis and arterial thrombus formation via Toll-like receptor-2. Blood. 2017;130(4):542-53.

34. Skye SM, Zhu W, Romano KA, Guo CJ, Wang Z, Jia X, et al. Microbial Transplantation With Human Gut Commensals Containing CutC Is Sufficient to Transmit Enhanced Platelet Reactivity and Thrombosis Potential. Circulation research. 2018;123(10):1164-76.

35. Waterhouse M, Hope B, Krause L, Morrison M, Protani MM, Zakrzewski M, et al. Vitamin D and the gut microbiome: a systematic review of in vivo studies. European journal of nutrition. 2018.

36. Ticinesi A, Lauretani F, Milani C, Nouvenne A, Tana C, Del Rio D, et al. Aging Gut Microbiota at the Cross-Road between Nutrition, Physical Frailty, and Sarcopenia: Is There a Gut-Muscle Axis? Nutrients. 2017;9(12).

37. Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. Journal of the American College of Cardiology. 2008;52(19):1527-39.

38. Rangaswami J, Bhalla V, Blair JEA, Chang TI, Costa S, Lentine KL, et al. Cardiorenal Syndrome: Classification, Pathophysiology, Diagnosis, and Treatment Strategies: A Scientific Statement From the American Heart Association. Circulation. 2019:Cir0000000000000664.

39. Spaak J. Novel combined management approaches to patients with diabetes, chronic kidney disease and cardiovascular disease. The journal of the Royal College of Physicians of Edinburgh. 2017;47(1):83-7.

40. Graham T. The Bakerian Lecture - On the Diffusion of Liquids. PHILOSOPHICAL TRANSACTIONS. 1849.

41. Graham T. The Bakerian Lecture - On Osmotic Force. PHILOSOPHICAL TRANSACTIONS. 1854.

42. Kolff WJ. The artificial kidney. Journal of the Mount Sinai Hospital, New York. 1947;14(2):71-9.

43. Gottschalk CW, Fellner SK. History of the science of dialysis. American journal of nephrology. 1997;17(3-4):289-98.

44. Kjellstrand CM. Dedication to Nils Alwall. Nephron. 1985;39(2):71-2.

45. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet (London, England). 2015;385(9981):1975-82.

46. Robinson BM, Akizawa T, Jager KJ, Kerr PG, Saran R, Pisoni RL. Factors affecting outcomes in patients reaching end-stage kidney disease worldwide: differences in access to renal replacement therapy, modality use, and haemodialysis practices. Lancet (London, England). 2016;388(10041):294-306.

47. Davenport A. Moving beyond small solute clearance: What evidence is there for more permeable dialyzers and haemodiafiltration? Hemodialysis international International Symposium on Home Hemodialysis. 2018;22(S2):S24-s8.

48. Morton RL, Sellars M. From Patient-Centered to Person-Centered Care for Kidney Diseases. Clinical journal of the American Society of Nephrology : CJASN. 2019.

49. Sjoberg B, Snaedal S, Stenvinkel P, Qureshi AR, Heimburger O, Barany P. Three-month variation of plasma pentraxin 3 compared with C-reactive protein, albumin and homocysteine levels in haemodialysis patients. Clinical kidney journal. 2014;7(4):373-9.

50. Windahl K, Faxen Irving G, Almquist T, Liden MK, van de Luijtgaarden M, Chesnaye NC, et al. Prevalence and Risk of Protein-Energy Wasting Assessed by Subjective Global Assessment in Older Adults With Advanced Chronic Kidney Disease: Results From the EQUAL Study. Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2018;28(3):165-74.

51. Casserly LF, Dember LM. Thrombosis in end-stage renal disease. Seminars in dialysis. 2003;16(3):245-56.

52. Goncharov NV, Nadeev AD, Jenkins RO, Avdonin PV. Markers and Biomarkers of Endothelium: When Something Is Rotten in the State. Oxidative medicine and cellular longevity. 2017;2017:9759735.

53. Daiber A, Steven S, Weber A, Shuvaev VV, Muzykantov VR, Laher I, et al. Targeting vascular (endothelial) dysfunction. British journal of pharmacology. 2017;174(12):1591-619.

54. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clinica chimica acta; international journal of clinical chemistry. 2006;368(1-2):33-47.

55. Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. Atherosclerosis. 2012;222(1):8-14.

56. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. Vascular pharmacology. 2016;77:1-7.

57. Kazmi RS, Boyce S, Lwaleed BA. Homeostasis of Hemostasis: The Role of Endothelium. Seminars in thrombosis and hemostasis. 2015;41(6):549-55.

58. Floege J, Ketteler M. Vascular calcification in patients with end-stage renal disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2004;19 Suppl 5:V59-66.

59. Olson M, Chambers M, Shaibi G. Pediatric Markers of Adult Cardiovascular Disease. Current pediatric reviews. 2017;13(4):255-9.

60. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97(18):1837-47.

61. Floege J, Kim J, Ireland E, Chazot C, Drueke T, de Francisco A, et al. Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(6):1948-55.

62. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. Kidney international. 2017;92(1):26-36.

63. Levin A, Tang M, Perry T, Zalunardo N, Beaulieu M, Dubland JA, et al. Randomized Controlled Trial for the Effect of Vitamin D Supplementation on Vascular Stiffness in CKD. Clinical journal of the American Society of Nephrology : CJASN. 2017;12(9):1447-60.

64. Lundwall K, Jorneskog G, Jacobson SH, Spaak J. Paricalcitol, Microvascular and Endothelial Function in Non-Diabetic Chronic Kidney Disease: A Randomized Trial. American journal of nephrology. 2015;42(4):265-73.

65. Fishbane S, Chittineni H, Packman M, Dutka P, Ali N, Durie N. Oral paricalcitol in the treatment of patients with CKD and proteinuria: a randomized trial. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2009;54(4):647-52.

66. Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, et al. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. Jama. 2012;307(7):674-84.

67. Wang AY, Fang F, Chan J, Wen YY, Qing S, Chan IH, et al. Effect of paricalcitol on left ventricular mass and function in CKD--the OPERA trial. Journal of the American Society of Nephrology : JASN. 2014;25(1):175-86.

68. Broos K, Feys HB, De Meyer SF, Vanhoorelbeke K, Deckmyn H. Platelets at work in primary hemostasis. Blood reviews. 2011;25(4):155-67.

69. Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. Blood reviews. 2015;29(3):153-62.

70. Au AE, Josefsson EC. Regulation of platelet membrane protein shedding in health and disease. Platelets. 2016:1-12.

71. Hamilos M, Petousis S, Parthenakis F. Interaction between platelets and endothelium: from pathophysiology to new therapeutic options. Cardiovascular diagnosis and therapy. 2018;8(5):568-80.

72. Estevez B, Du X. New Concepts and Mechanisms of Platelet Activation Signaling. Physiology (Bethesda, Md). 2017;32(2):162-77.

73. Iannacone M. Platelet-mediated modulation of adaptive immunity. Seminars in immunology. 2016;28(6):555-60.

74. Pamukcu B, Lip GY, Snezhitskiy V, Shantsila E. The CD40-CD40L system in cardiovascular disease. Annals of medicine. 2011;43(5):331-40.

75. Stepniewska J, Dolegowska B, Chrusciana M, Golembiewska E, Malinowska-Jedraszczyk A, Marchelek-Mysliwiec M, et al. Platelet - derived CD154 antigen in patients with chronic kidney disease. Clinical biochemistry. 2016;49(3):243-7.

76. Pan H, Boucher M, Kaunelis D. PAR-1 Antagonists: An Emerging Antiplatelet Drug Class. CADTH Issues in Emerging Health Technologies. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2016.

77. Chen YC, Lin FY, Lin YW, Cheng SM, Chang CC, Lin RH, et al. Platelet MicroRNA 365-3p Expression Correlates with High On-treatment Platelet Reactivity in Coronary Artery Disease Patients. Cardiovascular drugs and therapy. 2019.

78. Adams MJ, Irish AB, Watts GF, Oostryck R, Dogra GK. Hypercoagulability in chronic kidney disease is associated with coagulation activation but not endothelial function. Thrombosis research. 2008;123(2):374-80.

79. Opatrny K, Jr., Zemanova P, Opatrna S, Vit L. Fibrinolysis in chronic renal failure, dialysis and renal transplantation. Annals of transplantation. 2002;7(1):34-43.

80. Lutz J, Menke J, Sollinger D, Schinzel H, Thurmel K. Haemostasis in chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2014;29(1):29-40.

81. Dubin R, Cushman M, Folsom AR, Fried LF, Palmas W, Peralta CA, et al. Kidney function and multiple hemostatic markers: cross sectional associations in the multi-ethnic study of atherosclerosis. BMC nephrology. 2011;12:3.

82. Jalal DI, Chonchol M, Targher G. Disorders of hemostasis associated with chronic kidney disease. Seminars in thrombosis and hemostasis. 2010;36(1):34-40.

83. Huang MJ, Wei RB, Wang Y, Su TY, Di P, Li QP, et al. Blood coagulation system in patients with chronic kidney disease: a prospective observational study. BMJ open. 2017;7(5):e014294.

84. Segarra A, Chacon P, Martinez-Eyarre C, Argelaguer X, Vila J, Ruiz P, et al. Circulating levels of plasminogen activator inhibitor type-1, tissue plasminogen activator, and thrombomodulin in hemodialysis patients: biochemical correlations and role as independent predictors of coronary artery stenosis. Journal of the American Society of Nephrology : JASN. 2001;12(6):1255-63.

85. Molino D, De Lucia D, Gaspare De Santo N. Coagulation disorders in uremia. Seminars in nephrology. 2006;26(1):46-51.

86. Mezzano D, Tagle R, Pais E, Panes O, Perez M, Downey P, et al. Endothelial cell markers in chronic uremia: relationship with hemostatic defects and severity of renal failure. Thrombosis research. 1997;88(6):465-72.

87. Mortberg J, Blomback M, Wallen A, He S, Jacobson SH, Spaak J. Increased fibrin formation and impaired fibrinolytic capacity in severe chronic kidney disease. Blood Coagul Fibrinolysis. 2016;27(4):401-7.

88. Shashar M, Francis J, Chitalia V. Thrombosis in the uremic milieu--emerging role of "thrombolome". Seminars in dialysis. 2015;28(2):198-205.

89. Cheung KL, Bouchard BA, Cushman M. Venous thromboembolism, factor VIII and chronic kidney disease. Thrombosis research. 2018;170:10-9.

90. Bao YS, Jia XB, Wang D, Liu RC, Zou CB, Na SP. Characterization of soluble thrombomodulin levels in patients with stage 3-5 chronic kidney disease. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2014;19(4):275-80.

91. Undas A, Nycz K, Pastuszczak M, Stompor T, Zmudka K. The effect of chronic kidney disease on fibrin clot properties in patients with acute coronary syndrome. Blood Coagul Fibrinolysis. 2010;21(6):522-7.

92. Undas A, Kolarz M, Kopec G, Tracz W. Altered fibrin clot properties in patients on long-term haemodialysis: relation to cardiovascular mortality. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2008;23(6):2010-5.

93. Schuett K, Savvaidis A, Maxeiner S, Lysaja K, Jankowski V, Schirmer SH, et al. Clot Structure: A Potent Mortality Risk Factor in Patients on Hemodialysis. Journal of the American Society of Nephrology : JASN. 2017;28(5):1622-30.

94. Matika RW, Nielsen VG, Steinbrenner EB, Sussman AN, Madhrira M. Hemodialysis patients have plasmatic hypercoagulability and decreased fibrinolytic vulnerability: role of carbon monoxide. ASAIO journal (American Society for Artificial Internal Organs : 1992). 2014;60(6):716-21.

95. Sharma S, Farrington K, Kozarski R, Christopoulos C, Niespialowska-Steuden M, Moffat D, et al. Impaired thrombolysis: a novel cardiovascular risk factor in end-stage renal disease. European heart journal. 2013;34(5):354-63.

96. O'Donnell JS, O'Sullivan JM, Preston RJS. Advances in understanding the molecular mechanisms that maintain normal haemostasis. British journal of haematology. 2019.

97. Hoffman M. Remodeling the blood coagulation cascade. Journal of thrombosis and thrombolysis. 2003;16(1-2):17-20.

98. Lu HY, Liao KM. Increased risk of deep vein thrombosis in end-stage renal disease patients. BMC nephrology. 2018;19(1):204.

99. Cheung KL, Zakai NA, Folsom AR, Kurella Tamura M, Peralta CA, Judd SE, et al. Measures of Kidney Disease and the Risk of Venous Thromboembolism in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2017;70(2):182-90.

100. Rijken DC, Uitte de Willige S. Inhibition of Fibrinolysis by Coagulation Factor XIII. BioMed research international. 2017;2017:1209676.

101. Foley JH, Kim PY, Mutch NJ, Gils A. Insights into thrombin activatable fibrinolysis inhibitor function and regulation. Journal of thrombosis and haemostasis : JTH. 2013;11 Suppl 1:306-15.

102. GB: M. Opera Omnia, Venice, Italy, 1764. Ex Typographia Remondiniana. 1764.

103. Noris M, Remuzzi G. Uremic bleeding: closing the circle after 30 years of controversies? Blood. 1999;94(8):2569-74.

104. Lewis JH, Ferguson JH, Zucker MB. Bleeding tendency in uremia. Blood. 1956;11(12):1073-6.

105. Boccardo P, Remuzzi G, Galbusera M. Platelet dysfunction in renal failure. Seminars in thrombosis and hemostasis. 2004;30(5):579-89.

106. Remuzzi G, Benigni A, Dodesini P, Schieppati A, Livio M, Poletti E, et al. Parathyroid hormone inhibits human platelet function. Lancet (London, England). 1981;2(8259):1321-3.

107. Yorulmaz G, Akalin A, Akay OM, Sahin G, Bal C. The Effect of Hyperparathyroid State on Platelet Functions and Bone Loss. Turkish journal of haematology : official journal of Turkish Society of Haematology. 2016;33(4):293-8.

108. Noris M, Benigni A, Boccardo P, Aiello S, Gaspari F, Todeschini M, et al. Enhanced nitric oxide synthesis in uremia: implications for platelet dysfunction and dialysis hypotension. Kidney international. 1993;44(2):445-50.

109. Pavord S, Myers B. Bleeding and thrombotic complications of kidney disease. Blood reviews. 2011;25(6):271-8.

110. Rabelink TJ, Zwaginga JJ, Koomans HA, Sixma JJ. Thrombosis and hemostasis in renal disease. Kidney international. 1994;46(2):287-96.

111. Montoro-Garcia S, Shantsila E, Tapp LD, Lopez-Cuenca A, Romero AI, Hernandez-Romero D, et al. Small-size circulating microparticles in acute coronary syndromes: relevance to fibrinolytic status, reparative markers and outcomes. Atherosclerosis. 2013;227(2):313-22.

112. Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Panteleev MA, Krymskaya OV, et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. Thrombosis and haemostasis. 2007;97(3):425-34.

113. Mobarrez F, He S, Broijersen A, Wiklund B, Antovic A, Antovic J, et al. Atorvastatin reduces thrombin generation and expression of tissue factor, P-selectin and GPIIIa on platelet-derived microparticles in patients with peripheral arterial occlusive disease. Thrombosis and haemostasis. 2011;106(2):344-52.

114. Burton JO, Hamali HA, Singh R, Abbasian N, Parsons R, Patel AK, et al. Elevated levels of procoagulant plasma microvesicles in dialysis patients. PloS one. 2013;8(8):e72663.

115. Chargaff E, West R. The biological significance of the thromboplastic protein of blood. The Journal of biological chemistry. 1946;166(1):189-97.

116. Wolf P. The nature and significance of platelet products in human plasma. British journal of haematology. 1967;13(3):269-88.

117. Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of extracellular vesicles. 2018;7(1):1535750.

118. Boulanger CM, Loyer X, Rautou PE, Amabile N. Extracellular vesicles in coronary artery disease. Nature reviews Cardiology. 2017;14(5):259-72.

119. Slomka A, Urban SK, Lukacs-Kornek V, Zekanowska E, Kornek M. Large Extracellular Vesicles: Have We Found the Holy Grail of Inflammation? Frontiers in immunology. 2018;9:2723.

120. Badimon L, Suades R, Fuentes E, Palomo I, Padro T. Role of Platelet-Derived Microvesicles As Crosstalk Mediators in Atherothrombosis and Future Pharmacology Targets: A Link between Inflammation, Atherosclerosis, and Thrombosis. Frontiers in pharmacology. 2016;7:293.

121. Nomura S. Microparticle and Atherothrombotic Diseases. Journal of atherosclerosis and thrombosis. 2016;23(1):1-9.

122. Mortberg J, Lundwall K, Mobarrez F, Wallen H, Jacobson SH, Spaak J. Increased concentrations of platelet- and endothelial-derived microparticles in patients with myocardial infarction and reduced renal function- a descriptive study. BMC nephrology. 2019;20(1):71.

123. Almquist T, Mobarrez F, Jacobson SH, Wallen H, Hjemdahl P. Effects of lipid-lowering treatment on circulating microparticles in patients with diabetes mellitus and chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2016;31(6):944-52.

124. Chen YL, Chen CH, Wallace CG, Wang HT, Yang CC, Yip HK. Levels of circulating microparticles in patients with chronic cardiorenal disease. Journal of atherosclerosis and thrombosis. 2015;22(3):247-56.

125. Trappenburg MC, Van Schilfgaarde M, Frerichs FCP, Spronk HMH, Ten Cate H, De Fijter CWH, et al. Chronic renal failure is accompanied by endothelial activation and a large increase in microparticle numbers with reduced procoagulant capacity. Nephrology Dialysis Transplantation. 2012;27(4):1446-53.

126. Amabile N, Guerin AP, Tedgui A, Boulanger CM, London GM. Predictive value of circulating endothelial microparticles for cardiovascular mortality in end-stage renal failure: a pilot study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(5):1873-80.

127. Dursun I. The relationship between circulating endothelial microparticles and arterial stiffness and atherosclerosis in children with chronic kidney disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2009;24:2511–8.

128. Boulanger CM, Amabile N, Guerin AP, Pannier B, Leroyer AS, Mallat CN, et al. In vivo shear stress determines circulating levels of endothelial microparticles in end-stage renal disease. Hypertension (Dallas, Tex : 1979). 2007;49(4):902-8.

129. Faure V, Dou L, Sabatier F, Cerini C, Sampol J, Berland Y, et al. Elevation of circulating endothelial microparticles in patients with chronic renal failure. Journal of thrombosis and haemostasis : JTH. 2006;4(3):566-73.

130. Amabile N, Guerin AP, Leroyer A, Mallat Z, Nguyen C, Boddaert J, et al. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. Journal of the American Society of Nephrology : JASN. 2005;16(11):3381-8.

131. Ando M, Iwata A, Ozeki Y, Tsuchiya K, Akiba T, Nihei H. Circulating platelet-derived microparticles with procoagulant activity may be a potential cause of thrombosis in uremic patients. Kidney international. 2002;62(5):1757-63.

132. Connor DE, Exner T, Ma DD, Joseph JE. The majority of circulating platelet-derived microparticles fail to bind annexin V, lack phospholipid-dependent procoagulant activity and demonstrate greater expression of glycoprotein Ib. Thrombosis and haemostasis. 2010;103(5):1044-52.

133. Arraud N, Linares R, Tan S, Gounou C, Pasquet JM, Mornet S, et al. Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. Journal of thrombosis and haemostasis : JTH. 2014;12(5):614-27.

134. Suades R, Padro T, Vilahur G, Badimon L. Circulating and platelet-derived microparticles in human blood enhance thrombosis on atherosclerotic plaques. Thrombosis and haemostasis. 2012;108(6):1208-19.

135. Tehrani S, Mobarrez F, Antovic A, Santesson P, Lins PE, Adamson U, et al. Atorvastatin has antithrombotic effects in patients with type 1 diabetes and dyslipidemia. Thrombosis research. 2010;126(3):e225-31.

136. Trappenburg MC, van Schilfgaarde M, Frerichs FC, Spronk HM, ten Cate H, de Fijter CW, et al. Chronic renal failure is accompanied by endothelial activation and a large increase in microparticle numbers with reduced procoagulant capacity. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(4):1446-53.

137. Stepien E, Stankiewicz E, Zalewski J, Godlewski J, Zmudka K, Wybranska I. Number of microparticles generated during acute myocardial infarction and stable angina correlates with platelet activation. Archives of medical research. 2012;43(1):31-5.

138. Skeppholm M, Mobarrez F, Malmqvist K, Wallen H. Platelet-derived microparticles during and after acute coronary syndrome. Thrombosis and haemostasis. 2012;107(6):1122-9.

139. Christersson C, Thulin A, Siegbahn A. Microparticles during long-term follow-up after acute myocardial infarction. Association to atherosclerotic burden and risk of cardiovascular events. Thrombosis and haemostasis. 2017;117(8):1571-81.

140. Giannopoulos G, Oudatzis G, Paterakis G, Synetos A, Tampaki E, Bouras G, et al. Red blood cell and platelet microparticles in myocardial infarction patients treated with primary angioplasty. International journal of cardiology. 2014;176(1):145-50.

141. Chiva-Blanch G, Laake K, Myhre P, Bratseth V, Arnesen H, Solheim S, et al. Platelet-, monocyte-derived and tissue factor-carrying circulating microparticles are related to acute myocardial infarction severity. PloS one. 2017;12(2):e0172558.

142. Kafian S, Mobarrez F, Wallen H, Samad B. Association between platelet reactivity and circulating platelet-derived microvesicles in patients with acute coronary syndrome. Platelets. 2015;26(5):467-73.

143. Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. Cell and tissue research. 2009;335(1):143-51.

144. Gistera A, Hansson GK. The immunology of atherosclerosis. Nature reviews Nephrology. 2017;13(6):368-80.

145. Berezin A, Zulli A, Kerrigan S, Petrovic D, Kruzliak P. Predictive role of circulating endothelial-derived microparticles in cardiovascular diseases. Clinical biochemistry. 2015;48(9):562-8.

146. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. The New England journal of medicine. 2005;353(22):2373-83.

147. Montinari MR, Minelli S, De Caterina R. The first 3500years of aspirin history from its roots - A concise summary. Vascular pharmacology. 2018.

148. Preliminary report: Findings from the aspirin component of the ongoing Physicians' Health Study. The New England journal of medicine. 1988;318(4):262-4.

149. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second

International Study of Infarct Survival) Collaborative Group. Lancet (London, England). 1988;2(8607):349-60.

150. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. The New England journal of medicine. 2001;345(7):494-502.

151. Cattaneo M. P2Y12 receptors: structure and function. Journal of thrombosis and haemostasis : JTH. 2015;13 Suppl 1:S10-6.

152. Barbieri L, Pergolini P, Verdoia M, Rolla R, Nardin M, Marino P, et al. Platelet reactivity in patients with impaired renal function receiving dual antiplatelet therapy with clopidogrel or ticagrelor. Vascular pharmacology. 2016;79:11-5.

153. Zhu P, Tang XF, Xu JJ, Song Y, Liu R, Zhang Y, et al. Platelet reactivity in patients with chronic kidney disease undergoing percutaneous coronary intervention. Platelets. 2018:1-7.

154. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. The New England journal of medicine. 2009;361(11):1045-57.

155. Boersma E, Harrington RA, Moliterno DJ, White H, Theroux P, Van de Werf F, et al. Platelet glycoprotein IIb/IIIa inhibitors in acute coronary syndromes: a meta-analysis of all major randomised clinical trials. Lancet (London, England). 2002;359(9302):189-98.

156. Bosch X, Marrugat J, Sanchis J. Platelet glycoprotein IIb/IIIa blockers during percutaneous coronary intervention and as the initial medical treatment of non-ST segment elevation acute coronary syndromes. The Cochrane database of systematic reviews. 2013(11):Cd002130.

157. Chew DP, Bhatt DL, Sapp S, Topol EJ. Increased mortality with oral platelet glycoprotein IIb/IIIa antagonists: a meta-analysis of phase III multicenter randomized trials. Circulation. 2001;103(2):201-6.

158. Newby LK, Califf RM, White HD, Harrington RA, Van de Werf F, Granger CB, et al. The failure of orally administered glycoprotein IIb/IIIa inhibitors to prevent recurrent cardiac events. The American journal of medicine. 2002;112(8):647-58.

159. Flaumenhaft R, De Ceunynck K. Targeting PAR1: Now What? Trends in pharmacological sciences. 2017;38(8):701-16.

160. Morrow DA, Braunwald E, Bonaca MP, Ameriso SF, Dalby AJ, Fish MP, et al. Vorapaxar in the secondary prevention of atherothrombotic events. The New England journal of medicine. 2012;366(15):1404-13.

161. Tricoci P, Lokhnygina Y, Huang Z, Van de Werf F, Cornel JH, Chen E, et al. Vorapaxar with or without clopidogrel after non-ST-segment elevation acute coronary syndromes: results from the thrombin receptor antagonist for clinical event reduction in acute coronary syndrome trial. American heart journal. 2014;168(6):869-77.e1.

162. Balakumar P, Nyo YH, Renushia R, Raaginey D, Oh AN, Varatharajan R, et al. Classical and pleiotropic actions of dipyridamole: Not enough light to illuminate the dark tunnel? Pharmacological research. 2014;87:144-50.

163. Diener HC, Sacco RL, Yusuf S, Cotton D, Ounpuu S, Lawton WA, et al. Effects of aspirin plus extended-release dipyridamole versus clopidogrel and telmisartan on disability and cognitive function after recurrent stroke in patients with ischaemic stroke in the Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial: a

double-blind, active and placebo-controlled study. The Lancet Neurology. 2008;7(10):875-84.

164. Kuo KL, Hung SC, Tseng WC, Liu JS, Lin MH, Hsu CC, et al. Dipyridamole decreases dialysis risk and improves survival in patients with pre-dialysis advanced chronic kidney disease. Oncotarget. 2018;9(4):5368-77.

165. Szummer K, Lundman P, Jacobson SH, Schon S, Lindback J, Stenestrand U, et al. Relation between renal function, presentation, use of therapies and in-hospital complications in acute coronary syndrome: data from the SWEDEHEART register. Journal of internal medicine. 2010;268(1):40-9.

166. Lundwall K, Jörneskog G, Jacobson SH, Spaak J. Paricalcitol, Microvascular and Endothelial Function in Non-Diabetic Chronic Kidney Disease: A Randomized Trial. American journal of nephrology. 2015;42(4):265-73.

167. Mansouri L, Lundwall K, Moshfegh A, Jacobson SH, Lundahl J, Spaak J. Vitamin D receptor activation reduces inflammatory cytokines and plasma MicroRNAs in moderate chronic kidney disease - a randomized trial. BMC nephrology. 2017;18(1):161.

168. He S, Zhu K, Skeppholm M, Vedin J, Svensson J, Egberg N, et al. A global assay of haemostasis which uses recombinant tissue factor and tissue-type plasminogen activator to measure the rate of fibrin formation and fibrin degradation in plasma. Thrombosis and haemostasis. 2007;98(4):871-82.

169. Rezeli M, Vegvari A, Donnarumma F, Gidlof O, Smith JG, Erlinge D, et al. Development of an MRM assay panel with application to biobank samples from patients with myocardial infarction. J Proteomics. 2013;87:16-25.

170. Rezeli M, Sjodin K, Lindberg H, Gidlof O, Lindahl B, Jernberg T, et al. Quantitation of 87 Proteins by nLC-MRM/MS in Human Plasma: Workflow for Large-Scale Analysis of Biobank Samples. Journal of proteome research. 2017;16(9):3242-54.

171. Lind L, Arnlov J, Lindahl B, Siegbahn A, Sundstrom J, Ingelsson E. Use of a proximity extension assay proteomics chip to discover new biomarkers for human atherosclerosis. Atherosclerosis. 2015;242(1):205-10.

172. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PloS one. 2014;9(4):e95192.

173. Collet JP, Park D, Lesty C, Soria J, Soria C, Montalescot G, et al. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. Arteriosclerosis, thrombosis, and vascular biology. 2000;20(5):1354-61.

174. Leander K, Blomback M, Wallen H, He S. Impaired fibrinolytic capacity and increased fibrin formation associate with myocardial infarction. Thrombosis and haemostasis. 2012;107(6):1092-9.

175. Angiolillo DJ, Bernardo E, Capodanno D, Vivas D, Sabate M, Ferreiro JL, et al. Impact of chronic kidney disease on platelet function profiles in diabetes mellitus patients with coronary artery disease taking dual antiplatelet therapy. Journal of the American College of Cardiology. 2010;55(11):1139-46.

176. Mobarrez F, Antovic J, Egberg N, Hansson M, Jorneskog G, Hultenby K, et al. A multicolor flow cytometric assay for measurement of platelet-derived microparticles. Thrombosis research. 2010;125(3):e110-6.

177. Stenvinkel P, Lindholm B, Heimburger M, Heimburger O. Elevated serum levels of soluble adhesion molecules predict death in pre-dialysis patients: association with malnutrition, inflammation, and cardiovascular disease. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2000;15(10):1624-30.

178. Jimenez JJ, Jy W, Mauro LM, Soderland C, Horstman LL, Ahn YS. Endothelial cells release phenotypically and quantitatively distinct microparticles in activation and apoptosis. Thrombosis research. 2003;109(4):175-80.

179. Jia X, Xu J, Gu Y, Gu X, Li W, Wang Y. Vitamin D suppresses oxidative stress-induced microparticle release by human umbilical vein endothelial cells. Biology of Reproduction. 2017;96(September):199-210.

180. Xu J, Jia X, Gu Y. Vitamin D Reduces Oxidative Stress – Induced Procaspase-3/ROCK1 Activation and MP Release by Placental Trophoblasts. J Clin Endocrinol Metab. 2017;102(6):2100-10.

181. Amabile N, Guerin AP, Leroyer AP, Mallat Z, Nguyen C, Boddaert J, et al. Circulating Endothelial Microparticles Are Associated with Vascular Dysfunction in Patients with End-Stage Renal Failure. Journal of the American Society of Nephrology. 2005;16(11):3381-8.

182. Amabile N, Guérin AP, Tedgui A, Boulanger CM, London GM. Predictive value of circulating endothelial microparticles for cardiovascular mortality in end-stage renal failure: a pilot study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(5):1873-80.

183. Esposito K, Ciotola M, Schisano B, Gualdiero R, Sardelli L, Misso L, et al. Endothelial microparticles correlate with endothelial dysfunction in obese women. Journal of Clinical Endocrinology and Metabolism. 2006;91(9):3676-9.

184. Nozaki T, Sugiyama S, Sugamura K, Ohba K, Matsuzawa Y, Konishi M, et al. Prognostic value of endothelial microparticles in patients with heart failure. European journal of heart failure. 2010;12(11):1223-8.

185. Wang JM, Su C, Wang Y, Huang YJ, Yang Z, Chen L, et al. Elevated circulating endothelial microparticles and brachial-ankle pulse wave velocity in well-controlled hypertensive patients. Journal of human hypertension. 2009;23(5):307-15.

186. Sinning JM, Losch J, Walenta K, Böhm M, Nickenig G, Werner N. Circulating CD31 +/Annexin V + microparticles correlate with cardiovascular outcomes. European heart journal. 2011;32(16):2034-41.

187. Amabile N, Heiss C, Chang V, Angeli FS, Damon L, Rame EJ, et al. Increased CD62e+ Endothelial Microparticle Levels Predict Poor Outcome in Pulmonary Hypertension Patients. Journal of Heart and Lung Transplantation. 2009;28(10):1081-6.

188. Sansone R, Baaken M, Horn P, Schuler D, Westenfeld R, Amabile N, et al. Release of endothelial microparticles in patients with arterial hypertension, hypertensive emergencies and catheter-related injury. Atherosclerosis. 2018;273:67-74.

189. Mobarrez F, Egberg N, Antovic J, Bröijersen A, Jörneskog G, Wallén H. Release of endothelial microparticles in vivo during atorvastatin treatment; A randomized double-blind placebo-controlled study. Thrombosis research. 2012;129(1):95-7.

190. Tehrani S, Mobarrez F, Lins P-E, Adamson U, Wallén HN, Jörneskog G. Impaired endothelium-dependent skin microvascular function during high-dose atorvastatin

treatment in patients with type 1 diabetes. Diabetes & vascular disease research : official journal of the International Society of Diabetes and Vascular Disease. 2013;10(6):483-8.

191. Steppich BA, Braun SL, Stein A, Demetz G, Groha P, Schomig A, et al. Plasma TF activity predicts cardiovascular mortality in patients with acute myocardial infarction. Thrombosis journal. 2009;7:11.

192. Morange PE, Blankenberg S, Alessi MC, Bickel C, Rupprecht HJ, Schnabel R, et al. Prognostic value of plasma tissue factor and tissue factor pathway inhibitor for cardiovascular death in patients with coronary artery disease: the AtheroGene study. Journal of thrombosis and haemostasis : JTH. 2007;5(3):475-82.

193. Pawlak K, Tankiewicz J, Mysliwiec M, Pawlak D. Tissue factor/its pathway inhibitor system and kynurenines in chronic kidney disease patients on conservative treatment. Blood Coagul Fibrinolysis. 2009;20(7):590-4.

194. Xie JX, Alderson H, Ritchie J, Kalra PA, Xie Y, Ren K, et al. Circulating CD40 and sCD40L Predict Changes in Renal Function in Subjects with Chronic Kidney Disease. Scientific reports. 2017;7(1):7942.

195. Engstrom G, Zoller B, Svensson PJ, Melander O, Persson M. Soluble urokinase plasminogen activator receptor and incidence of venous thromboembolism. Thrombosis and haemostasis. 2016;115(3):657-62.

196. Hayek SS, Sever S, Ko YA, Trachtman H, Awad M, Wadhwani S, et al. Soluble Urokinase Receptor and Chronic Kidney Disease. The New England journal of medicine. 2015;373(20):1916-25.

197. Dal Monte M, Cammalleri M, Pecci V, Carmosino M, Procino G, Pini A, et al. Inhibiting the urokinase-type plasminogen activator receptor system recovers STZ-induced diabetic nephropathy. Journal of cellular and molecular medicine. 2019;23(2):1034-49.

198. Sommerer C, Zeier M, Morath C, Reiser J, Scharnagl H, Stojakovic T, et al. Soluble urokinase plasminogen activation receptor and long-term outcomes in persons undergoing coronary angiography. Scientific reports. 2019;9(1):475.

199. Borne Y, Persson M, Melander O, Smith JG, Engstrom G. Increased plasma level of soluble urokinase plasminogen activator receptor is associated with incidence of heart failure but not atrial fibrillation. European journal of heart failure. 2014;16(4):377-83.

200. Kaminski TW, Pawlak K, Karbowska M, Mysliwiec M, Grzegorzewski W, Kuna J, et al. Association between uremic toxin-anthranilic acid and fibrinolytic system activity in predialysis patients at different stages of chronic kidney disease. International urology and nephrology. 2018;50(1):127-35.

201. Loghmani H, Conway EM. Exploring traditional and nontraditional roles for thrombomodulin. Blood. 2018;132(2):148-58.

202. Martin FA, Murphy RP, Cummins PM. Thrombomodulin and the vascular endothelium: insights into functional, regulatory, and therapeutic aspects. American journal of physiology Heart and circulatory physiology. 2013;304(12):H1585-97.

203. Chong AY, Freestone B, Patel J, Lim HS, Hughes E, Blann AD, et al. Endothelial activation, dysfunction, and damage in congestive heart failure and the relation to brain natriuretic peptide and outcomes. The American journal of cardiology. 2006;97(5):671-5.

204. Salomaa V, Matei C, Aleksic N, Sansores-Garcia L, Folsom AR, Juneja H, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and

symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. Lancet (London, England). 1999;353(9166):1729-34.

205. Kosaki R, Minoura Y, Ogura K, Oishi Y, Tanaka L, Arai K, et al. Thrombomodulin can predict the incidence of second events in patients with acute coronary syndrome: Single-center, retrospective cohort study. Journal of cardiology. 2018;72(6):494-500.

206. Ishimitsu T, Ono H, Minami J, Matsuoka H. Pathophysiologic and therapeutic implications of adrenomedullin in cardiovascular disorders. Pharmacology & therapeutics. 2006;111(3):909-27.

207. Yuyun MF, Narayan HK, Ng LL. Prognostic significance of adrenomedullin in patients with heart failure and with myocardial infarction. The American journal of cardiology. 2015;115(7):986-91.

208. Persson F, Rossing P, Parving HH. Direct renin inhibition in chronic kidney disease. British journal of clinical pharmacology. 2013;76(4):580-6.

209. Volpe M, Battistoni A, Chin D, Rubattu S, Tocci G. Renin as a biomarker of cardiovascular disease in clinical practice. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2012;22(4):312-7.

210. Satou R, Shao W, Navar LG. Role of stimulated intrarenal angiotensinogen in hypertension. Therapeutic advances in cardiovascular disease. 2015;9(4):181-90.

211. Rooth E, Wallen NH, Blomback M, He S. Decreased fibrin network permeability and impaired fibrinolysis in the acute and convalescent phase of ischemic stroke. Thrombosis research. 2011;127(1):51-6.

212. Larsson P, Alwis I, Niego B, Sashindranath M, Fogelstrand P, Wu MC, et al. Valproic acid selectively increases vascular endothelial tissue-type plasminogen activator production and reduces thrombus formation in the mouse. Journal of thrombosis and haemostasis : JTH. 2016;14(12):2496-508.

213. Lee ST, Chu K, Jung KH, Kim JM, Moon HJ, Bahn JJ, et al. Circulating CD62E+ microparticles and cardiovascular outcomes. PloS one. 2012;7(4):e35713.

214. deFilippi CR, Herzog CA. Interpreting Cardiac Biomarkers in the Setting of Chronic Kidney Disease. Clinical chemistry. 2017;63(1):59-65.

215. Siriwardhana C, Lim E, Davis J, Chen JJ. Progression of diabetes, ischemic heart disease, and chronic kidney disease in a three chronic conditions multistate model. BMC public health. 2018;18(1):752.

216. Serebruany VL, Tomek A, Pokov AN, Kim MH. Clopidogrel, prasugrel, ticagrelor or vorapaxar in patients with renal impairment: do we have a winner? Expert review of cardiovascular therapy. 2015;13(12):1333-44.