

## Full Review

# Haemostasis in chronic kidney disease

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## ABSTRACT

The coagulation system has gained much interest again as new anticoagulatory substances have been introduced into clinical practice. Especially patients with renal failure are likely candidates for such a therapy as they often experience significant comorbidity including cardiovascular diseases that require anticoagulation. Patients with renal failure on new anticoagulants have experienced excessive bleeding which can be related to a changed pharmacokinetic profile of the compounds. However, the coagulation system itself, even without any interference with coagulation modifying drugs, is already profoundly changed during renal failure. Coagulation disorders with either episodes of severe bleeding or thrombosis represent an important cause for the morbidity and mortality of such patients. The underlying reasons for these coagulation disorders involve the changed interaction of different components of the coagulation system such as the coagulation cascade, the platelets and the vessel wall in the metabolic conditions of renal failure. Recent work provides evidence that new factors such as microparticles (MPs) can influence the coagulation system in patients with renal insufficiency through their potent procoagulatory effects. Interestingly, MPs may also contain microRNAs thus inhibiting the function of platelets, resulting in bleeding episodes. This review comprises the findings on the complex pathophysiology of coagulation disorders including new factors such as MPs and microRNAs in patients with renal insufficiency.

## INTRODUCTION

The introduction of new anticoagulants into clinical practice has again shed light on the problem of coagulation disorders in patients with chronic renal failure as these patients can experience severe bleeding disorders during a therapy with such new compounds. A major problem is the prolonged half-life of some new substances due to pharmacokinetic changes namely accumulation of the compounds during renal failure. Moreover, even without coagulation-modifying compounds, the function of the coagulation system itself is already profoundly changed in patients with renal failure, as they are prone to episodes of prolonged bleeding. On the other hand, they may also develop excessive formation of thrombi [1]. Bleeding disorders are the result of insufficient function of platelets, the coagulation cascade and/or activation of the fibrinolytic system, while hypercoagulability is rather the result of disorders of the coagulation regulatory factors as well as platelet hyperreactivity [1, 2]. Little is known so far about the reasons why one patient develops bleeding problems, while another tends to head towards excessive thrombus formation. However, both problems are of significant clinical relevance as some patients can be endangered by fatal bleeding episodes such as prolonged bleeding from the dialysis fistula, gastrointestinal bleeding or cerebral haemorrhage, while other patients experience a prothrombotic status associated with an increased number of cardiovascular events or

recurrent thrombosis of the dialysis access with insufficient dialysis quality [2]. The reasons for these disorders are complex and involve the coagulation cascade, the fibrinolytic system, the platelets, the endothelium, or, the vessel wall with its extracellular matrix. The relationship between these components is influenced by uraemic toxins and metabolic compounds accumulating during renal insufficiency [3]. Furthermore, patients with renal insufficiency suffer from a varying degree of inflammation, which also influences haemostasis [4]. Structural changes in the vessel wall related to arteriosclerosis may also influence coagulation [5]. Thus, patients with renal failure have a complex disturbance of their coagulation system, which makes them prone to severe bleeding episodes or thromboembolic events. If this already heavily disturbed system is further deranged by anticoagulants, potentially fatal sequelae can result particularly if one considers that anticoagulants may accumulate in patients with renal insufficiency due to their reduced renal elimination [6].

Altogether, patients with renal insufficiency are prone to coagulation disorders due to the complex interactions of uraemic toxins, morphologically changed vessel walls including the endothelium with the coagulation cascade and platelets, which is further complicated by anticoagulants that have the potential to accumulate in these patients.

## INCREASED RISK OF BLEEDING

Bleeding has been reported in 40–50% of patients with chronic renal failure or on haemodialysis (HD) [7, 8]. Another study reported bleeding events in 24% of patients on HD [9]. A hospital-based study showed that the risk of bleeding episodes is increased ~2-fold in patients with renal failure [10]. Clinically, an increased bleeding tendency in patients with renal failure may present as gastrointestinal bleeding, bleeding from cannulation sites, retinal haemorrhage, subdural haematoma, epistaxis, haematuria, ecchymosis, purpura, bleeding from the gums, gingival bleeding, genital bleeding, haemoptysis, telangiectasia, haemarthrosis and petechiae (Table 1) [7, 8].

What could be the pathophysiological basis for the increased risk of bleeding in patients with renal failure?

### Platelets

It has been shown that the platelet function is insufficient in patients with severe renal impairment [1, 5]. A well-recognized abnormality in platelet physiology contributing to platelet dysfunction with bleeding problems in patients with renal failure is the disturbance of the platelet  $\alpha$ -granules [11, 12]. They contain platelet factor 4, transforming growth factor- $\beta$ 1, platelet-derived growth factor, fibronectin, B-thromboglobulin, von Willebrand factor (vWF), fibrinogen, serotonin and coagulation factors V and XIII. In uraemic patients, the  $\alpha$ -granules have an increased ATP/ADP ratio and a reduced content of serotonin. Furthermore, the thrombin-triggered release of ATP together with an increased calcium content and a disturbed intracellular calcium flux upon several stimuli has been related to platelet dysfunction and bleeding in uraemic patients [11]. Platelets of uraemic patients also demonstrate a

**Table 1. Clinical presentation of bleeding and thrombosis in patients with renal insufficiency**

Bleeding	Gastrointestinal bleeding Bleeding from cannulation sites Retinal haemorrhage Subdural haematoma Epistaxis Haematuria Ecchymosis Purpura Bleeding from the gums Gingival bleeding Genital bleeding Haemoptysis Telangiectasia Haemarthrosis Petechiae
Thrombosis	Deep venous thrombosis PE HD vascular access thrombosis Central venous catheter thrombosis Central vein thrombosis Right atrial thrombus Acute coronary syndrome Cerebrovascular event Peripheral artery occlusion

deregulated arachidonic acid and prostaglandin metabolism with an impaired synthesis and/or release of thromboxane  $A_2$  resulting in a reduced adhesion and aggregation of platelets with following bleeding disorders [11, 13], which can be reversed by dialysis, suggesting that uraemic toxins are related to this effect [14]. In addition, ultrafiltrates collected from uraemic patients inhibited platelet-activating factor synthesis that could account for the decreased platelet activity [15].

Furthermore, circulating fibrinogen fragments have been demonstrated that can also interfere with haemostasis as they competitively bind to the glycoprotein (GP) IIb/IIIa receptor on platelets resulting in a decreased adhesion and aggregation potential of platelets [16].

Haemodialysis represents a particular situation that influences the whole organism including platelets. First, a number of uraemic toxins, such as phenol, phenolic acid (impairment of primary aggregation to ADP), guanidinosuccinic acid (inhibition of the second wave of ADP-induced platelet aggregation), influence platelet function [17–19]. However, a correlation between the bleeding time and the concentration of the dialysable uraemic metabolites was not detected [20]. Interestingly, HD itself can contribute to the bleeding disorder, not only due to the administered heparin but also due to continuous platelet activation at the dialyser membrane with following decreased activity dialyser [21]. On the other hand, HD has been shown to improve platelet abnormalities resulting in a reduced risk of bleeding due to the removal of uraemic toxins [22].

An increased calcium content together with an abnormal calcium mobilization upon different stimuli can also contribute to the decreased activity of platelets in uraemic patients. Due to disturbances in the calcium metabolism, parathyroid hormone was assumed to be involved in these disturbances. Although parathyroid hormone has been shown to inhibit platelet aggregation *in vitro*, clinical investigations demonstrated no effect of parathyroid hormone on the bleeding time in patients with renal failure [23].

Furthermore, oxidative stress as well as inflammation, both conditions known to be present in patients with renal failure, have a profound effect on platelet function [24].

### Platelet–vessel wall interactions

Binding of platelets to the vessel wall is mediated by the adhesion proteins fibrinogen and vWF and the receptors GP Ib as well as the GP IIb/IIIa complex [1]. A decreased amount of GP Ib on platelets have been observed in uraemic patients [7], which was attributed to the probable high proteolysis of GP Ib under these circumstances [25]. The insufficient binding of vWF and fibrinogen to activated platelets from uraemic patients can be responsible for the decreased function of the GP IIb/IIIa complex. The binding problems can be related to a uraemic toxin interfering with binding since this defect improves with dialysis [26–28].

In addition, a functional defect in vWF–platelet interaction can be related to an increased bleeding tendency since cryoprecipitates (rich in vWF) and desmopressin (release of vWF from storage sites in platelets) can correct this problem in uraemic patients [29, 30].

Moreover, vasoactive substances such as nitric oxide (NO), inhibiting platelet aggregation through the formation of cGMP, or prostacyclin, which modulates vascular tone, can also play a role in defective haemostasis in renal failure. Plasma levels of prostacyclin, NO generation of platelets and the concentration of NO metabolites are increased in the plasma of uraemic patients, thus contributing to dysfunctional haemostasis with an increased bleeding risk [31, 32]. All these changes can be related to a factor present in uraemic plasma.

### Anaemia

An important factor in the development of bleeding disorders in uraemic patients is renal anaemia itself [33]. In patients with renal failure, anaemia directly influences the bleeding time [34, 35].

Erythrocytes lead to a concentration of platelets along the vessel walls within the blood flow together with a stimulation of platelet ADP release and an inactivation of PGI<sub>2</sub>, thus stimulating platelet function [36]. Furthermore, haemoglobin is a scavenger of NO [37]. Thus, anaemia reduces platelet function by a reduced platelet–vessel wall interaction, reduced ADP release/inactivation of PGI<sub>2</sub> as well as reduced scavenging of NO.

These findings are supported by observations in uraemic patients where the administration of erythrocytes [38] or erythropoietin [39, 40] reduced the bleeding time. However, a normal haematocrit was associated with an increased incidence of myocardial infarction and a higher mortality [41]. So

far, the optimal haematocrit in patients on dialysis needs to be determined in order to avoid bleeding disorders on one hand and thrombotic events on the other hand.

### Drugs

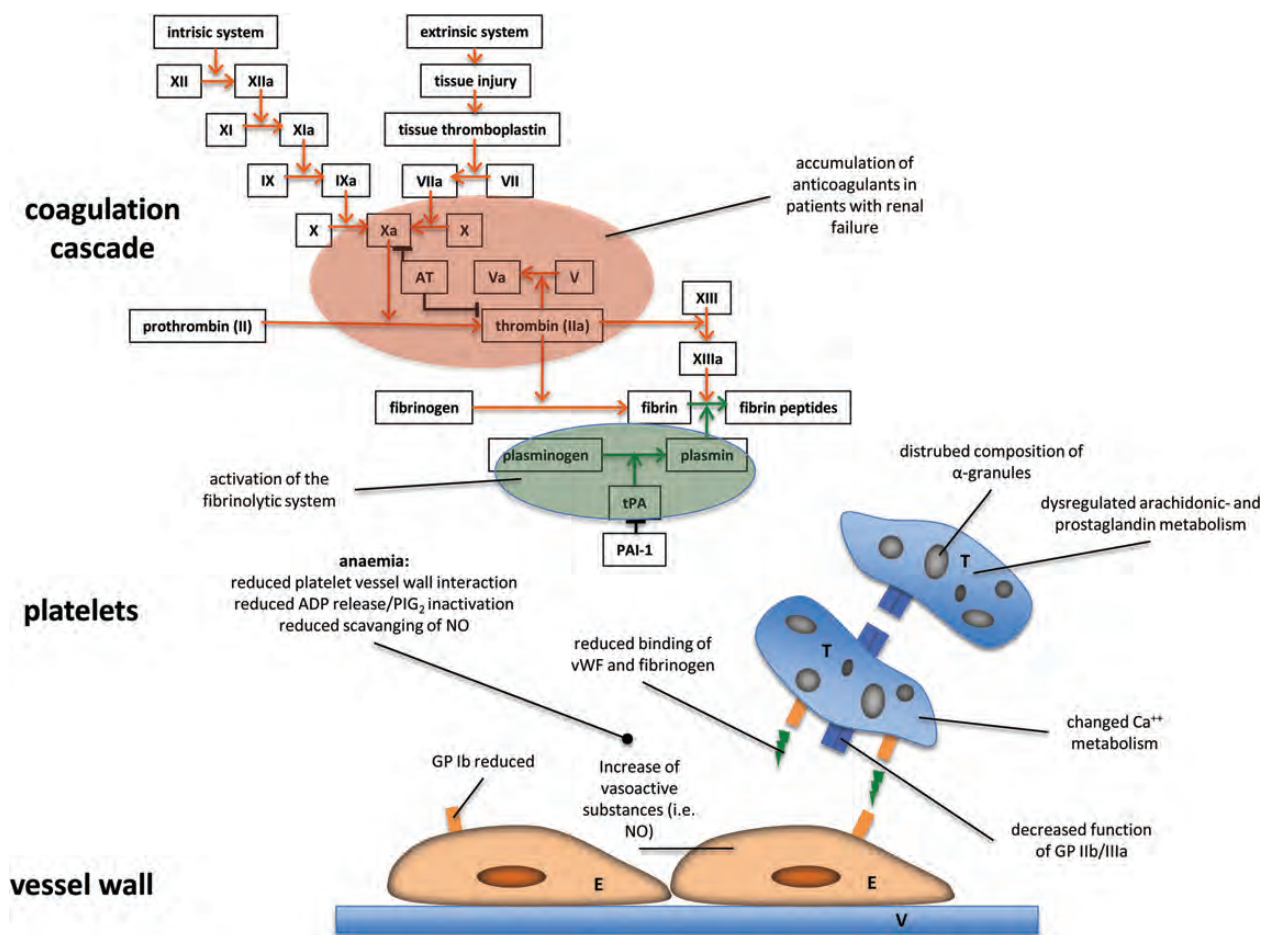
Drug interactions with platelets have fundamental effects on platelet function and thus on bleeding disorders or abnormal aggregation of platelets in patients with renal failure. Antibiotics such as third-generation cephalosporines and  $\beta$ -lactam antibiotics play an important role under these circumstances [42, 43].  $\beta$ -Lactam antibiotics can interact with the function of platelet membranes through interference with ADP receptors. These effects are related to dose and duration of the therapy. Aspirin is also a common drug used in patients with renal failure due to the high prevalence of cardiovascular disorders and the prevention of vascular dialysis access thrombosis. Aspirin has been shown to prolong the bleeding time more pronounced in patients with renal failure when compared with a control group with normal kidney function [44]. Furthermore, other non-steroidal anti-inflammatory drugs also alter platelet function through the inhibition of cyclooxygenase although this is promptly reversible after discontinuation of the drug.

As many anticoagulants are eliminated by the kidney, it is clear that they can accumulate if their dose is not properly adapted to the renal function of the patient [6]. Anticoagulants that can accumulate in patients with renal impairment include low-molecular weight heparins (LMWHs), direct factor Xa inhibitors like danaparoid and fondaparinux as well as the direct thrombin inhibitors refudan and dabigatran. So far, one has to be cautious with dosing of new anticoagulants, as only limited information regarding renal elimination is available.

In summary, the increased risk of bleeding in patients with renal failure is the result of changes in platelets related to the composition of  $\alpha$ -granules, a deregulation of arachidonic acid and prostaglandin metabolism, circulating fibrinogen fragments, changed calcium content and calcium mobilization, as well as oxidative stress (Figure 1). A direct link between an enhanced activation of the fibrinolytic system and oxidative stress could be demonstrated in dialysis patients which could be a sign of a counter reaction against the activation of blood coagulation [45]. During dialysis, an additional activation of platelets within the dialysis filter contributes to the increased risk of bleeding.

Furthermore, the changed platelet–vessel wall interaction with a reduced amount of GP Ib receptor for adhesion molecules on endothelial cells, a reduced binding of vWF and fibrinogen with decreased function of the GP IIb/IIIa complex and an increased concentration of vasoactive substances (i.e. NO) also contribute to the risk of bleeding. In addition, anaemia contributes to the increased risk of bleeding in renal failure through a reduced platelet–vessel wall interaction together with a reduced ADP release/inactivation of PGI<sub>2</sub>, and a reduced scavenging of NO by haemoglobin. Moreover, bleeding episodes can be the result of an accumulation of anticoagulants in patients with renal failure.





**FIGURE 1:** Factors involved in the increased risk of bleeding in patients with renal failure (for details see the text).

## INCREASED RISK OF THROMBOSIS

The risk of venous thromboembolism is increased in patients with renal failure [8, 10, 46]. The mortality related to pulmonary embolism (PE) is greater in patients with renal failure when compared with those without renal disease [47, 48]. Thromboembolism itself has a 2-fold increased risk in patients with advanced kidney disease [49–51], while a higher risk has been shown in hospitalized patients with renal impairment [52]. The risk begins to rise when the estimated glomerular filtration rate (eGFR) falls  $<75 \text{ mL/min/1.73 m}^2$ . During early stages of chronic kidney disease (CKD), the risk of thrombosis seems to be related to albuminuria [53].

Clinical relevant thrombosis in patients with renal failure may present as deep venous thrombosis with/without PE, HD vascular access-associated thrombosis including arteriovenous graft thrombosis as well as native AV fistula thrombosis, central venous catheter thrombosis with/without central vein thrombosis, right atrial thrombus. Furthermore, thrombus formation may also occur within arteries that are often atherosclerosis-associated, and could present as acute coronary syndrome, cerebrovascular event or peripheral artery occlusion (Table 1) [46].

What are the pathophysiological pathways associated with an increased risk of thrombosis?

## Coagulation cascade

Patients with CKD have increased levels of fibrinogen that directly contribute to a hypercoagulable state. This is associated with increased levels of pro-inflammatory markers such as C-reactive protein and interleukin-6 [54, 55]. Furthermore, increased levels of plasma tissue factor (TF) have been observed in patients with renal failure [56, 57]. Apart from coagulation it can contribute also to inflammation as it can induce the proinflammatory transcription factor  $\text{Nf-}\kappa\text{B}$  as well as protease-activated receptor-1 [58]. It has also been shown that the concentrations of the coagulation factors XIIa and VIIa as well as activated protein C complex and thrombin-anti thrombin complexes are increased in patients with renal failure [59–61]. On the other hand, the activity of antithrombin is reduced [61].

A clinically important system that may be involved in the hypercoagulable state of patients with renal failure can be the renin–angiotensin–aldosterone system as its activation has been associated with increased levels of plasma fibrinogen, D-dimer and plasminogen activator inhibitor (PAI)-1 [62]. PAI-1 has been associated with an inhibition of extracellular matrix turnover, stimulation of macrophage and myofibroblast infiltration as well as the regulation of  $\text{TGF-}\beta$ , thus promoting tissue fibrosis with progression of CKD [63]. Furthermore, PAI-1 inhibits the activation of the fibrinolytic system through

inhibition of the tissue plasminogen activator (t-PA) and urokinase.

### Platelets

In patients performing peritoneal dialysis, platelets can be activated which is thought to be related to hypoalbuminaemia [64]. It has been demonstrated that in catabolic uraemic patients, reduced plasma levels of L-arginine and NO were associated with an increased platelet aggregability [24]. This can be supported through an accumulation of phenyl acetic acid, a uraemic toxin, inhibiting inducible NO synthase (iNOS) resulting in a reduced production of NO [65].

In patients with renal failure, increased levels of phosphatidylserine can be observed at the surface of platelets [66] that is related to caspase-3 activation. Phosphatidylserine binds to activated factor V that promotes binding of factor X leading to the formation of thrombin [66] with thrombus formation. Platelets of uraemic patients contain increased levels of p-selectin as well as the fibrinogen receptor PAC-1 resulting in platelet/leucocyte aggregates, followed by an increased reactivity of platelets. In addition, this mediates the formation of platelet/leucocyte aggregates, related to the formation of free oxygen radicals by neutrophil granulocytes leading to thrombus formation in patients with renal failure.

### Endothelium

The endothelium is of crucial importance for haemostasis. It is responsible for the secretion of factors modulating the coagulation cascade such as PAI-1 and vWF, participates in the regulation of the vascular tone, regulates oxidant stress and thus also inflammatory responses and produces endothelial microparticles (MPs) [3, 67]. Furthermore, it influences haemostasis through proliferation/repair processes that also include endothelial progenitor cells (EPCs) [68, 69]. The endothelium may lose its anti-thrombogenic properties if it is stimulated by thrombin, hypoxia, shear stress, oxidants, interleukin-1, tumour necrosis factor,  $\gamma$ -interferon, desmopressin acetate and endotoxin [3]. In patients with end-stage renal disease (ESRD), endothelial cell damage can lead to coagulation disorders together with thrombophilia. Homocysteine can play a role as a mediator between renal dysfunction and endothelial cell damage. It can inhibit the thrombomodulin-dependent activated protein C system that results in permanent activation of thrombin with subsequent formation of fibrin. It also interferes with endothelial release of t-PA predisposing to hypofibrinolysis. This can also be due to an impaired release of t-PA from the endothelium with an intact endothelium-dependent vasodilation.

Hyperhomocysteinaemia also interferes with subendothelial cell proliferation through metalloproteinase-inducible genes as it leads to an activation of matrix metalloproteinase-9. Again, also in this setting, increased levels of PAI-1 have been suggested as markers of endothelial cell activation. However, high plasma concentration of fibrinogen, D-dimer, thrombin-antithrombin complex, coagulation factor VII, vWF, thrombomodulin and PAI-1 can all indicate endothelial cell damage and a thrombophilic state in uraemic patients [3].

Atherosclerosis itself seems to be associated with an increased risk of the development of venous thrombosis in patients with renal failure [70]. The reason for this phenomenon could be an overlap of the respective risk factors such as obesity, hypertension, smoking, diabetes and dyslipidaemia. Furthermore, in patients with renal failure, platelets and the coagulation system could be activated in atherosclerotic vessels contributing to the formation of venous thrombosis at different vessel sites. In a recent population-based study, 26% of patients with venous thrombosis also had a history of symptomatic atherosclerosis [71]. Interestingly, microalbuminuria is also associated with the development of venous thrombosis. This could be related to the fact that microalbuminuria reflects the severity of endothelial damage which in turn can promote thrombosis [72].

### Microparticles

MPs have recently been discovered to have potent procoagulatory capacity and thus could play an important role in coagulation [67, 73]. MPs are formed from plasma membranes of many cells including endothelial cells, platelets as well as monocytes/macrophages [67]. They are the result of cell activation during inflammatory processes but also occur during physiological processes such as cell differentiation and senescence. Increased levels of MPs have been described in diseases with an increased procoagulant state such as chronic kidney insufficiency as well as cancer [67]. Their procoagulatory effects are derived from the presentation of phosphatidylserine facilitating the conversion from prothrombin to thrombin as well as the presence of TF on their surface [74–76]. Apart from membrane bound TF, the MPs also release soluble TFs further promoting coagulation resulting in excessive thrombus formation. Furthermore, MPs could influence coagulation by another mechanism, which is through the recently discovered microRNAs (miRNAs) [67]. miRNAs are small non-coding single-strand RNAs that modulate target gene expression by post-transcriptional modulation and are expressed in the majority of cells. The connection between miRNAs and the coagulation system is not clear so far. However, some data exist, linking miRNAs to the function of platelets through regulation of platelet mRNA translation. Here, the expression of the P2Y<sub>12</sub> receptor, that is important for the ADP-stimulated activation of the GP IIb/IIIa receptor resulting in prolonged platelet aggregation, is regulated through miRNAs [77]. Vesicle-associated membrane protein 8 (VAMP8) is important for the secretion process of platelets with hyperreactive platelets demonstrating increased VAMP8 levels, while hyporeactive platelets show decreased levels [78]. Thus, it could be shown that the concentration of miRNA96 was 2.6 times higher in hyporeactive platelets. How such regulation processes are influenced in renal failure is not known so far but it is tempting to speculate that an important influence exists.

As MPs and miRNAs could regulate platelet activity, this also brings the platelet proteome/transcriptome into focus. Contact of platelets to uraemic toxins and artificial surfaces during HD could lead to a change in the platelet proteome, which could be a result of an altered platelet transcriptome. Interestingly, analysis of the platelet mRNA and miRNA

transcriptome revealed alterations when compared with healthy subjects. Dialysis seemed to correct the levels of some mRNAs and most miRNAs. Here, analysis of hsa-miR-19b, a miRNA involved in the regulation of platelet reactivity through phosphatidylcholine transfer protein and WD repeat-containing protein 1, was increased in platelets of uraemic patients. This suggests that altered miRNA-based mRNA regulatory mechanisms could influence the platelet response to uraemia leading to platelet-related complications in CKD [79].

### Antiphospholipid antibodies

Antiphospholipid antibodies (lupus anticoagulant, cardiolipin antibodies) can be detected in many patients on HD [80, 81]. Their significance in patients with renal failure with or without HD is not clear so far. They could represent a risk factor for thrombosis, particularly vascular access thrombosis in this group of patients. One group detected an increased prevalence of IgG anticardiolipin antibodies in patients with recurrent vascular access thrombosis [82]. On the other hand, the presence of antiphospholipid antibodies could be an epiphenomenon of aspirin use or simply HD in these patients. Even vascular access stenosis but not thrombosis has been associated with the presence of such antibodies [83]. Furthermore, an increased prevalence of anti-protein C and anti-protein S has been observed in HD patients with vascular access thrombosis [84]. Again, the pathogenic significance of this observation is not clear so far. Anti-protein C antibodies would result in an increased activity of factor VII and potentially also factor Va resulting in an increased formation of thrombin leading to thrombus formation [3].

In conclusion, the increased risk of thrombosis in patients with renal failure is related to changes in the coagulation cascade with increased levels of fibrinogen and plasma TF, factor XIIa and VIIa, F VIII, activated protein C complex, thrombin–antithrombin complexes, D-dimers, prothrombin fragments 1 + 2 (F1 + 2) and a reduced activity of antithrombin (Figure 2). Furthermore, the activity of platelets is increased by a reduced amount of vasoactive substances (i.e. NO), increased levels of phosphatidylserine, p-selectin, and fibrinogen receptor PAC-1. Changes of the endothelium also contribute to the risk of thrombosis by increased thrombin, hypoxia, shear stress, oxidants and cytokines. A new mechanism is related to MPs that promote the presentation of phosphatidylserine and TF, and also contain miRNAs potentially regulating the function of platelets. In addition, antiphospholipid antibodies can also promote thrombosis in patients with renal insufficiency (Figure 2).

## THE HAEMOSTATIC SYSTEM IN PARTICULAR CLINICAL SETTINGS OF RENAL DISEASES

### Vascular access thrombosis

Vascular access thrombosis is an important clinical problem in patients on chronic HD. The frequency of thrombosis is higher in arterial-venous grafts when compared with native arterial-venous fistulas. One of the key pathogenic

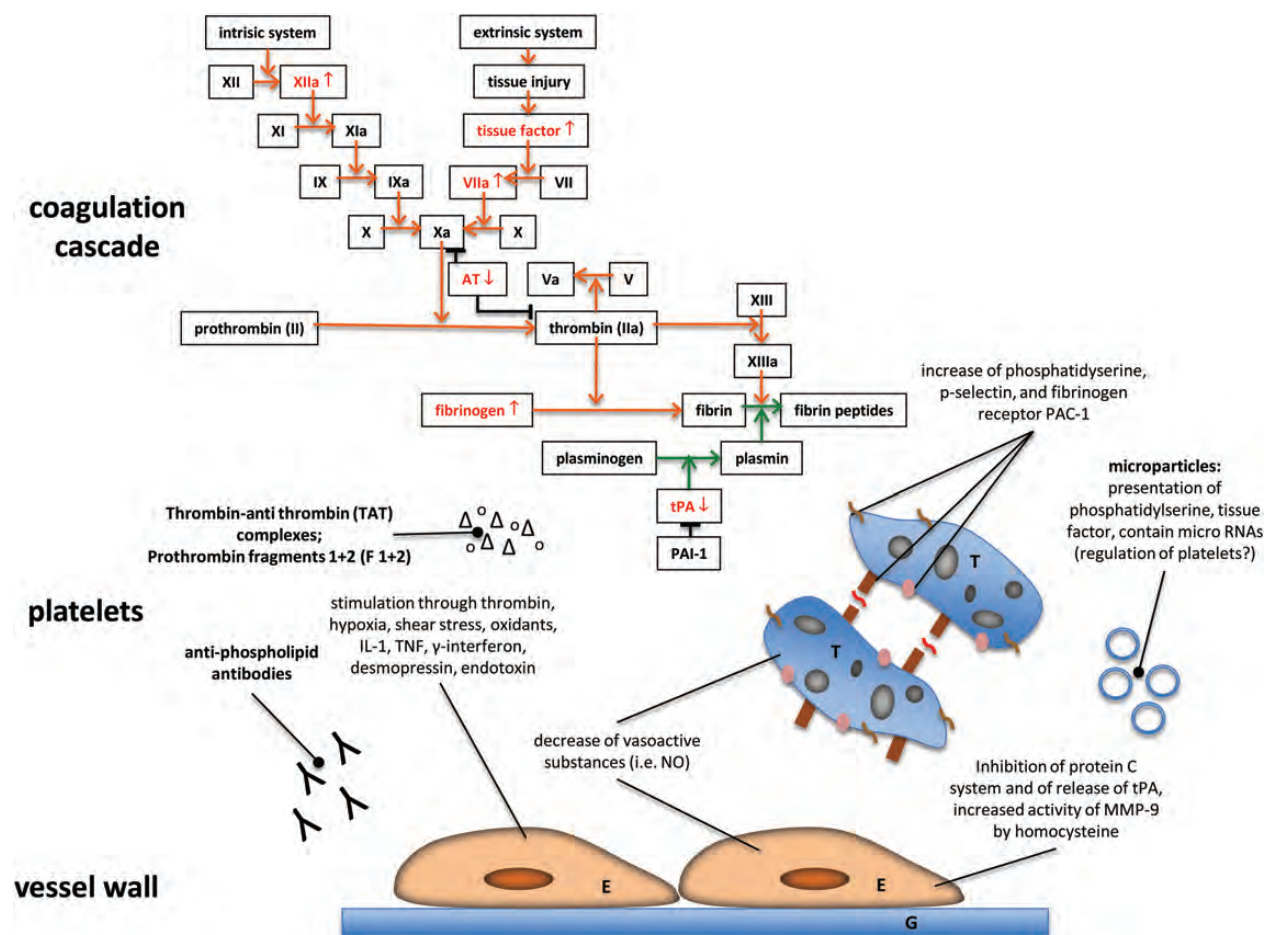
factors for the development of vascular access thrombosis is a stenosis either in the anastomosis region or the draining veins, rather than a particular alteration of the haemostatic system. The flow should be >500 mL/min for native fistulas and >600 mL/min for grafts.

A stenosis related to the vascular access can be found in 84–92% of patients with vascular access thrombosis [85]. Neointimal hyperplasia resulting from endothelial injury at the time of graft placement, shear stress from irregular blood flow and platelet activation after needle punctures add additional risk for the development of access thrombosis. However, acquired and inherited factors also play an important role such as prothrombin G20210A, antiphospholipid antibodies (lupus anticoagulants, cardiolipin antibodies), lack in antithrombin, protein C, protein S, factor V Leiden mutation, as well as polymorphisms in the TGF- $\beta$ 1 gene, NO synthase, PAI-1, angiotensin-converting enzyme, methylene tetrahydrofolate reductase and HO-1. Moreover, cardiovascular risk factors such as diabetes mellitus, obesity, nicotine abuse, arterial hypertension, hyperhomocysteinaemia, hyperlipoproteinaemia including elevated lipoprotein a. Other factors include low serum albumin, erythropoietin administration, malnutrition, calcium/phosphate deposition, cytomegalovirus infection [86, 87]. These factors act together with an endothelial cell damage, blood stasis and hypercoagulability resulting in vascular access thrombosis. Prognostic factors affecting the patency of vascular accesses include mechanical factors such as the operation technique, mode of puncture of the access as well as shear stress on the endothelium. Furthermore, clinical factors such as stasis (influenced by hypotension, hypoalbuminaemia and compression) influence the generation of vascular access thrombosis. Furthermore, medications such as angiotensin converting enzyme inhibitors, calcium channel blockers, platelet inhibitors (e.g. aspirin, dipyridamol, clopidogrel, prasugrel, ticlopidine, ticagrelor, IIb/IIIa receptor antagonists) vitamin K-antagonists (e.g. warfarin) influence vascular access patency.

### Nephrotic syndrome

Nephrotic syndrome is a particular disorder that is not directly related to renal failure but rather to a structural damage of the glomerular basement membrane with damage to podocytes in certain glomerulopathies leading to severe proteinuria >3.5 g/day. Patients present with proteinuria, oedema, hypercholesterinaemia/hypertriglyceridaemia and hypoalbuminaemia. The patients have an increased risk of thrombosis (particularly renal vein thrombosis, deep vein thrombosis) leading to an increased risk of PE as well as an increased rate of infectious complications due to a deficit in antibodies that are lost through the injured glomeruli. The increased risk of thrombosis is related to an imbalance between pro- and anti-coagulatory factors with pro-coagulatory factors outweighing the anti-coagulatory factors related to an imbalanced production in the liver leading to an increased formation of thrombosis [88]. Therapy consists of the application of heparins or LMWHs. However, this is only effective if the AT (anti-thrombin) is >60%. On a long-term basis, the patients should be treated with warfarin. Basically, the renal





**FIGURE 2:** Factors involved in the increased risk of thrombosis in patients with renal failure (for details see the text).

histomorphologic diagnosis should be confirmed by biopsy. A definitive treatment can be achieved if treatment of the underlying renal disease with a substantial reduction of the proteinuria is successful.

### Heparin-induced thrombocytopenia type II (HIT II)

HIT II is a prothrombotic state related to the formation of platelet-activating antibodies that are directed against complexes of platelet factor 4 and heparin. Despite low platelets HIT II leads to thromboembolic events (venous > arterial thromboembolism) in 50–60%. Patients on dialysis are at particular risk for the development of HIT II as they receive heparin as an anticoagulant during dialysis at a regular basis. However, HIT II is not as frequent among patients receiving HD as one might suggest with a reported frequency between 0.5 and 3–5% [89]. The incidence is strongly influenced by the nature of the heparin fraction, the dosage and the duration of administration. After administration of LMWH, the HIT II-incidence is 10 times lower than after administration of unfractionated heparin. The HIT II-incidence depends furthermore on patient-related factors (surgical patients > medical patients). Interestingly, the risk for development of HIT II is low in non-surgical populations (critically ill patients and patients receiving HD) and very low in pregnant women [89, 90]. Platelet-activating antibodies (HIT-II-antibodies) do

not necessarily lead to clinically apparent HIT II. So far, it is not clear what makes an individual susceptible for the development of platelet-activating antibodies and furthermore, why do only a minority of these patients with HIT II-antibodies develop a clinical manifest HIT II [91]. However, it does not seem that the renal insufficiency itself or uraemic toxins play a role in this respect. If anticoagulation must be initiated in patients with HIT, argatroban or regional citrate anticoagulation may be used as alternative anticoagulants instead of heparins. Danaparoid (HIT II could progress under therapy with danaparoid), lepirudin (has been taken from the market) or fondaparinux (not licensed for HIT II therapy) should not be used in patients with renal failure as they also may heavily accumulate in these patients. The new oral anticoagulants dabigatran, rivaroxaban and apixaban are contraindicated in patients with ESRD (ESC Clinical Practice Guidelines update 2012). Dabigatran can be eliminated via HD in cases of overdosing with severe bleeding complications.

### Anticoagulation strategies during haemodialysis

Unfractionated heparin (UFH) is not a single molecule but a composition of more than 120 different molecules among them different glycosaminoglycans consisting of sulphated D-glucosamine and D-glucuronic acid units. Thus, pharmacological purity cannot be determined. Its action is rapid

(between several minutes), while its half-life ranges from 0.5 to 2 h. As UFH is highly negatively charged, it can bind non-specifically to plastic tubes or the dialyser surface. Usually, UFH is administered with an initial bolus followed by a maintenance dose. Its dose should be reduced and only the maintenance dose be administered in case of a bleeding risk. If overdosing occurs, UFH can be antagonized with protamine chloride or sulphate.

LMWHs have a significantly lower binding activity against thrombin (factor IIa), while their anti-Xa activity is not impaired. Their half-lives are longer than that of UFH due to their renal clearance. Usually, they are administered as single boli; whether a bolus needs to be repeated during dialysis depends upon the particular LMWH and the particular half-life. In contrast to UFH, only 50% of the LMWH can be antagonized by protamine. The incidence of HIT II might be lower with LMWH when compared with UFH. Heparinoids such as danaparoid or fondaparinux should not be used during dialysis as they massively accumulate in patients with renal failure leading to bleeding complications.

Direct thrombin inhibitors such as argatroban and formerly lepirudin (has been removed from the market recently) are alternatives to heparins particularly in patients with HIT II [90, 92]. Again, argatroban should be preferred as it can be monitored by the aPTT, while lepirudin accumulates in patients with renal failure. However, dose adjustment can also be necessary with argatroban in patients with renal failure who additionally develop cardiac failure [93]. As it is hepatically eliminated, its dose must also be adjusted in patients with liver insufficiency.

Regional citrate anticoagulation is an alternative to the systemic administration of other anticoagulants particularly UFH or LMWH [94, 95]. It leads to an anticoagulation effect only in the dialyser circuit while no anticoagulation occurs in the patient. This is particularly suitable for patients at risk for bleeding (i.e. after surgery, biopsies, gastric ulcer, oesophageal varicose) or with HIT. The problems related to citrate anticoagulation are metabolic alkalosis (reduce bicarbonate concentration in the dialysis fluid) and non-metabolism of citrate (i.e. hepatic failure, isolated ultrafiltration) that should be treated with a reduced administration of citrate or a termination of the citrate administration.

### Impact of peritoneal dialysis on the haemostatic system

As more proteins including also factors regulating haemostasis are lost via the peritoneum in peritoneal dialysis, patients on peritoneal dialysis could be affected by disturbances of the haemostatic system in another way as patients on HD. Indeed, thrombosis seems to occur more often in peritoneal dialysis when compared with HD [96, 97].

Continuous ambulatory peritoneal dialysis (CAPD) patients have a lower activity of plasma tPA, together with an increase in PAI-1 levels in dialysis solutions. This could contribute to the development of thrombosis together with the formation of fibrin on the peritoneal epithelium promoting the development of peritoneal fibrosis [98]. A further study analysed CAPD patients together with healthy controls. CAPD patients demonstrated elevated levels of prothrombin

fragments, disclosing an activation of coagulation. The t-PA activity, PAI activity and plasminogen were similar to that in controls, suggesting that slight secondary activation of fibrinolysis due to coagulation activation happened [99]. Platelet aggregation was not increased in CAPD patients. Altogether, a pro-thrombotic tendency due to chronic low-grade activation of the coagulation system is present in the plasma of CAPD patients. Here, the fibrinolytic system and platelets did not contribute to the pro-thrombotic state.

Coagulation inhibitors and fibrinolytic parameters were studied in 12 patients on CAPD and 10 patients on HD. Patients on CAPD exhibited higher levels of ATIII and proteins C and S than those on HD. No significant differences were noted in tPA and PAI levels. Both groups of patients showed higher levels of tPA than controls. Besides, patients on HD had significantly lower levels of ATIII and protein C than controls. PAI levels in both patient groups were similar to those of the controls, but tPA levels were higher in patients than in controls. These results indicate that HD is associated with marked diminution in the circulating levels of coagulation inhibitors. This is in contrast to CAPD patients who showed elevated levels of these inhibitors, despite their significant loss in the dialysate. The finding of enhanced fibrinolysis in both patient groups may be a natural protective mechanism against the development of a thrombotic tendency [100].

Altogether, comparing peritoneal dialysis with HD in terms of haemostasis is difficult as only few studies directly compared patients on peritoneal dialysis with those on HD.

## DIAGNOSTIC TESTS

### Skin bleeding time

The skin bleeding time can be evaluated by the cutaneous bleeding time test, thus identifying uraemic patients at risk for bleeding problems. Under standardized conditions with a sphygmomanometer at the forearm inflated to 40 mmHg to control for the venous pressure two standardized incisions are set at the volar aspect of the forearm [101]. The time until the bleeding stops is assessed by blotting of the blood that emerges from the wounds with a filter paper. An analysis on uraemic patients in whom dialysis was initiated revealed that the skin bleeding time was significantly shorter after the initiation of dialysis when compared with the values before dialysis [101].

### Platelet function analyser (*in vitro* closure time test)

Platelet function can easily be evaluated using a platelet function analyser that quantitatively measures platelet-dependent haemostasis in relation to shear stress [102, 103]. In dialysis patients, this method has been proven safe and reliable as it does not depend on many factors like the tests measuring the skin bleeding time [102, 103]. The analysis of platelet function with the platelet function analyser in 45 dialysis patients showed that platelet function was significantly improved after HD [102]. However, predicting the individual bleeding risk of a particular patient is difficult as so far no analysis of bleeding episodes in relation to the results of particular diagnostic tests



exist. Altogether, it is possible to identify patients with disturbed haemostasis and renal failure but what the results mean in terms of a specific risk of bleeding related to a certain intervention (i.e. kidney biopsy) is not clear so far.

### Platelet aggregation test

Platelet aggregation has also been successfully measured with a whole-blood aggregometer based on the screen filtration pressure method in HD patients [104]. The analysis included non-diabetic and diabetic patients as well as a control group of healthy persons. Non-diabetic HD patients had a significantly reduced platelet aggregation than the controls. As expected, the use of antiplatelet agents again reduced platelet aggregation. Thus, analysis of platelet function using an aggregometer could be a method to identify patients with renal failure at risk for bleeding complications.

### Activating clotting time (ACT)

The activating clotting time (ACT) is a point of care test for monitoring anticoagulant effects of UFH, LMWH and argatroban. In this test, fresh, whole blood is added to a tube containing a surface activator. The results represent the activation of coagulation via the intrinsic pathway (FXII). The ACT is less precise than the aPTT or anti-Xa values [105, 106]. However, as many patients with renal failure experience disturbances at the level of platelets or platelet–vessel wall interaction, only coagulation problems at the levels of the intrinsic pathway would be discovered which would represent only a minor proportion of patients.

## PROCOAGULATORY VERSUS ANTICOAGULATORY EFFECTS OF CHRONIC RENAL INSUFFICIENCY

It is not clear so far which patient with renal insufficiency is more prone to bleeding problems and which patient has an increased risk of developing thrombosis. Furthermore, it is also difficult to define a main or superior pathogenic factor responsible for a bleeding or clotting tendency in patients with renal failure. Certainly further factors apart from the coagulation system itself play a role in the development of these disorders in patients with renal insufficiency. Comorbid conditions such as high blood pressure together with thrombopenia and the administration of anticoagulants can result in an increased risk of bleeding. On the other hand, stenosis within dialysis access fistulas as well as in central veins particularly as a result of a previous placement of a subclavian catheter may result in an increased risk of thrombus formation.

## CONCLUSIONS

Chronic renal insufficiency influences haemostasis through different mechanisms which result either in an anticoagulatory state characterized by recurrent thrombosis or in a procoagulatory state characterized by episodes of bleeding. The risk of developing either thrombosis or bleeding might be associated

with the severity of renal dysfunction with advanced renal failure being associated with bleeding, while renal failure with moderately preserved renal function is more associated with thrombosis. The picture is further complicated through anticoagulatory drugs that need to be administered in patients with renal insufficiency as they suffer from significant cardiovascular comorbidity requiring anticoagulation. Here, some direct and indirect (i.e. fondaparinux) Xa inhibitors as well as thrombin inhibitors can accumulate particularly in patients with renal impairment that makes dose adaptation mandatory. Further research is necessary to identify the factors accumulating in patients with CKDs that interfere with haemostasis. With respect to this, the role of MPs and miRNAs must also be evaluated in order to identify specific targets to restore haemostasis in patients with CKDs.

These findings suggest that bleeding events in patients with impaired renal function receiving anticoagulant treatment may involve both pharmacokinetic alterations for such agents and intrinsic alterations of coagulation associated with an impaired renal function. While the relative role of both components remains to be determined, anticoagulant drugs should be well chosen and carefully dosed in patients with renal impairment. Monitoring is helpful particularly to detect accumulation and to optimize anticoagulant therapy.

Future research should be directed towards the identification of the factors and their specific site of interaction within the haemostatic system in order to specifically treat the related clinical problems. Furthermore, research is needed to better understand under which conditions patients with renal failure develop bleeding disorders or are more prone to thrombotic complications while others experience bleeding disorders and thrombotic complications during a short period of time. Moreover, the role of miRNAs and MPs for the development of disorders of the coagulation cascade in renal failure warrants further research.

## CONFLICT OF INTEREST STATEMENT

The results presented in this paper have been previously published.

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