Chapter 15
Effects of Hemodiafiltration on Platelets and Coagulation

Menso J. Nubé and Auguste Sturk

Abstract In patients with chronic kidney disease (CKD) the hemostatic balance is disturbed due to alterations in the coagulation cascade, inhibitors of the coagulation system, the fibrinolytic pathway, platelets and endothelial cells. As a result, CKD is characterized by both a bleeding tendency and a pro-thrombotic state. In hemodialysis (HD) patients, coagulation is activated during the passage of blood through the extra-corporeal circuit, which depends largely on the type of dialyzer and individual patient characteristics. Furthermore, platelets are stimulated and release their granule contents. With the exception of the platelet degranulation product beta-thromboglobulin, treatment-induced alterations in hemostatic parameters are more pronounced during HDF than HD, most probably due to a higher trans-membrane pressure (TMP) and increased hemoconcentration. In clinical practice, the required doses of both unfractionated heparin and low molecular weight heparin are higher during HDF as compared to HD.

Keywords Hemodialysis • Hemodiafiltration • Clotting • Platelets • Platelet surface marker CD62p • Thromboglobulin • Platelet factor 4 • Heparin

Introduction

Patients with chronic kidney diseases (CKD) develop a variety of hemostatic disorders during the course of their disease. Actually, derangements in opposite directions occur, as both the tendency for bleeding and clotting are increased in these patients. The underlying abnormalities include all components of the hemostatic system, i.e. the coagulation cascade, inhibitors of coagulation, the fibrinolytic

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pathway, platelets and endothelial cells, which are influenced by uremic toxins and metabolic substances accumulating in renal insufficiency [1–3]. When renal failure progresses to end-stage kidney-disease (ESKD), renal replacement therapy becomes necessary for survival. During treatment with hemodialysis (HD), the blood of the patients is exposed to the foreign materials of the extra-corporeal circuit (ECC), consisting of needles, blood lines, air bubble trap and dialyzer. In addition, blood components are subjected to the mechanical forces of the roller pump and the transmembrane pressure (TMP) within the dialyzer needed for ultrafiltration (UF). Finally, anticoagulants are administered intravenously to prevent clotting over the entire length of the ECC, especially at the dialyzer because of the large surface area and high TMP. As a result of UF, blood viscosity progressively increases during the treatment session, which may also contribute to the susceptibility for clotting. All these factors may further influence the already disturbed hemostatic balance in patients with CKD. As both TMP and hemoconcentration during postdilution hemodiafiltration (HDF) are considerable higher as compared to HD [4], it is conceivable that hemostasis is even further deranged during HDF.

Platelet Activation

Compared to healthy controls, ESKD patients have a low platelet count at the start of HD treatment. This declines even further during first passage through the extracorporeal circuit (ECC), most probably due to adherence of platelets onto the dialyzer membrane [5, 6]. Especially the fraction of immature platelets is decreased, suggesting reduced megakaryopoiesis as a cause for the low platelet number, which will be further reduced by HD.

During HD, platelets are activated, as indicated by an early upregulation of the platelet surface marker p-selectin (CD62p) [7]. Moreover, small deviations are observed in mean platelet volume, platelet distribution width and platelet large cell ration, suggesting degranulation and/or an altered balance between old (small) and young (large) platelets [6]. Electron microscopic evaluation of a blood sample from a chronic HD patient taken before the start of treatment indeed showed that the surface area of platelets is considerably smaller as compared to platelets from a healthy subject. The total area of the platelet granules called dense bodies, which contain serotonin, histamine, pyrophosphate and other low molecular weight secretion products, was also noticeably reduced as quantified by application of digital image masks (Fig. 15.1) [8]. Together, these findings suggest severe depletion of the platelet granular content, most probably as a result of the repeated dialysis treatment, which reduces their capability to function in the hemostatic process.

Release of the platelet granule content can also be estimated by measuring platelet degranulation products, such as platelet factor 4 (PF4) and beta-thromboglobulin (BTG), in the plasma. These constituents as well as all other proteins secreted by the platelet upon their activation, are localized in the so-called α-granules of the platelet. From a cross-over analysis in eight chronic HD patients who were treated

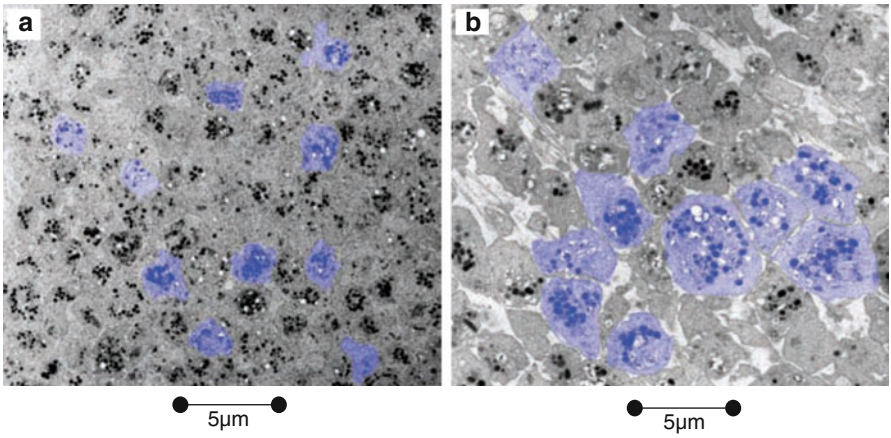

Fig. 15.1 Digital masks of electron microscopic platelet evaluation of (**a**) a HD subject and (**b**) a healthy subject. Masks are shown in *blue*. Magnification: 2500× (Reprinted from Schoorl et al. [8]. With permission from Page Press)

alternatively with unfractionated heparin (UHep), low molecular weight heparin (LMWH) or citrate during the HD procedure, it appeared that degranulation was absent during treatment with citrate but did occur with UHep and LMWH, while differences between UHep and LMWH were not observed [9]. Of note, when LMWH was administered 10 min before the start of HD, a considerable increase in PF4 was observed, while CD62p expression and BTG levels remained unaltered. After starting dialysis, CD62p expression and BTG levels showed a marked rise, while PF4 values hardly increased any further. From this study it also appeared that the increase in CD62p expression correlated with both the drop in platelet count after first passage and BTG release. Thus, treatment-induced PF4 release depends rather on heparin administration than on extracorporeal circulation and originates primarily from non-platelet derived sources, such as endothelial proteoglycans [10], and is therefore not correlated with CD62p expression on the platelet surface. By contrast, the HD-induced increase in plasma BTG levels is not affected by the administration of LMWH, but originates from platelets partially releasing their granular content during their activation in the extracorporeal circuit and is therefore highly correlated with the expression of CD62p. This platelet surface marker originates from the membrane of the α-granules and becomes exposed on the platelet surface by the fusion of that membrane with the outer membrane of the platelet during the secretory process.

Taking these findings in mind, in a subgroup of CONTRAST, platelet activation was compared between nine patients who were treated with online post-dilution HDF and ten with low-flux HD [4]. From this study it appeared that the expression of CD62p was more pronounced and more protracted during HDF (Fig. 15.2). Moreover, the drop in platelet counts in the first 30 min and the rebound after 1 h was significantly greater during this mode of treatment.

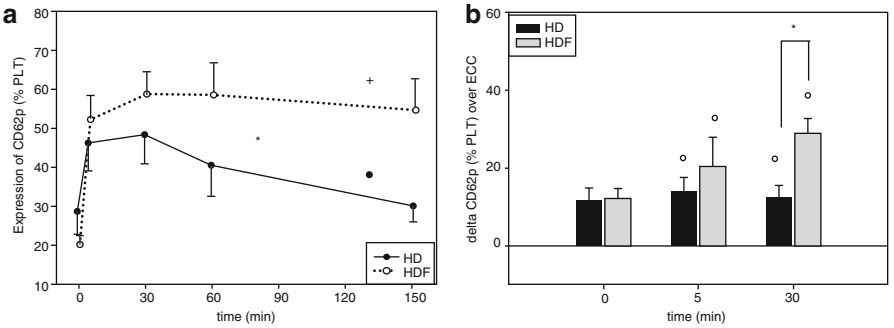

Fig. 15.2 (**a**) Platelet CD62p expression (% platelets with increased surface expression, PLT, mean ± SE) in the efferent line during HD and HDF. Changes over time were observed during both treatments: • (HD) and + (HDF): P<0.001. Difference between HD and HDF: ∗P=0.002. (**b**) Changes in PLT CD62p expression (% PLT, mean ± SE) over the ECC (delta: efferent value – afferent value) during HD and HDF: °P<0.05. Difference between HD and HDF: ∗P=0.039 (Reprinted from Gritters-van den Oever et al. [4]. With permission from Oxford University Press)

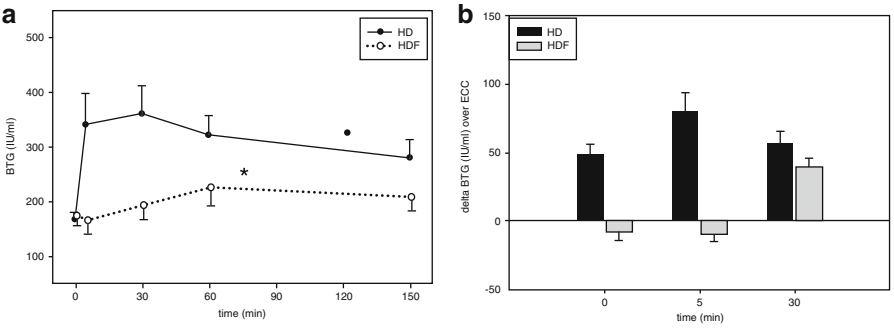

Fig. 15.3 (**a**) Plasma BTG (IU/ml, mean ± SE) concentrations in the efferent line during HD and HDF. Changes over time were observed during HD: • P=0.013. Difference between HD and HDF: *P=0.030. (**b**) Changes in BTG (IU/ml, mean ± SE) concentrations over the ECC (delta: efferent value –afferent value) during HD and HDF (Reprinted from Gritters-van den Oever et al. [4]. With permission from Oxford University Press)

As for the degranulation product PF4 (MW 29 kD), only minor differences were observed between HD and HDF, which is in line with the observation that its increase during the treatment is mainly due to LMWH-induced detachment from the endothelium. Considering BTG, however, a completely different picture emerged. In a previous study in patients who were dialyzed with low-flux HD, it was already found that BTG is almost exclusively released within the ECC [11]. In that study, BTG levels rose almost twofold over the dialyzer, with a maximum after 30 min. At the end of HD, blood levels in the arterial line were still elevated. During HDF however, BTG levels hardly changed, both over the ECC and over time (Fig. 15.3).

As the molecular weight of BTG is 36 kilo Dalton (kD), it is plausible that this substance is removed by convection during HDF, which is obvious not the case in

low-flux HD. Alternatively, it is conceivable that platelets are exhausted as a result of the repetitive stimulation by HDF treatment, and hence, incapable of further secreting granule contents. The combined data from HD and HDF patients showed a highly significant correlation between the hemoconcentration (during HDF up to 49 %) on the one hand and a drop in platelet numbers ($r=-0.82$) or platelet activation ($r=0.68$), as measured by CD62p expression, at the other. Lastly, as TMP values appeared approximately three times higher in HDF than in HD, the high pressure gradient over the dialyzer may also contribute to platelet activation [4].

Altogether, from these data it appears that, while platelet activation occurs to a similar extent during low-flux and high-flux HD [12], it is more pronounced during HDF. Both activation and trapping within the ECC are correlated with the extent of hemoconcentration within the dialyzer. It is currently unknown, however, whether the chronic platelet activation by HDF treatment is more harmful to the patients than the degree of platelet stimulation by HD. As activated platelets adhere to both intact and activated endothelium and may thus contribute to the process of atherosclerosis [13], depletion by exhaustion may prevent or delay platelets from their involvement in this process.

Activation of Coagulation

Introduction

Coagulation is generally divided into the intrinsic pathway, initiated by contact of the blood with negatively charged surfaces and leading to activated coagulation factor XII (XIIa), and the extrinsic pathway initiated by tissue factor (TF), which is exposed at sites of vascular injury and activates the coagulation system via factor VII (VIIa). Both pathways activate factor X (Xa), which together with activated factor V (Va) is capable of converting prothrombin into thrombin. Once formed, thrombin converts fibrinogen to fibrin monomers, which polymerize to form stable fibrin strands. Finally, a three dimensional network is formed by interaction of thrombin-induced factor XIII activation (XIIIa). Calcium is a cofactor required at several phases of these activation processes. The coagulation cascade is regulated by several inhibitory mechanisms, including antithrombin (AT), the protein C system and the TF pathway inhibitor (TFPI). Once a clot is formed its proteolytic degradation occurs, exerted by the plasmin system, finally leading to fibrin and thrombus dissolution (Fig. 15.4).

In HD patients, the clotting cascade is activated once the blood interacts with the ECC. Without anticoagulation, this would lead to clot formation, obstruction of the dialyzer and a reduction in the effectiveness of the dialysis procedure. In clinical practice, the tendency for clotting is reduced by the intravenous administration of unfractionated heparin (UHep) or low molecular weight heparin (LMWH). UHep inhibits the coagulation cascade by inducing a conformational change in the enzyme inhibitor AT that results in its binding to thrombin, leading to thrombin-antithrombin

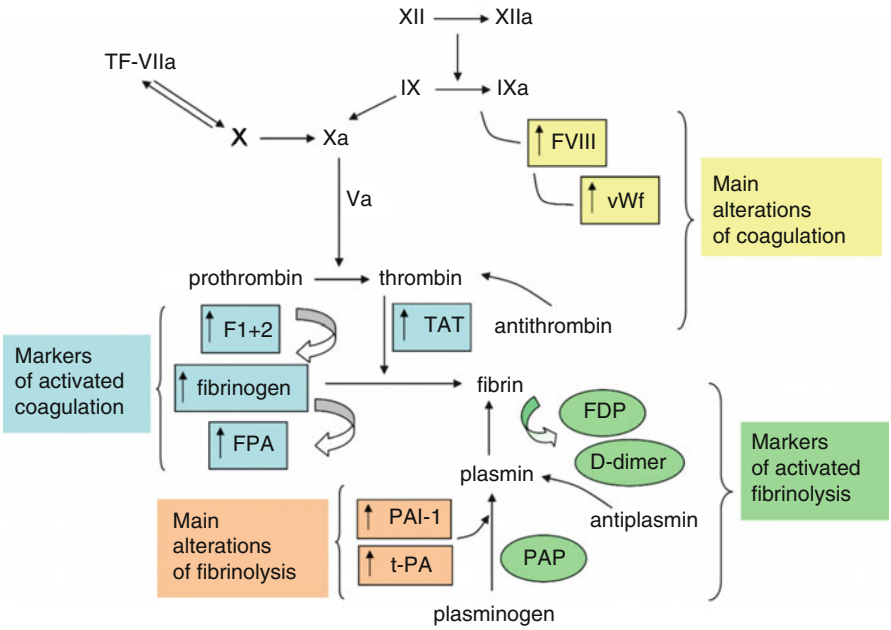

Fig. 15.4 Markers of activated coagulation and fibrinolysis together with main alterations of coagulation and fibrinolysis in the cause of HD treatment [14]. Abbreviations: *F1 + 2* prothrombin fragment FI+2, the peptide originating from the prothrombin molecule upon its proteolytic conversion into thrombin, *FPA* fibrinopeptide A, one of the peptides originating from fibrinogen upon its proteolytic conversion into the fibrin monomr, *PAI-1* plasminogen activator inhibitor, *PAP* plasmin-antiplasmin complexes, *TAT* thrombin-antithrombin complexes, *t-PA* tissue type plasminogen activator, *vWf* von Willebrand factor (Modified from Miljic et al. [14]. With permission from Hormones Journal)

complexes (TAT) with inactivated thrombin, and thereby inhibition of fibrin formation. By contrast, LMWH almost exclusively inhibits activation of factor X to Xa. In the following subparagraphs, the status of the coagulation system is generally estimated by measuring different steps in the coagulation cascade, such as factor X and Xa, TAT complexes, the prothrombin fragment F1+2, the immediate precursor of insoluble fibrin named thrombus precursor protein (TpP) and the fibrin degradation product D-dimer.

Activation of Coagulation in End-Stage Kidney Disease (ESKD)

Despite a compromised platelet function and a hemorrhagic tendency, uremic patients not yet on dialysis are generally characterized by a pro-coagulant state, as measured by increased plasma concentrations of fibrinogen, D-dimer, increased prothrombin fragment 1+2, tissue factor (TF) antigen, coagulation factor VII, and reductions in the levels of antithrombine, protein S and factor X [13, 15–17].

During HD a complex interaction occurs between the hemostatic system and the ECC, which depends on individual characteristics of the patient and the membrane material of the dialyzer. As already mentioned, anticoagulants are administered directly at the start of the treatment to prevent clotting. From a number of studies in HD patients who were treated with different membranes, it was concluded that HD patients already show signs of coagulation activation before treatment, as measured by increased concentrations of the prothrombin fragment F1+2. Studies on dialyzers with different membranes [polyacrylonitrile (AN69), polymethylmethacrylate (PMMA) and polysulphon (PS)], all showed a decrease of factor XII levels over time, which, however, depended highly on the material of the membranes used (PMMA: mean decrease 80 % of the initial value, PS: mean 40 % and AN69: mean 28 %). Plasma TpP levels, a measure of fibrin clot formation, remained unaltered during HD with PS membranes and showed an increase during treatment with both AN69 and PMMA dialyzers [18, 19]. Comparable findings were obtained for the coagulation activation marker F1+2. TAT complexes remained unaltered during HD with PMMA, but peaked sharply after 30 min during treatment with both AN69 and PS. All measurements showed large inter-individual variations. Of note, some of these intra-dialytic changes (TAT, F1+2) can easily be missed when measuring only before and after a session, as return of these parameters to pre-dialytic values may occur towards the end of the treatment [16, 20].

Altogether, it can be concluded that the coagulation system in patients with ESKD is already activated prior to the start of HD. During treatment with various types of dialyzers further stimulation occurs, which, however, is highly dependent on the individuals under study and the material of the membranes used.

Only a few studies have addressed the question whether coagulation activity differs between HD and HDF. From a comparative analysis between high-flux HD and pre-dilution HDF it appeared that TAT and D-dimer generation at identical anti-Xa levels was considerably higher during HDF [21]. On the other hand, when the dose of the LMWH enoxaparin was kept constant and anti-Xa activity was compared between online postdilution HDF (substitution volume 18 L), low flux HD and high-flux HD, anticoagulant activity was significantly lower during HDF than during both high-flux HD and low-flux HD (low-flux HD: 0.71 ± 0.17, high-flux HD: 0.35 ± 0.17 and HDF: 0.19 ± 0.11 IU/ml, $p < 0.005$) [22]. UHep and LMWH dose-finding studies are described in the paragraph below.

Anti-coagulation During Hemodialysis and Hemodiafiltration

Unfractionated heparin (UHep) has been the standard anticoagulant in HD for decades [23]. It has both anti-Xa and anti-IIa activity and its presence can be estimated by the activated prothrombin time (aPTT). More recently, low molecular heparins (LMWH) have emerged as an alternative anticoagulant, because of a more simple mechanism of action (more powerful inhibition of the conversion of factor X to factor Xa without

an effect on thrombin, which reduces the bleeding risk in patients on this anticoagulant therapy), a more rapid onset of action, less platelet activation [24] and less fibrin deposition onto the dialyzer [25]. In addition, a single bolus injection at the start of HD is usually sufficient to obtain adequate anticoagulation during the entire duration of the dialysis session [26]. A detailed discussion of the merits and disadvantages of both types of anticoagulants in HD is beyond the scope of this chapter [27, 28].

Data comparing UHep and LMWH use in HDF are limited. From a study in patients who were treated with low-flux HD (n=28) and HDF (n=26), it appeared that both treatments induced adequate anticoagulation without major risk of bleeding [29]. Plasma anti-Xa activities were similar. Laboratory measurements in HDF patients indicated that aPTT and TAT (MW 90 kD) values were lower during treatment with the LMWH nadroparin (MW 1.7 kD), while plasma anti-Xa activity was higher. In addition, platelet-derived BTG (MW 36 kD) and PF4 (MW 358 kD) were lower after administration of LMWH. Other differences between HD and HDF were not described in this study.

From a dose-finding study in patients who underwent either high-flux HD (n=22) or online post-dilution HDF (n=33) it appeared that the optimal dalteparin (MW 6 kD) dose is approximately 60±10 IU/kg, the desirable target range of anti-Xa activity at 1 h 0.4–0.75 IU/ml and <0.4 IU/ml at the end of a session [30]. Differences in efficacy and side effects were not found between HD and HDF while accumulation did not occur in either modality. As dialysis time is an important determinant of end-dialysis anti-Xa activity, it was concluded that patients with a shorter dialysis time may need dosing in the lower range and individuals with longer dialysis time near the upper range. Comparable results were described with the synthetic LMWH fondaparinux (MW 1.7 kD) in five patients suffering from heparin-induced thrombocytopenia (HIT), who were treated with online postdilution HDF [31]. Despite its almost complete renal excretion and a terminal half-life of 17–20 h, accumulation did not occur during this type of treatment, most likely due to removal by convection. To obtain the desired anti-Xa activity both after 1 h (0.4–0.6 IU/ml) and at the end of the session (<0.4 IU/ml), 0.03 mg/kg appeared to be the optimal bolus at the start of HDF after a number of adjustments. At this dose no bleeding episodes were observed and only minor clotting occurred. Thrombocytopenia did not occur in 160 sessions.

Two large randomized, controlled trials, comparing HDF with HD, reported anticoagulant use during treatment with the two modalities. In the Turkish HDF Study the dose of UHep was approximately 25 % higher in HDF patients as compared to individuals who were treated with high-flux HD [32]. In CONTRAST [33], the dose of LMWH (mainly nadroparin and dalteparin) was approximately 10 % higher in the HDF group as compared to patients who were treated with low-flux HD [34]. As the MW of UHep is 5–30 kD and of LMWH 2–8 kD, removal by convection, as demonstrated for the LMWH enoxaparin during HD with high-flux membranes, [35] is a potential option for both substances. Of note, protein binding, which determines the free fraction available for elimination and bioavialability, differs considerably between the commercially available LMWHs, varying between 80 % for enoxaparin and <10 % for dalteparin [36]. For UHep protein binding is approximately 90 %. Other explanations for the requirement of high doses of anticoagulants during HDF include the elevated TMP and marked hemoconcentration.

Hemodiafiltration with Reduced Doses or No Anticoagulation in the Extra-Corporeal Circuit

As the administration of both UHep and LMWH has been related to unfavorable side effects, including platelet activation and hypertriglyceridemia, attempts have been made to reduce their doses. From a recent study in online postdilution HDF with a heparin-grafted polyacrylonitril (HeprAN) membrane, it appeared that the dose of the LMWH nadroparin (MW 1.7 kD) could be reduced by 60 % without noticeable clinical side-effects but with similar concentrations of TAT complexes, i.e. coagulation activation, as found during standard anticoagulation [37]. Other attempts to reduce or even avoid the use of UHep or LMWH during HDF include the application of pre-dilution HDF and regional citrate anticoagulation without [38] or with calcium-containing dialysate [39]. While especially the latter modality was characterized by a high incidence of clotting in the venous bubble trap, a study on pre-dilution HDF with reduced doses of the LMWH enoxaparin (MW 4.5 kD) was prematurely discontinued because of a significantly higher occurrence of major clotting incidents compared to standard regimes [40]. In CONTRAST, a small substudy was performed in patients with a temporarily contra-indication for systemic anticoagulation. Patients who were randomized to postdilution HDF were switched to the predilution mode and those randomized to HD were treated with regional citrate anticoagulation. A total of 14 patients (HDF 9; HD 5) underwent 29 treatments. Preliminary data indicate that 74 % of the HDF patients successfully completed the sessions compared to 90 % in the HD group.

Interestingly, the use of a citrate-based dialysate in online postdilution HDF appeared safe and allowed the exclusion of both the LMWH enoxaparin and UHep in most cases [41]. As ionized calcium diffuses from the blood into the dialysate and the calcium-citrate-complex from dialysate into the blood, ionized calcium decreased while total calcium remained unaltered, if compared to treatment with standard dialysate. The maximum plasma citrate concentration only slightly exceeded the upper limit of the normal range. In 120 sessions, side effects were not described.

Summary and Practical Advice

During the course of CKD, the hemostatic balance is disturbed due to alterations in the coagulation cascade, inhibitors of the coagulation system, the fibrinolytic pathway, platelets and endothelial cells. Hence, CKD is characterized by both a bleeding and a pro-thrombotic tendency.

During dialysis the blood of the patients is exposed to the foreign materials of the ECC, mechanical forces within the ECC and the anticoagulation administered during treatment. As a result, coagulation activation, as measured particularly by an increase in plasma TAT complexes and a decrease of individual clotting factors,

increases depending on the type of dialysis membrane used and individual patient characteristics. In addition, activation of platelets, as measured by an increase in the expression of the platelet surface marker CD62p and release of platelet granulation products, occurs. With the exception of the platelet degranulation product BTG, treatment-induced alterations in hemostatic parameters are more pronounced during HDF than HD, most probably due to a higher TMP and increased hemoconcentration.

Clinical studies revealed that anti-Xa activity is lower during HDF than during HD, at similar LMWH dosage. When anti-Xa was kept constant, higher doses of LMWH were necessary during treatment with HDF. In two randomized controlled trials comparing HDF with HD, post hoc analysis showed that both heparin and LMWH doses were higher in patients treated with HDF. Therefore, visible clotting of the dialyzer and air bubble trap, and pressure monitoring at the arterial line is of utmost importance. To avoid clot formation within the dialyzer and guarantee an undisrupted HDF procedure as much as possible, a higher loading dose may be advisable. When measuring anti-Xa activity, a concentration of 0.4–0.6 IU/ml after 1 h and 0.4 IU/ml at the end of the session appears sufficient. When using the LMWH fondaparinux, a dose of 0.3 mg/kg appeared the optimal bolus at the start of treatment while 60 IU/kg appears appropriate for dalteparin. In case of a contraindication for intravenous anticoagulation, use of pre-dilution HDF without administration of UHep or LMWH is an option, although published results are somewhat disappointing. Depending on the experience of the medical and technical staff, application of online post-dilution HDF with citrate-based dialysate may offer a suitable option.

Future studies are needed to assess first, whether the higher doses of UHep and LMWH during HDF are a reflection of the more bio-incompatible conditions within the dialyzer or result from removal by convection. Second, to assess whether higher doses of anticoagulants are correlated with clinical outcome (either favorable or adverse) or just an innocent bystander of HDF treatment.

Teaching Points

- Patients with chronic kidney disease (CKD) develop a variety of hemostatic disorders over time
- As a result, CKD patients suffer from both a bleeding tendency and a predisposition for clotting
- Abnormalities include the coagulation cascade, inhibitors of coagulation, fibrinolytic pathway, platelets and endothelial cells
- In hemodialysis (HD) patients, both the accumulation of uremic toxins and the bio-incompatibility of the extracorporeal circuit (ECC) play an important role in the hemostatic abnormalities
- Not only the dialyzer, but also other components of the ECC, intra-dialyzer hemoconcentration, mechanical forces and administration of intravenous anticoagulants contribute to this process

- Evaluation by electron microscopy revealed that platelets from a HD patient are smaller and hold less granule contents
- As both hemoconcentration and transmembrane pressure (TMP) are considerably increased in HDF, even further derangements occur in hemodiafiltration (HDF)
- Both in Turkish HDF Study and CONvective TRAnsport Study, higher doses of anticoagulants were administered to HDF patients
- Since middle molecular weight uremic toxins, such as platelet-derived beta-thromboglobulin, are removed during HDF, it is unclear whether the more bio-incompatible conditions within the ECC during HDF are harmful or not

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