Activation of platelets and coagulation during haemodialysis

Schoorl, Marianne

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GENERAL DISCUSSION AND FUTURE PERSPECTIVES
1. INTRODUCTION

In end-stage kidney disease, haemodialysis treatment is applied to remove excess fluid and waste products, such as urea and creatinine, from the blood. During haemodialysis, blood of a patient flows through an extracorporeal circuit, which consists of needles, blood lines, dialyzer, bubble trap and roller pump. Although all of these factors may play a role in the bio(in)compatibility of the system, it has been well documented that the dialyzer - with a surface area of 1-2 m² – is crucial in this respect. In addition, both the administered anticoagulant, usually low molecular weight heparin, and microbial contamination of the dialysate may contribute to the level of bio(in)compatibility.

Despite the efficiency of modern dialyzers and major improvements in the bio-compatibility of the materials used, various side effects occur during haemodialysis, including activation of several types of blood cells (platelets, leucocytes), protein systems (coagulation and complement) and the endothelium.

Haemodialysis affects the haemostatic system in two ways:
1. By contact of blood constituents with the entire inner surface of the extracorporeal circuit.
2. By the anticoagulation procedure to prevent clotting in the extracorporeal circuit.

Effects of the roller pump and the bubble trap as well as the role of the anticoagulation procedure preventing platelet activation have been studied by others in our research group. It was demonstrated that the increase in platelet factor 4 occurs only to a limited extent within the dialyzer, but primarily as a result of detachment from the endothelium due to the administered heparin. From these studies it also appeared that anticoagulation with trisodiumcitrate completely inhibits haemodialysis-induced platelet activation and platelet degranulation.

In this thesis, attention is focussed on activation of platelets and the coagulation system using several types of dialyzer membranes (Table I). A variety of parameters regarding platelet morphology, platelet activation, platelet degranulation and markers for activation of coagulation were analyzed both before and during haemodialysis treatment with low-flux polysulphon (F-8), high-flux polysulphon (F-60), high flux acrylonitrile (AN-69) and low-flux poly-methyl-metacrylaat (PMMA) dialyzer membranes. At the start of all sessions a bolus injection of low molecular weight heparin was applied. An overview of the results reflecting activation of the coagulation system, platelet morphology, platelet activation and degranulation of platelets before and during a haemodialysis session is represented in Table II.

Markedly elevated plasma levels of soluble cell adhesion molecules (sICAM-1, sVCAM-1) and von Willebrand factor have been observed in individuals with chronic kidney disease. Moreover, during haemodialysis treatment an increase in the levels of vascular cell surface molecules was observed. Therefore, it is conceivable that haemodialysis treatment promotes vascular injury, thus contributing to the increased cardiovascular risk in end-stage kidney disease. With regard to endothelial cell activation, the biomarker
### TABLE I. Overview of the studies presented in Chapters 2 to 8.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Type of anticoagulation</th>
<th>Dialyzer membrane</th>
<th>Blood sampling: before/during HD treatment</th>
<th>Platelet haemocytometry</th>
<th>Platelet morphology</th>
<th>Platelet activation or degranulation</th>
<th>Activation of coagulation</th>
<th>Endothelial integrity</th>
<th>Reference groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>AN-69 high flux</td>
<td>Before / t= 1, 5, 30, 150 minutes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>FXII, TAT, F1+2, TpP, Fibrinogen</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>PMMA / F-60 high flux</td>
<td>Before / t= 5, 30, 150 minutes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>FXII, TAT, F1+2, TpP, Fibrinogen</td>
<td>-</td>
<td>healthy / uraemia</td>
</tr>
<tr>
<td>4</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>F8 low flux</td>
<td>Before / t=1, 5, 30, 150 minutes</td>
<td>PLT count, PDW, MPV, p-LCR, IPF</td>
<td>Light-microscopy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>healthy</td>
</tr>
<tr>
<td>5</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>F8 low flux</td>
<td>Before / t=1, 5, 30, 60, 150 minutes (efferent line) + t=5, 30 minutes (afferent line)</td>
<td>PLT count, PDW, MPV, p-LCR, IPF</td>
<td>-</td>
<td>Platelet CD62p expression, plasma PF4, β-TG</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>F8 low flux</td>
<td>Before / t= 5, 30, 150 minutes</td>
<td>PLT count, IPF</td>
<td>Light-microscopy</td>
<td>Platelet CD62p expression, serotonin content; plasma PF4, β-TG</td>
<td>TAT, F1+2</td>
<td>-</td>
<td>healthy</td>
</tr>
<tr>
<td>7</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>F8 low flux</td>
<td>Before / t= 5, 30, 150 minutes</td>
<td>PLT count, PDW, MPV, p-LCR</td>
<td>Light-microscopy; electron microscopy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>healthy</td>
</tr>
<tr>
<td>8</td>
<td>Bolus LMWH, at start HD treatment</td>
<td>F8 low flux</td>
<td>Before</td>
<td>-</td>
<td>Light-microscopy</td>
<td>-</td>
<td>TAT, Fibrinogen</td>
<td>ProET-1</td>
<td>healthy / uraemia</td>
</tr>
</tbody>
</table>

PLT, platelet; p-LCR, platelet large cell ratio; PF4, platelet factor 4; TAT, thrombin-antithrombin complexes; PDW, platelet distribution width; IPF, immature platelet fraction; β-TG, β-thromboglobulin; F1+2 = prothrombin fragment 1+2; TpP, thrombus precursor protein; MPV, mean platelet volume; proET-1,proendothelin-1; HD, haemodialysis; LMWH, low molecular weight heparin.
proendothelin-1 was investigated together with activation of the coagulation system and platelet granule depletion both in patients not yet on haemodialysis and in patients with haemodialysis treatment (Table II).

2. HAEMODIALYSIS-INDUCED CHANGES REGARDING THE COAGULATION SYSTEM

The effects of several types of dialysis membranes on activation of the coagulation system were evaluated before and during a session of haemodialysis treatment (Chapters 2, 3, 6).

**High flux AN-69 dialyzer membrane**
Already before the start of haemodialysis treatment increased concentrations of fibrinogen and prothrombin fragment F1+2 were established. During a haemodialysis session a FXII decrease amounting to 25% of the initial FXII level occurred within the first minute. Subsequently, only a slight increase towards the initial FXII level was detected. At the end of haemodialysis treatment moderately increased concentrations of thrombin-antithrombin complexes and prothrombin fragment F1+2 were established, whereas changes in fibrinogen concentrations were not observed.

**Low flux PMMA dialyzer membrane**
Concentrations of prothrombin fragment F1+2 were already increased before the haemodialysis treatment. During haemodialysis treatment FXII levels decreased. Slightly increased concentrations of prothrombin fragment F1+2 and fibrinogen were established, whereas increased generation of thrombin-antithrombin complexes was not detected.

**High-flux F-60 dialyzer membrane**
Obvious decreases of FXII exceeding the lower level of the reference range were observed within 5 minutes after starting haemodialysis treatment. Subsequently, only a slight increase amounting to 20% of the initial FXII level was detected. Changes in plasma concentrations of prothrombin fragment F1+2 and fibrinogen were not observed. In 40% of the patients slightly increased concentrations of thrombin-antithrombin complexes were established during a haemodialysis session.

**Low-flux F-8 dialyzer membrane**
Concentrations of prothrombin fragment F1+2 were already increased before the start of a haemodialysis session. During haemodialysis steadily ongoing increases of prothrombin fragment F1+2 and thrombin-antithrombin complexes were established.

From our data the following conclusions can be drawn. Patients with end-stage kidney disease already have signs of activation of the coagulation system prior to a haemodialysis session. During haemodialysis with various types of dialysis membranes even further stimulation of the coagulation system occurs, which, however, appears highly dependent on the individuals under study.

As the above mentioned investigations were performed over a time span exceeding a pe-
TABLE II. Overview of findings for laboratory markers of platelet (PLT) haemocytometry, morphology, activation or degranulation as well as activation of coagulation before, during and at the end of haemodialysis session presented in Chapters 2 to 7.

<table>
<thead>
<tr>
<th>Blood sampling</th>
<th>Before HD (T=0)</th>
<th>During HD (T=1, 5, 30, 60 minutes)</th>
<th>End HD (T=150 minutes)</th>
<th>Uræmia reference group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AN-69 high flux</td>
<td>PMMA high flux</td>
<td>F-60 high flux</td>
<td>F8 low flux</td>
</tr>
<tr>
<td>PLT haemocytometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT count</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PDW</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>MPV</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>P-LCR</td>
<td>N - ↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLT morphology</td>
<td>% PLTs with a staining density of granule-containing cytoplasm of - &gt;75%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLT activation or degranulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD62p</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PF4</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>β-TG</td>
<td>N - ↑</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serotonin in PRP</td>
<td>N - ↓</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Activation of coagulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FXII</td>
<td>N</td>
<td>N - ↓</td>
<td>N - ↓</td>
<td>-</td>
</tr>
<tr>
<td>F1+2</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>TAT</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>N - ↑</td>
<td>N - ↑</td>
<td>N - ↑</td>
<td>N - ↑</td>
</tr>
<tr>
<td>TpP</td>
<td>N - ↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

PLT, platelet; p-LCR, platelet large cell ratio; PF4, platelet factor 4; TAT, thrombin-antithrombin complexes; PDW, platelet distribution width; IPF, immature platelet fraction; β-TG, β-thromboglobulin; F1+2, prothrombin fragment 1+2; MPV, mean platelet volume; PRP, platelet-rich plasma; HD, haemodialysis
period of 10 years and comparative studies are lacking, it is hardly possible to pass judgment on the differences between the various types of dialysis membranes used. If anything, it appears that high-flux polysulphon (F-60) and poly-methyl-metacrylaat (PMMA) dialysis membranes are less bio-incompatible than low-flux polysulphon (F-8) and high flux AN-69, a copolymer of acrylonitrile and sodium methallylsulfonate, dialysis membranes.

3. HAEMODIALYSIS-INDUCED CHANGES REGARDING PLATELETS

Studies concerning platelet number and activation status were performed with low-flux polysulphon F-8 dialyzer membranes (Chapters 4-8). Altered characteristics of platelets, indicated by deviations in RNA content, volume and morphology, were established before and during haemodialysis sessions (Chapters 4, 5, 6, 7) to investigate whether the number of circulating platelets in the low normal range in the patients on haemodialysis treatment is due to platelet disappearance during the haemodialysis session, whether this disappearance is a balance between an increase in the number of new platelets versus a higher overall removal, and whether the disappearance is associated with platelet activation during the haemodialysis session. In Chapter 8 effects of uraemia and haemodialysis-induced changes, with particular emphasis on platelet granule depletion were investigated.

3.1 Platelet haemocytometry and platelet morphology

The data on platelet count, morphology and granular staining in the various studies are presented in Table III.

Table III. Overview of changes in platelet numbers and characteristics during a haemodialysis session.

<table>
<thead>
<tr>
<th></th>
<th>Before HD (mean value)</th>
<th>End HD</th>
<th>% increase / decrease</th>
<th>PLT number increase / decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (150 - 400 x10⁹/L)</td>
<td>198</td>
<td>182</td>
<td>-8</td>
<td>-16</td>
</tr>
<tr>
<td>IPF (4 -17 x10⁹/L)</td>
<td>6.7</td>
<td>6.1</td>
<td>-9</td>
<td>-0.6</td>
</tr>
<tr>
<td>MPV (9 - 11.5 fl)</td>
<td>10.5</td>
<td>9.5</td>
<td>-10</td>
<td></td>
</tr>
<tr>
<td>PDW (10.5 - 14 fl)</td>
<td>12.3</td>
<td>11.0</td>
<td>-11</td>
<td></td>
</tr>
<tr>
<td>p-LCR (&lt;36 %)</td>
<td>29</td>
<td>27.2</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td>% PLTS with 75% staining density (&gt;50%)</td>
<td>19</td>
<td>29</td>
<td>+50</td>
<td>+15</td>
</tr>
<tr>
<td>% PLTS with 25% staining density (&lt;20%)</td>
<td>36</td>
<td>29</td>
<td>-20</td>
<td>-18</td>
</tr>
</tbody>
</table>

PLT, platelet; IPF, immature platelet fraction; MPV, mean platelet volume; PDW, platelet distribution width, p-LCR, platelet large cell ratio; HD, haemodialysis; Reference ranges are presented between the parentheses.
On average, patients on haemodialysis treatment already had a low normal platelet count. The platelet count decreased slightly during a haemodialysis session. The number of immature platelets in the circulation was also low normal prior to haemodialysis and further decreased during the haemodialysis session. This implies a reduced megakaryopoiesis, increased removal of young platelets or a combination thereof in the period between the haemodialysis sessions.

Compared to apparently healthy subjects, minor deviations in mean platelet volume, platelet distribution width and platelet large cell ratio were established during but not prior to the haemodialysis sessions. Electron microscopy studies revealed that the platelet surface area in a haemodialysis patient was considerably smaller if compared with an apparently healthy subject. It is not elucidated why the reduced mean platelet volume, as derived from digital image analyses of the electron microscopy findings, markedly exceeded the small alterations in platelet volume as assessed by haemocytometry. Possibly, platelets in an uraemic environment may be extremely sensitive to the fixation procedure applied for electron microscopy imaging, resulting in abnormal dehydration and shrinkage.

Platelets with a granule staining density of less than 25% have been arbitrarily defined as depleted. The reference range amounts to a maximum number of 20% of the platelets in the circulation.

In patients with chronic kidney disease (without haemodialysis treatment) depleted granule staining density was present in 25% of the platelets, in patients with end stage kidney disease 36% of the platelets.

Also, the granularity of platelets determined as the area of dense bodies per platelet was substantially less (31%) in comparison with platelets of a healthy subject. The observations obtained by electron microscopy support the findings of increased numbers of platelets with decreased staining density of granule-containing cytoplasm by light microscopic evaluation and the hypothesis of granular depletion of platelets in patients with end stage kidney disease.

### 3.2 Platelet activation

Before the start of a haemodialysis session, results for platelet surface expression of p-selectin (CD62p), concentrations of serotonin in platelets and concentrations of platelet factor 4 in plasma were within the reference range. β-Thromboglobulin concentrations were already markedly increased. This finding is interpreted as a result from ongoing platelet activation. In chronic kidney disease the normal half life of β-thromboglobulin of approximately 100 minutes is considerably increased which may cause the increased β-thromboglobulin concentration. Therefore, the increased β-thromboglobulin concentrations prior to haemodialysis should not be interpreted as ongoing platelet activation between the haemodialysis sessions. Platelet factor 4 concentrations in the plasma prior to the haemodialysis session were normal, but this protein binds to the endothelium and is therefore not a suitable marker for ongoing platelet activation. Overall, based upon the absent platelet surface expression of p-selectin we conclude that persistant activation of platelets between the haemodialysis sessions is not substantial.

To investigate the effect of the dialysis membranes on platelet activation, platelet characteristics, p-selectin, β-thromboglobulin and platelet factor 4 were estimated in samples from
the afferent and efferent lines of the dialyzer (Chapter 5). Platelet numbers decreased immediately after the first passage of blood through the dialyzer, most probably due to adherence to the foreign material of the dialyzer membrane. Platelet activation, as detected by increased p-selectin, also occurred at the first passage. The p-selectin expression and plasma concentrations of β-thromboglobulin further increased in the extracorporeal circuit within 30 minutes. Platelet factor 4 concentrations demonstrated an immediate steep increase after the start of the haemodialysis sessions. This is ascribed to its detachment from the endothelium by the low molecular weight heparin bolus administration.

Before the start of a haemodialysis session, concentrations of serotonin in platelet-rich plasma, i.e. plasma plus platelets, were situated in the lower quartile of the reference range. Plasma concentrations of serotonin before the start of a haemodialysis session were increased, possibly due to its reduced clearance. The platelet serotonin content was decreased. During a haemodialysis session platelet serotonin concentrations steadily decreased further to 35% of the initial level, indicating ongoing platelet secretion of their granular content into the plasma. Plasma serotonin concentrations decreased to 45% of the initial level. Considering the low molecular weight (176 D), serotonin is most likely efficiently removed from the plasma during a haemodialysis session.

3.3 Conclusions

The data on the platelet morphology, immaturity, granular content and activation parameters may indicate the following. Prior to haemodialysis the patients had, on average, a low normal number of platelets in their circulation. The number of immature platelets was also low normal, which reflect an impaired megakaryopoiesis, an increased removal or a combination thereof. The platelet serotonin content was reduced prior to a haemodialysis session, but the serotonin content decreased dramatically during the session, indicating granular release. The period between the haemodialysis sessions may be too short to refill the platelets with serotonin. We do not consider the low serotonin content of the platelets to indicate ongoing platelet activation between the haemodialysis sessions, because the platelets do not express p-selectin on their surface prior to the haemodialysis session.

During haemodialysis, the number of platelets decreased already at the first passage of the blood through the dialyzer (from 198 to 173 x10⁹/L) and subsequently increased somewhat to 182 at t=150 min. The initial decrease is likely to be caused by the adherence of the platelets to the dialyzer. The subsequent increase in platelet count may be due to platelets detaching from the dialyzer, but also due to a release from platelet stores in the body. The platelet granular density staining indicates removal of less density stained platelets and enrichment of density stained platelets after the haemodialysis session. These data indicate that at least some of the platelet increase during is haemodialysis is due to platelet release from some storage compartments, e.g. the bone marrow. The reduction in the number of immature platelets after the haemodialysis would counterdict this conclusion, but the decrease may be due to increased removal of such platelets in the dialyzer. This is supported by our finding of the reduction in MPV after the haemodialysis session, as immature platelets are larger than older platelets. Thus, the slight reduction in platelet count after a haemodialysis session is a result of platelet removal, especially within the dialyzer, and simultaneously some release of new platelets from platelet storage sites.
4. HAEMODIALYSIS-INDUCED CHANGES REGARDING ENDOTHELIAL INTEGRITY

An essential function of the endothelium is to provide a surface which prohibits activation of the coagulation cascade in the resting state. Another function is to promote coagulation activation by the activated endothelial cells. Tissue factor expression on the surface of the endothelial cells plays an important role in the transformation of their anticoagulant into the procoagulant state. The endothelial cells of patients with chronic kidney disease are continuously exposed to uraemic toxins, which may cause their activation and manifestation of features associated with systemic inflammation. However, the mechanisms by which uraemia might activate endothelial cells have not yet been elucidated.

During haemodialysis various blood cell types and protein systems are stimulated, depending on the type of dialyzer and the degree of dialysate contamination. As a result, pro-inflammatory cytokines, such as IL-6 and TNF-α are released. Degranulation of granulocytes and platelets leads to the discharge of various granule products, such as myeloperoxidase and platelet factor 4, respectively. Stimulated white blood cells and activated platelets leave the dialyzer, enter the systemic circulation of the patients, and may activate and/or damage endothelial cells. As a consequence, haemodialysis treatment induces oxidative stress, prothrombotic changes, and signs of inflammation.

Proendothelin-1 concentrations in patients with chronic kidney disease and end stage kidney disease were investigated to establish whether patients on haemodialysis have a reduced endothelial integrity (Chapter 8). The concentration of the in vivo inactive biomarker proendothelin-1 reflects the level of the bioactive peptide endothelin-1. Proendothelin-1 is the precursor of endothelin-1, which in contrast to endothelin-1 reveals stability ex vivo. Plasma concentrations of proendothelin-1 in chronic kidney disease and end stage kidney disease were some five-fold increased compared to a reference group from apparently healthy individuals. In subjects 65 years of age the concentrations of proendothelin-1 are some 20% increased as compared to individuals 25 years of age, and this increase is gradual over the years. Even taking the age-related increase into account, the increase in proendothelin-1 plasma concentrations in the subjects with chronic kidney disease and end stage kidney disease was obviously higher, indicating reduced endothelial integrity.

Regarding the markedly increased concentrations of proendothelin-1 and reduction of endothelial integrity, a study of Grooteman et al demonstrated that endothelial cell adhesion molecules (sICAM-1 and sVCAM-1) and von Willebrand factor are also markedly increased in patients with end-stage kidney disease and do not change after 4 weeks on haemodialysis treatment. It was demonstrated that each haemodialysis session results in a marked increase in von Willebrand factor within 24 hours, whereas the concentration of endothelial cell adhesion molecules (sICAM-1) did not change. In addition, concentrations of cell adhesion molecules and von Willebrand factor varied notably between individual patients. It was concluded that endothelial dysfunction appears to be far more dependent on patient-related factors, such as co-morbidity, chronic kidney dysfunction and prescribed medication, than on the haemodialysis treatment itself.
5. CONCLUSION

Patients with end-stage kidney disease are prone to develop complications due to derangements in two opposite directions of the haemostatic process: bleeding and clotting. Clinically relevant are the prolonged bleeding from the dialysis fistula and the prothrombotic state. To summarize the findings of the studies we performed, new insights have been obtained with respect to haemodialysis-induced activation of platelets and coagulation:

- Already before the start of a haemodialysis session the patients have, on average, a low normal platelet count. This may reflect impaired megakaryopoiesis, but also a lack of ability of the body to replenish the removed platelets during the frequent haemodialysis sessions.
- Because platelet surface expression of p-selectin prior to the haemodialysis is absent, we hypothesize that ongoing platelet activation between the haemodialysis sessions does not occur.
- The staining density of the platelet granule content prior to haemodialysis reveals a marked decrease below the reference range. Platelets in patients with end stage kidney disease thus demonstrate chronic depletion of their granular content, not due to ongoing activation between the haemodialysis sessions. Most likely the reduced granular content is due to their repeated activation during the haemodialysis treatment.
- The overall slight decrease in the platelet count after a haemodialysis session is most likely due to platelet adherence to the dialyzer membrane, which is not fully compensated by an influx of platelets from storage pools.
- Prior to haemodialysis, the patients already reveal an activated coagulation system, as indicated by slightly increased plasma concentrations of thrombin-antithrombin complexes and prothrombin fragment F1+2.
- During a haemodialysis session, extensive activation of the coagulation system is observed in spite of the anticoagulant regime and the relative bio-compatibility of current dialyzer membranes. This seems to be particularly initiated via the factor XII dependent pathway.
- Some evidence of an impaired integrity of the endothelium was obtained as indicated by the increased plasma concentrations of pro-endothelin 1.
- Activation of platelets during haemodialysis sessions and activation of the coagulation system during and between haemodialysis sessions evidently yields a continuous prothrombotic state of the patients.

6. FUTURE PERSPECTIVES

Introduction
As described in the preceding paragraphs, both the uremic state and the haemodialysis procedure itself are associated with a variety of platelet abnormalities and disorders of the coagulation system. Clinically these alterations may contribute to the seemingly paradoxical findings of both a pro-thrombotic state and a bleeding tendency in patients with chronic kidney disease. Moreover, as the haemostatic system has been implicated in the development of atherosclerosis in non-renal patients, it is conceivable that the patho-
physiological derangements of haemostasis in uraemic patients contribute to the extremely high burden of cardiovascular disease in this patient group. Therefore, alleviation of these abnormalities may have a favorable influence on both short and long-term complications in patients with chronic kidney disease not yet on haemodialysis and patients with haemodialysis treatment.

**Short term complications**

As mentioned before, a bolus of un-fractionated heparin or low-molecular weight heparin is administered to the patients shortly before the start of a haemodialysis session in order to prevent clotting in the extracorporeal circuit. In every day clinical practice, the starting dose of the anticoagulant is mainly based on the weight of the individuals, and modified during consecutive sessions in case of visible clotting in the bubble trap or an increasing pressure in the afferent line. However, whereas a higher dose (or a second bolus halfway the session) of the anticoagulant may temper clotting and lower the intra-dialyzer pressure, the chance on fistula bleeding at the end of the haemodialysis session may increase. Therefore, anti-factor Xa monitoring in high risk patients may diminish or even prevent this unwanted side-effect of chronic haemodialysis treatment. Other strategies include the more liberal use of trisodium citrate, which prevents platelet factor 4 release from the endothelium, a combination of citrate containing dialysate with low dose low molecular weight heparin, administration of nafostat mesylate (NFM), and pre-dilution haemodiafiltration (HDF). As these alternative approaches may alleviate or circumvent the above mentioned problems, further research in this field is mandatory.

**Long term complications**

*Fistula stenosis*

A relatively frequent complication of haemodialysis treatment is occlusion of the arteriovenous access, either a graft when synthetic materials are used or a fistula when an artery is directly connected with a vein. Whereas a graft is most frequently used in the United States of America, in the Netherlands in 90% of the patients a fistula is created as arteriovenous access. Thrombosis that occurs within one month after fistula construction is most often due to technical errors or premature use. The major predisposing factor for fistula thrombosis is a stenosis in the venous outflow tract, which is the result of progressive, fibromuscular intimal hyperplasia and perivenous fibrosis. Clinically, fistula thrombosis is characterized by a diminished blood flow, recirculation and hence a decreased efficiency of dialysis. Whether the compromised haemostasis, as described in this thesis, contributes to its development is not yet elucidated. Although treatment with aspirin and dipyridamole has been shown to reduce the time to first thrombosis in PTFE grafts, neither treatment with coumarins nor prescription of anti-platelet agents, showed a reduction in the occurrence rate of fistula failure. In this respect it is interesting to note that vascular remodeling and neointimal formation was reduced after targeted delivery of nonspecific factor Xa inhibitors, such as heparin and low molecular weight heparin, coupled to an antifibrin antibody. Whether non-heparin containing modulators of haemostasis can prevent or delay the development of fistula stenosis is currently unknown and demands further research.
Cardiovascular disease
The risk of mortality is extremely high in haemodialysis patients, cardiovascular causes accounting for 40%-50%. Apart from classical risk factors, such as increased cholesterol and hypertension, several non-classical risk factors have been identified which aggravate or contribute to the vascular abnormalities in chronic kidney disease. Whereas in the previous decade most attention was paid to an abnormal homocysteine metabolism, oxidative stress and chronic micro-inflammation, currently, disorders of mineral metabolism are the main research topics in this area.

However, both experimental and clinical data indicate that platelets and the coagulation system are involved in classical atherogenesis and atherothrombosis. In numerous clinical trials, administration of antiplatelet or anticoagulant therapy has been associated with attenuation or even regression of plaque growth. A systemic micro-inflammatory environment, as described in patients with chronic kidney disease, has been shown to induce a phenotypic switch to a pro-atherogenic endothelium. As discussed in chapter 8, in patients with chronic kidney disease not only elevated levels of pro-endothelin were found, but also high levels of soluble adhesion molecules (sICAM-1, sVCAM-1), suggesting endothelial activation. The resulting enhanced expression of cell-adhesion molecules induces adherence of platelets and the secretion of various atherogenic mediators, including cytokines. Binding between adherent platelets and leucocytes support activation and transmigration of monocytes, which is considered critical for plaque formation.

In patients with chronic kidney disease various uraemia specific pro-atherogenic conditions have been identified, such as hyperhomocysteinemia, oxidative stress, micro-inflammation and disorders of mineral metabolism. Whether uraemic derangements in platelets characteristics and disorders of coagulation contribute to the vascular abnormalities, which are commonly observed in patients with chronic kidney disease, is conceivable but incompletely understood. On the other hand, depletion and exhaustion of platelets may protect against platelet-induced damage to vascular wall. Future research in this field may help to unravel the complex interactions between disorders of haemostasis and other inducers of uraemic vasculopathy. Whether interventions in the haemostatic system can alleviate the high burden of cardiovascular disease in this population is also a subject for future research.
REFERENCES


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