Chapter 4

Bleeding Diathesis in Hemodialysis Patients

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Additional information is available at the end of the chapter

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1. Introduction

End-stage renal disease patients, particularly those treated with hemodialysis (HD), suffer from complex hemostatic disorders. Patients with uremia may experience two opposite hemostatic complications: bleeding diathesis and thrombotic tendencies. Bleeding diathesis in uremic patients is primarily seen due to abnormalities in primary hemostasis, particularly platelet function disorder and impairment of the platelet-wall interaction. Anemia, abnormal nitric oxide production and some drugs employed also contribute to bleeding diathesis.

In addition to bleeding diathesis, thrombotic complications are also frequently seen in uremic patients. Thrombotic complications play a significant role in cardiovascular events, the main cause of mortality in this patient group. Thrombotic hemostatic changes include increased platelet aggregability, increased plasma fibrinogen, factor VIII:C and vWF levels, a decrease in protein C and protein S anticoagulant activity, changes in fibrinolytic system activity and a rise in plasma lipoprotein and homocysteine levels.

In addition to hemostatic changes caused by uremia in the HD patient group, HD therapy itself leads to various hemostatic changes. These include coagulation cascade activation as a result of contact between the dialysis membrane and blood elements, the effect of anticoagulants used to prevent coagulation developing due to this cascade activation and a decrease in the negative effects on platelet functions of middle molecule uremic toxins, thought to be eliminated during HD.

Both hemorrhagic and thrombotic changes in this patient group can give rise to life-threatening consequences. For that reason, research is still continuing into identification and treatment of hemostatic abnormalities in this patient group. Here, we shall be discussing the pathogenesis and treatment of hemorrhagic and thrombotic complications in the light of new research.

2. Normal hemostasis

A knowledge of normal hemostasis is needed in order to understand hemostatic disorders in uremic patients. The normal hemostatic process establishes blood viscosity inside the vessel and rapid plaque formation as a result of vascular injury. Hemostasis consists of three phases; primary hemostasis, coagulation and fibrinolysis (Galbusera et al., 2009). Platelets assume the main role in primary hemostasis. Under normal conditions, it prevents vascular endothelium platelet aggregation and adhesion. In the event of vascular injury, platelet-mediated hemostatic plaque formation begins (Stassen et al., 2004). Two main platelet receptors, glycoprotein Ib (GPIb) and activation-dependent glycoprotein IIb–IIIa (GPIIb–IIIa) complex, and the adhesion molecule von Willebrand factor (VWF) and fibrinogen are involved in the adhesion process in hemostatic plaque formation. Various modifications take place in the platelets after the adhesion phase, and molecules assisting platelet activation and adhesion, such as ADP, serotonin, epinephrine, fibrinogen, thromboxane and VWF, are released from the platelet granules (Ruggeri et al., 2003). The coagulation phase consists of intrinsic and extrinsic coagulation pathways. A number of coagulation proteins are involved in these coagulation pathways, including Tissue Factor (TF), and factors VII, IX, X, V, VIII, XI and XIII. Natural inhibitors of the coagulation cascade are protein C, Tissue factor (TF) pathway inhibitor and antithrombin III (Stassen et al., 2004). The fibrinolytic system leads to plasmin-mediated dissolution of fibrin. Molecules serving in this system are the plasminogen activator inhibitors PAI-1 and PAI-2, the plasmin inhibitor alpha-1-antiplasmin, alpha-2-macroglobulin and thrombin activatable fibrinolysis inhibitor (TAFI) (Fay et al., 2007).

3. Bleeding diathesis in uremic patients

The relation between uremia and bleeding diathesis has been known for many years. Uremic patients used to be lost from bleeding from vital organs. Despite today's improvement in anemia with modern HD techniques and erythropoietin therapy, bleeding diathesis continues to represent a significant problem. There may be serious, life-threatening bleeding, and surgical procedures may be delayed or not performed at all out of concern over bleeding diathesis. This causes a rise in patient morbidity. The most common cause of uremic bleeding diathesis is impaired primary hemostasis. The most frequent complications seen as a reflection of primary hemostasis disorders are petechiae, purpura, and bleeding in the arteriovenous fistula puncture site and regions where the HD catheter is inserted (Galbusera et al., 2009; Remuzzi et al., 1989). In addition, bleeding in vital organs may also be seen in uremia, leading to less frequently observed but fatal complications. In HD patients in particular, various HD therapy-related factors mean that bleeding complications to be seen more frequently. Although various rates have been citied in HD patients in different publications, the bleeding diathesis rate is around 10%-15%, and bleeding-associated morbidity around 15% (Davenport et al., 1994, Martin et al., 1994; van de Wetering et al., 1996). Gastrointestinal (GIS) bleeding, particularly upper GIS bleeding, is seen in one third of uremic patients (Galbusera et al., 2009). Kutsumi et al. showed that 17% of patients presenting to the emer-

gency department with GIS bleeding received HD therapy (Kutsumi et al., 1998). Other examples of vital organ bleeding include hemorrhagic stroke, subdural hematoma, spontaneous retroperitoneal hemorrhage, hepatic subcapsular hematoma intraocular hemorrhage and hemorrhagic pericarditis leading to cardiac tamponade (Galbusera et al., 2009; Remuzzi, 1989). Of these, hemorrhagic stroke and subdural hematoma are widely observed in HD patients. Incidence of hemorrhagic stroke is 5-10 times greater than in the normal population. (Seliger et al., 2003; Toyoda et al., 2005), while that of subdural hematoma has been put at 20 times greater. Mortality in this patient group has been determined at above 40% (Power et al., 2010). van de Wetering et al. observed 48 hemorrhagic complications in 78 HD patients. Forty of the patients with hemorrhagic complications had major bleeding and 8 minor bleeding. Six of the 40 major hemorrhages were intra-abdominal, 18 involved bleeding around the catheter, 3 were GIS bleeding, 12 were oronasopharyngeal and 1 intracerebral. One intracerebral case, 1 intra-abdominal case and 1 with gastrointestinal bleeding died (van de Wetering et al., 1996). As seen in all these studies, a not inconsiderable level of hemorrhagic complications with high mortality is seen in HD patients. It is therefore important to understand the pathogenesis of and treatment approaches toward bleeding diathesis in the HD patient group. While bleeding diathesis in HD patients is associated with uremia-related factors, HD therapy itself also creates a tendency to bleeding.

3.1. The pathogenesis of uremia-associated bleeding diathesis

Predisposition to bleeding in uremic patients has been known for years. While the pathogenesis of bleeding diathesis is not fully established, multifactorial causes are thought to be responsible. The most important of these factors are structural and functional defects in platelets. Other factors are abnormal platelet-vessel wall interaction, anemia, abnormal Nitric oxide (NO) production, drug use and HD therapy itself.

3.1.1. Platelet dysfunction

3.1.1.1. Thrombocytopenia

Moderate level platelet function disorder not sufficient to cause life-threatening bleeding is frequently seen in uremic patients (Galbusera et al., 2009). The cause of thrombocytopenia is generally decreased platelet production or an increase in consumption (Boccardo et al., 2004; Panicucci et al., 1983). Thrombocytopenia may be related to HD therapy itself or develop due to primary renal disease or various accompanying comorbid conditions. These conditions include systemic lupus erythematosus, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome and disseminated intravascular coagulation (Barbour et al., 2012; Boccardo et al., 2004; Loirat et al., 2012). HD-related thrombocytopenia may be related to the membrane and anticoagulant used in dialysis therapy. Studies investigating the effect of HD on platelet numbers have generally looked at platelet numbers 15-30 min before HD and after HD (Daugirdas & Bernardo, 2012). Studies investigating the effect of type of HD membrane on platelet number have shown that greater thrombocytopenia develops in non-biocompatible cellulose membranes compared to biocompatible synthetic polymer

membranes. Thrombocytopenia observed in non-biocompatible membranes has been associated with complement activation (Gutierrez et al., 1994; Hoenich et al., 1997; Verbeelen et al., 1991). Few studies have investigated the effect of membrane sterilization technique on platelet number. One such study, by Miller et al. analyzed the biocompatibility of the high-flux membrane steam sterilized F60S and ethylene oxide sterilized F60 and the low-flux membrane ethylene oxide sterilized F6, in other words, their effect on platelet and leukocyte numbers. The three different membranes had no different effects on platelet numbers, while the steam-sterilized membrane was shown to reduce leukocyte numbers less (Müller et al., 1998). Another effect of HD therapy on platelet number concerns the heparin used during therapy. Because of its low cost and short half-life, heparin is a frequently used anticoagulant in HD therapy. However, heparin-induced thrombocytopenia (HIT) is a complication that may limit its use and cause mortality. HIT is classified into types I and II. Type-I HIT is a widely seen form. It arises as the result of a direct reaction between heparin and thrombocytes. There is generally a slight fall in platelet numbers soon after heparin administration, and platelet numbers return to normal despite repeated administrations of heparin. Type-II HIT is less common, with an incidence of between 0.5% and 5% (Jang & Hursting, 2005). Etiology is attributed to platelet factor 4 and antibodies developing against heparin complex (Suranyi & Chow, 2010; Visentin et al., 1994). HIT generally appears with the development of thrombocytopenia, thrombosis, skin necrosis and gangrene accompanied by acute systemic reactions 5-30 min after administration of bolus unfractionated heparin (Syed & Reilly, 2009). HIT diagnosis is established by scoring the following criteria: thrombocytopenia appearing 5-10 days after commencement of heparin therapy, the presence of any thrombotic event, a normal platelet number before heparin, a 50% fall in platelet numbers compared to basal values in the absence of any other cause of platelet decrease and platelet numbers returning to normal when heparin therapy is stopped (Warkentin, 2004). The first procedure in treatment is cessation of heparin until HIT antibody results are obtained and the use of alternative anticoagulant methods to heparin. The use of warfarin as an alternative anticoagulant until platelet numbers return to normal is not recommended (Syed & Reilly, 2009). Saline flush, citrate anticoagulation, the direct antithrombin inhibitors lepirudin and argatroban or the Factor Xa inhibitor danaparoid can be used in heparin-free dialysis (Matsuo & Wanaka, 2008, Syed & Reilly, 2009). In conclusion, in the light of current knowledge, generally, although uremia and HD-associated thrombocyte numbers may decline slightly, no fall in platelet numbers that might cause fatal bleeding is observed in association with uremia itself or in association with HD therapy (excluding HIT2).

3.1.1.2. Platelet function disorder

3.1.1.2.1. Structural and functional platelet disorder

Structural and functional disorders have long been known in uremic patients. One structural impairment is a decrease in mean platelet volume, and this contributes to bleeding diathesis by leading to a decrease in platelet mass (Galbusera et al., 2009; Michalak et al., 1991). There are three main types or granule in platelets; dense granules, α granules and lysosomes (Kaplan & Owen., 1986). Various substances in these granules are involved in the hemostatic

process by being released from platelets with activation. In uremic patients, both the content of these granules and defects in secretion during platelet activation contribute to bleeding diathesis. There are various studies of platelet granule content in uremic patients. Eknoyan et al. showed that a low level of adenosine diphosphate (ADP) and serotonin in chronic kidney disease patients parallels platelets' functional defects, and that these defects can be reversed with hemodialysis or transplantation (Eknoyan and Brown., 1981). Di Minno et al. determined a low platelet ADP level in uremic patients compared to normal individuals, and a decrease in thrombin-stimulated thromboxane B2 and adenosine triphosphate secretion (Di Minno et al., 1985). Vlachoyannis et al. determined a higher cyclic-AMP level in uremic patients compared to healthy individuals (Vlachoyannis & Schoeppe., 1982). There is a rise in cyclic-GMP (c-GMP) in uremic patients. The rise in c-GMP level is associated with increased production of NO, produced by platelets (Noris et al., 1993). A rise in platelet Ca content and abnormal Ca release as a response to stimuli is another structural defect (Gura et al., 1982; Ware et al., 1989). There are contradictory findings regarding defective arachidonic acid in uremic platelets (Boccardo et al., 2004). Remuzzi et al. reported defective arachidonic acid metabolism in uremic platelets and a decrease in thromboxane A2 production as a reflection of this (Remuzzi et al., 1983). Bloom et al. reported no cyclooxygenase defect in uremic patients, but determined a decrease in thrombin-stimulated thromboxane synthesis (Bloom et al., 1986). These structural and functional defects in uremic patients contribute to impairment in adhesion and aggregation and facilitate bleeding diathesis. In addition to these structural and functional defects, various uremic toxins are known to lead to platelet aggregation defect. The uremic toxins guanidosuccinic acid and phenolic acid lead to platelet aggregation defect by inhibiting ADP-induced platelet aggregation (Hedges et al., 2007).

3.1.1.2.2. Platelet-vessel wall interaction defect

Platelet-vessel wall interaction defect is one of the significant causes of bleeding diathesis, in addition to platelet morphology and function disorder. As discussed in the section on normal hemostasis, defective platelet adhesion to the vascular endothelium is mediated by two important proteins; fibrinogen and von Willebrand factor (vWF). Additionally, two important proteins on the platelet surface, GP Ib (vWF receptor) and activation-dependent GPIIb–IIIa complex (fibrinogen receptor), are also involved in adhesion. Platelet glycoprotein (GP) content is divided into the membrane and intracellular component. Studies of uremic patients have produced different results regarding GP content (Moal et al., 2003). Nomura et al. determined decreased GP Ib expression and normal GPIIb-IIIa expression in uremic patients (Nomura et al., 1994). In complete contrast, Sreedhara et al. determined normal GP Ib expression and a decrease in GPIIb–IIIa expression (Sreedhara et al., 1996). Salvati et al. determined low GP Ib expression and high GPIIb–IIIa expression (Salvati et al., 2001). Moal et al. determined low GP Ib expression in platelets at rest. They also reported higher GP Ib expression in stimulated platelets in the HD patient group compared to the control and CKD groups, while GPIIb–IIIa expression was lower in the CKD and HD patients compared to the controls (Moal et al., 2003). We think this may be attributed to the technique for measuring these differences in results, whether the patient group is pre-dialysis or dialysis, if dialy-

sis, then whether it is HD or Peritoneal dialysis (PD) and when blood specimen is collected (before or after HD). However, the majority of studies suggest that these two platelet GPs decrease in uremic patients or that there is a decrease in response to stimulation. This leads to platelet-wall interaction defect and bleeding diathesis.

vWF is one of the main important proteins in platelet adhesion. For that reason, there have been many studies on both vWF level and function in uremic patients. However, these have produced controversial results in terms of both levels and functions. One such study was that by Zwaginga et al., which showed that platelet adhesion defect was not associated with abnormal vWF (Zwaginga et al., 1990). Escolar et al. reported platelet adhesion defect in uremic patients and that the defect may stem from defective interaction between vWF and the receptor (Escolar et al., 1990). Despite these conflicting results, the fact that no response is obtained to cryoprecipitate and desmessoprin in uremic bleeding diathesis suggests defective vWF involvement (Boccardo et al., 2004; Galbusera et al., 2009). vWF defect in uremia arises because of reduced interaction with GPIIb–IIIa receptor or reduced expression of this receptor (Mohri et al., 1988). Defective vWF GPIIb–IIIa receptor interaction reduces TXA2 and ADP production by leading to defects in phosphatidylinositol bisphosphate breakdown and cytosolic calcium concentration. As a result, it leads to impaired adhesion (Hedges, 2007).

3.1.2. Anemia

Anemia is known to be widely seen in chronic kidney failure patients and to lead to morbidity and mortality. The most important reason why it leads to morbidity and mortality stems from its negative effect on myocardial functions. Another negative effect of anemia is that it contributes to bleeding diathesis. It is thought to do this in two ways. The first is that under normal conditions in a non-anemic individual the flow of formed elements of blood inside the vessel is regular. Therefore, in the event of injury, in order for the platelets to be quickly able to form a clot, they act in the periphery near the vascular endothelium. In anemic individuals, however, this rheological order is defective. And this leads to bleeding diathesis (Hedges et al., 2007). Another reason is that erythrocytes release ADP and TxA2. But this secretion decreases in anemic individuals. Decreased levels of ADP and TxA2 lead to a reduction in platelet aggregation (Valles et al., 1991). Another mechanism concentrated on is the effect of anemia on NO. Erythrocytes are known to have a high affinity for NO. In anemia, however, the NO scavenger role declines because the erythrocyte mass decreases. An increase in NO level, on the other hand, leads to a rise in cGMP level and a decline in platelet aggregation (Martin et al., 1985, Galbusera et al., 2009). Improvement of anemia with both blood transfusion and erythropoietin therapy reduces bleeding time. And these are findings that support the hypothesis that anemia has an effect on bleeding diathesis (Livio et al., 1982; Moia et al., 1987, Viganò et al., 1991).

3.1.3. Abnormal NO production

There is an accumulation of uremic toxins as well as guanidosuccinic acid in uremic patients. Guanidosuccinic acid accumulation is associated with guanidine transfer from L-arginine to aspartic acid. L-arginine is the most important precursor of NO. NO has a modulator effect on

vascular tonus. In addition, NO prevents platelet adhesion to the endothelium, lowers cAMP-mediated platelet aggregation and reduces platelet-platelet interaction by increasing cGMP levels. (Noris & Remuzzi, 1999). The administration of inhale has led to a prolongation of bleeding time in studies on healthy individuals (Högman et al., 1993). Abnormal NO production is therefore thought to contribute to bleeding diathesis in uremic patients.

3.2. Evaluation of bleeding diathesis

Evaluation of bleeding diathesis in uremia begins with the taking of a detailed history. The presence of other systemic disease, drugs used, if renal replacement therapy is administered whether this is HD or PD, and if HD what kind of membrane and which anticoagulant in what doses are used must all be established. At physical examination, petechiae, purpura, epistaxis and bleeding from the catheter or AVF puncture site, generally a reflection of platelet function effect, may all be seen. In addition, physical examination findings secondary to GIS bleeding or intracranial or subdural bleeding may also be encountered. Platelet numbers are generally normal or slightly low at laboratory analysis. The most frequently used clinical finding in evaluation of uremic bleeding diathesis is bleeding time (Steiner et al., 1979).

3.3. Treatment of bleeding diathesis

3.3.1. Hemodialysis

As previously discussed, while the HD process itself facilitates bleeding diathesis, in renal failure patients it is used as a therapeutic approach that reduces bleeding diathesis. HD's reductive effect on bleeding diathesis comes about through the removal of uremic toxins from the blood. More than 90 uremic toxins are known today. These are classified as small, middle or large molecular toxins based on their molecular weight. Large and/or protein-bound molecules in particular cannot be removed with HD techniques using classic low-flux membranes, but they can with high-flux membranes (Vanholder et al., 2005; Weissinger et al., 2004). Daily and long-term dialysis may be needed for the removal of uremic toxins and to reduce bleeding diathesis using traditional HD techniques (Hedges et al., 2007). Studies have shown that dialysis therapy produces an improvement in platelet functions by removing uremic toxins (Boccardo et al., 2004; Galbusera et al., 2009; Hedges et al., 2007). However, another point here that must not be forgotten is that HD therapy can lead to bleeding diathesis, due to both membrane interaction and to the anticoagulants used. Therefore, heparin-free dialysis must be performed, especially with patients with active bleeding or who have recently undergone major surgery. Various methods are currently applied for heparin-free HD. These include HD using low-dose heparin, regional heparinization with protamine, intermittent saline flush, regional citrate anticoagulation, prostacyclin infusion and other alternative techniques. Swartz et al. showed that regional heparinization had a lower bleeding reduction effect than low-dose heparinization (Swartz & Port, 1979). However, in another study by Swartz, a bleeding level as high as 26% was observed in patients with an active risk of bleeding using low-dose controlled heparinization (Swartz, 1981). In addition, there are studies showing that regional heparinization with protamine neutralization has an

increasing effect on bleeding, probably associated with the delayed heparin effect (Hampers et al., 1966). Nagarik et al. investigated the effects on bleeding diathesis episode of dialysis performed with intermittent saline flush and anticoagulant dialysis in patients administered intermittent renal replacement therapy in intensive care. They observed fewer bleeding episodes in patients administered intermittent saline flush (Nagarik et al., 2010). Sagedal et al. showed that intermittent saline flush did not reduce clot formation in dialyzers and intravascular coagulation in stable HD patients (Sagedal et al., 2006). Citrate has been used as an anticoagulant for many years because of its Ca-binding effect. Several studies have shown that citrate anticoagulant can be used safely in uremic patients with bleeding (Davies et al., 2011; Jarraya et al., 2010 Kreuzer et al., 2010; Park et al., 2011). However, it must not be forgotten that severe metabolic alkalosis may develop in patients receiving citrate anticoagulation, especially continuous renal replacement therapy (Alsabbagh et al., 2012). Additionally, there are various difficulties and side-effects in citrate anticoagulation beyond continuous renal replacement. Two reliable pumps are needed for citrate and Ca infusion, and these difficulties and side-effects are that serious metabolic alkalosis and hypocalcemia may result. Prostacyclin administration is another heparin-free dialysis technique. However, this technique is not much used, because it leads to headache, flushing and hypotension (Sagedal et al., 2006). Because regional anticoagulation with heparin-protamine or citrate-calcium infusion or intermittent saline flush lead to a loss of personnel time and have a number of side-effects, new techniques are under development. One of these is the use of citrate-enriched dialysate. Cheng et al. showed that citrate-enriched dialysate is more effective than intermittent saline flush (Cheng et al., 2011). However, studies showing efficacy in patients with bleeding are needed. Yixiong et al. showed that effective and safe anticoagulation is provided in high-risk bleeding patients with low-dose argatroban (direct thrombin inhibitor) saline flush (Yixiong et al., 2010). Providing effective dialysis in HD patients with bleeding continues to be a major problem. Techniques permitting safe and effective HD need to be developed with technological advances.

3.3.2. Desmopressin

Desmopressin (1-deamino-8-d-arginine vasopressin [DDAVP]) is a drug frequently used in HD patients with bleeding diathesis. While its reductive effect on bleeding diathesis is not fully understood, it is thought to function by increasing Factor VIII levels through release from where it is stored and by reducing the effect of vWF on dysfunction (Prowse et al., 1979). DDAVP's reducing effect on bleeding time starts within 1 h and continues for 4-8 h. Bleeding time returns to normal in 24 h (Mannucci et al., 1983; Galbusera et al., 2009). DDAVP has been shown to effectively reduce bleeding time when administered intravenously (0.3 microg⁄kg) by the subcutaneous and intranasal routes (Mannucci et al., 1983; Shapiro & Kelleher, 1984; Ulusoy et al.,2004; Viganò et al., 1989). One of the things that must not be forgotten in desmopressin therapy is that efficacy may decline with increasing use, probably in association with a decline in endothelial vWF stores (Canavese et al., 1985). Flushing, headache and tachycardia may be observed during desmopressin use. But not at such a level as to prevent its use in uremic bleeding diathesis.

3.3.3. Cryoprecipitate

Cryoprecipitate is a blood product rich in factor VII, vWF and fibrinogen. Use takes the form of 10 bags of American-Red-Cross-prepared cryoprecipitate infusion in 30 min. Its effect begins in 4-12 h. The effect mechanism is not fully understood, though it is thought to be associated with its concentrated coagulation factor content (Janson et al., 1980; Triulzi et al., 1990). Its advantage is that its effect appears early, the disadvantage that it involves a risk of transferring transfusion-related diseases. Hypocalcemia may also develop during cryoprecipitate transfusion, as with the transfusion of other blood products. Additionally, it may rarely lead to pulmonary edema and anaphylactic reaction. Factors requiring attention during blood and blood product transfusion, must also therefore not be forgotten in cryoprecipitate transfusion (Galbusera et al., 2009; Hedges et al., 2007; Spinella & Holcomb, 2009). Although cryoprecipitate works very quickly, other approaches are preferred because of the risk of transference of transfusion-related diseases.

3.3.4. Conjugated estrogen

The bleeding diathesis-reducing effect of conjugated estrogen emerged on the basis of observational data (Liu et al., 1980). Following these observational data, the effect on uremic bleeding diathesis began being investigated. It has been shown that use of 0.6 mg/kg ıv (4-5 days) in uremic patients lowers bleeding time (Viganò et al., 1988). Twenty-five milligrams of oral conjugated estrogen for 3-20 days has been shown to safely reduce bleeding time (Viganò et al., 1988). In addition, low-dose transdermal administrations two times a week (50–100 microg/24 h) have also been shown to effectively reduce bleeding (Sloand & Schiff., 1995). The bleeding diathesis-reductive effect is thought to come about by preventing NO synthesis (Zoja et al., 1991). In the light of these studies, since there is greater research into reducing uremic bleeding diathesis, iv administration of conjugated estrogen is recommended over the subcutaneous and transdermal routes (Hedges et al., 2006).

3.3.5. Erythropoietin

We have already discussed the effect of anemia on bleeding diathesis. Based on these data, researchers have investigated the effect on bleeding diathesis of erythropoietin (EPO), an indispensible element in anemia treatment in chronic kidney patients. Several studies have shown that correction of anemia with EPO therapy reduces uremic bleeding diathesis. EPO's bleeding diathesis-reductive effect may come about through several mechanisms. The first of these is that the erythrocyte mass that increases with EPO therapy serves as an NO scavenger and reduces NO's negative effect on platelet adhesion. Another is the disappearance of blood rheology impairment brought about by anemia (Martin et al., 1985; Viganò et al., 1991). EPO therapy is thought to reduce bleeding diathesis by increasing the number of reticulated platelets in bone marrow, with its greater metabolic efficacy, by increasing platelet aggregation and interaction with the vascular endothelium and, finally by increasing platelets' response to stimuli (Diaz-Ricart et al., 1999; Hedges et al., 2007; Tàssies et al., 1995; Zwaginga et al., 1991). In conclusion, anemia treatment brings about a significant improve-

ment in bleeding diathesis, especially Htc, at a level of 27%-32% (Galbusera et al., 2009; Viganò et al., 1991)

In conclusion, despite advances in technology, bleeding diathesis continues to be a life threatening condition in HD patients. Although bleeding diathesis is not fully understood, it is thought to be associated with primary hemostasis, in other words, platelet structure and functions. Anemia should be corrected with EPO therapy and, most important of all, effective dialysis must be performed in order to prevent bleeding diathesis in these patients. Since its effect starts quickly, we think that the use of DDAVP will be appropriate in acute, life-threatening bleeding; and because its effect is long-lasting, conjugated estrogen may be used in patients without life-threatening bleeding but requiring long-term monitoring.

4. Hypercoagulability in uremia

Thrombotic complications are encountered as frequently as bleeding diathesis and are life-threatening in uremic patients. Thrombotic complications lead to mortality giving rise to cardiovascular events and can also cause morbidity by leading to AVF thrombosis. Understanding the pathogenesis of thrombotic events in uremic patients and the treatment is therefore of vital importance. A rise in platelet hyperactivity, adhesion and aggregation, coagulation cascade activation and a decrease in fibrinolysis are held responsible in the pathogenesis of the thrombotic process.

4.1. Increased platelet activation, aggregation and adhesion

The findings of studies analyzing platelet activation in HD patients are inconsistent. These inconsistent results may stem from differences in the patient population and sampling techniques or from differences in the platelet activation markers used. Various molecules expressed on the surface of activated platelets or various substances known to be released into plasma in the event of platelet activation are today used as platelet activation markers. Platelet surface markers are generally evaluated using flow cytometry and monoclonal antibody-based measurement. CD41 is a flow cytometric marker of activation-dependent GPIIb/IIIa receptor. PAC-1 is used to determine this receptor in its activated state. CD42b or GPIb are used in the determination of vWF receptor. CD62P is used in the determination of p selectin found in the membrane of platelet alpha granules and given off during platelet activation. CD63 is used similarly to CD62P in the determination of degranulation of platelet dense granules (Daugirdas & Bernardo., 2012; Michelson, 1996). Many studies to date have evaluated the effect of the HD procedure on platelet activation using one or more of these markers. These studies have also evaluated the effects on activation markers of the membrane used in the HD procedure, the site of blood collection (where blood enters or leaves the HD membrane) and time of collection. Studies showing differences depending on blood collection site and time and that activation markers are higher in blood samples collected at the HD membrane exit are in the majority (Aggarwal et al., 2002; Daugirdas & Bernardo., 2012; Reverter et al., 1994;). Additionally, these studies have also shown higher platelet acti-

vation markers in patients using cuprophan membrane (Cases et al., 1993; Daugirdas & Bernardo., 2012; Reverter et al., 1994). Today, in addition to the analysis of platelet activation using flow cytometry, various markers found in platelet alpha granules and released into plasma during platelet activation are determined using ELISA. One such marker is sCD40L. This is a transmembrane protein structurally related to the tumor necrosis factor-α (TNF α) family. sCD40L is a form of CD40L released into plasma from the active thrombocyte surface (Henn et al., 1998). There are studies showing that sCD40L is correlated with platelet activation in both the normal population and HD patients. In a study of 103 HD patients in our clinic we determined a significantly higher predialysis sCD40 L level compared to healthy individuals. There was a rise in sCD40 L level in blood specimen taken at the end of HD, though this was not statistically significant. Our study supported the presence of platelet activation independent of the HD procedure and that the procedure had an enhancing effect on that activation (Ulusoy et al., 2012). Signal peptide-CUB (signal peptide–CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF(epidermal growth factor)- domain-containing protein 1 (SCUBE1) is a cell surface protein belonging to the SCUBE gene family. SCUBE1 has been shown to rise in parallel to platelet activation in acute ischemic events (Tu et al., 2006). However, the number of studies on this novel molecule is rather limited. The first of these limited studies in the HD patient group was performed in our clinic. We determined that a high SCUBE 1 level in HD patients, in a manner correlated with sCD40L, regarded as a platelet activation marker in HD patients. SCUBE 1 levels were significantly high in predialysis blood specimens and exhibited a significant rise in post-dialysis specimens. Gender, blood pressure, BUN, creatinine, hematocrit and high-sensitivity C-reactive protein (hsCRP) levels, hemodialysis membrane surface area, amount of ultrafiltration, blood flow rate, dialysis flow rate and carnitine use also significantly affected elevated SCUBE 1 in our study (Ulusoy et al., 2012). In conclusion, there are several studies showing platelet activation in HD patients, and it is a fact that the HD process affects this activation. Studies on the subject are continuing today. In addition to the effect of the HD procedure on platelet activation, there are also evaluating the effect on adhesion. Platelet adhesion during the HD procedure can be analyzed using the level of lactate dehydrogenase (LDH) released from the platelets (Daugirdas & Bernardo., 2012). Researchers have particularly evaluated membrane-specific platelet adhesion using this technique. One such study analyzed platelet adhesion in different membrane types by investigating LDH levels, and reported the lowest adhesion in a polysulfone membrane (Asai) (Hayama et al., 2004). In conclusion, HD therapy leads to an increase in platelet adhesion and degranulation. There is also an increase in platelet-platelet and platelet-leukocyte interaction during HD. For these reasons, as with uremic bleeding diathesis, platelets primarily involved in the hemostatic phase in the hypercoagulable process are held responsible for function defects.

4.2. Coagulation cascade activation and a decrease in fibrinolysis

A number of coagulation abnormalities associated with the HD procedure and uremia appear in HD patients. The effect of the HD procedure on coagulation cascade is by two routes. The first is blood passing through blood tubing sets and dialyzers coming into contact with the foreign surface during the procedure. The second is the anticoagulation used

during the procedure. As already discussed, a rise in platelet activation and adhesion comes about during the passage of blood through blood tubing sets and contact with the dialyzer during the HD procedure (Sabovic et al., 2005). The HD procedure also leads to neutrophils adhering to the dialysis membrane and release of granular content. The most important molecule in granular content is TF, one of the natural initiators of the coagulation cascade (Fischer, 2007). Endothelial damage may occur in chronic kidney patients due to uremia, elevated homocysteine, oxidative stress, inflammation and a number of traditional risk factors (HT, DM, hyperlipidemia, cigarettes, etc.). Endothelial damage or dysfunction may cause coagulation activation by leading to a rise in TF in particular, vWF and thrombomodulin (Gris et al., 1994; Gordge & Neild, 1991; Hergesell et al., 1993; Ishii et al., 1991) There are also studies showing a rise in plasmin and thrombin formation in uremic patients (Mezzano et al., 1996; Mezzano et al., 2001). The presence of endothelial damage in uremic patients has been evaluated using various markers. These include intracellular adhesion molecule-1 (ICAM-1), thrombin–antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2), plasmin–antiplasmin complex (PAP), fibrin degradation products (FDP), vWF and soluble thrombomodulin (Rios et al., 2010). A great many studies have shown a rise in these markers of endothelial dysfunction and coagulation cascade and alterations in the fibrinolytic system in uremic patients (Rabelink et al., 1994). One such study was performed by Kushiya et al., who demonstrated increased plasma levels of fibrinogen, plasmin-plasmin inhibitor complex (PIC), thrombomodulin (TM), and D-dimer pre-HD and decreased plasma levels of protein C (PC), antithrombin (AT), TAT and tissue plasminogen activator (tPA)-plasminogen activator inhibitor-I (PAI-I) complex (tPA-PAI-1 complex) (Kushiya et al., 2003). Vaziri et al. determined a decline in coagulation activities even though Factor XII, IX, X and II levels were normal or elevated. Additionally, they determined a significant increase in hyperfibrinogenemia and D-dimer, VWF, factor VII, and factor XIII antibody levels and a pronounced decrease in antithrombin III, free protein S, plasminogen and tissue-type plasminogen activator concentration in end-stage kidney failure patients (Vaziri et al., 1994). Sagripanti et al. determined TAT, fibrinopeptide A (FPA), D dimer, vWF, tumor necrosis factor alpha (TNF), beta-thromboglobulin (beta TG) and serotonin (5HT) levels in predialysis and HD patients compared to the controls. Erdem et al. determined high F1+2, TAT, t-PA, urokinase-plasminogen activator (u-PA), PAP, plasminogen and α2-antiplasmin and α2-macroglobulin levels in HD patients (Erdem et al., 1996). Studies have analyzed the clinical reflections of this coagulation cascade activation and decrease in fibrinolysis. One such determined high levels of fibrinogen, CRP, factor VIII, antiphospholipid antibody and anti-factor 4 platelet-heparin levels in patients with recurrent vascular access thrombosis (O'Shea et al., 2003). Knoll et al. showed that presence of FV Leiden and increased FVIII, Lp(a) and homocysteine levels were associated with vascular access thrombosis (Knoll et al., 2005). In conclusion, in addition to bleeding diathesis in HD patients, a decrease in fibrinolysis and Hypercoagulation, a diametrically opposed condition, is a fact that must not be ignored and continue to give rise to significant morbidity and mortality.

4.3. Treatment

As we have already discussed, in chronic kidney patients the HD procedure itself creates a tendency to thrombus formation due to formed elements in blood making contact with a foreign surface (blood tubing sets and dialyzers). Anticoagulant is used during HD in order to prevent clot formation and ensure the procedure can be completed. Anticoagulant has been used in HD for many years. Selection and dose adjustment of anticoagulant must be based on the patient's clinical condition. Classic unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are the most commonly preferred anticoagulant techniques. Direct thrombin inhibitor (danaparoid) can be used in some selected cases, but the cost is very high (Fischer et al., 2007). UFH and LMWH provide effective anticoagulation in patients with no contraindication in the HD procedure.

Thrombotic complication of vascular access is a frequently encountered condition in the HD patient group. Thrombotic complication is more common in patients using graft as vascular access route in particular. Studies low dose aspirin, sulfinpyrazone and ticlopidine in the prevention of vascular access thrombosis have produced good results (Fiskerstrand et al., 1985; Harter et al., 1979; Kaegi et al., 1975) But these drugs are not frequently used, both because of a lack of sufficient studies and out of a concern they may increase bleeding diathesis in this patient group with a tendency to bleeding. Another important thrombotic complication and main cause of mortality is cardiovascular thrombotic complications (myocardial infarction, cerebrovascular event). Preventive measures against these possibly fatal thrombotic complications in the HD patient group resemble those in the normal population. However, we think that the most important means of preventing various risk factors that facilitate the development of cardiovascular events particular to the HD patient group (hyperhomocysteinemia, inflammation, uremic toxins, Ca-P metabolism disorder) is with adequate dialysis. The provision of effective HD and that this effectiveness is being maintained should be checked at regular intervals.

In addition, as well as the contribution of uremia in HD patients, bleeding diathesis and thrombotic complications are associated with the HD procedure itself. Reduction of these complications with advances in HD technology (biocompatible membranes, new anticoagulant methods, etc.) will contribute to a decrease in mortality and morbidity in HD patients. As with complications of all kinds in HD patients, adequate dialysis plays a key role in this area.

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