Hemodialysis effect on platelet count and function and hemodialysis-associated thrombocytopenia

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Substantial activation of platelets can occur in the course of hemodialysis. Platelet surface markers show evidence of platelet degranulation. Some activation occurs due to exposure of blood to the roller pump segment and microbubbles may play a role. Platelet activation seems to be reduced with reused dialyzers or with those containing synthetic versus cellulosic membranes. Nevertheless, a substantial degree of platelet activation can be demonstrated with polysulfone and other synthetic membranes; the amount of activation may differ substantially among polysulfone membranes, depending on the manufacturer and the polyvinylpyrrolidone content. Platelet–platelet and platelet–leukocyte aggregates have been detected in the dialyzer blood outflow line and the consequences of these to the microcirculation are unknown. Typically, the platelet count decreases slightly during the first hour of dialysis, but mostly returns to initial values by the end of dialysis. A number of chronic hemodialysis patient cases have been reported in which a marked decrease in platelet count (50% or more) during dialysis was observed, resulting in mild degrees of predialysis thrombocytopenia. In only one case was the decrease in platelet count associated with bleeding. Dialyzer hypersensitivity symptoms are infrequently associated with a fall in platelet count. Most recent cases of dialysis-associated thrombocytopenia have been with polysulfone membranes, especially polysulfone membranes sterilized by electron beam. The exact cause of these reactions remains unknown.

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The goal of this review is to summarize the more recent literature pertaining to the effects of hemodialysis on platelet count and function, with an emphasis on the role of different dialysis membranes, as well as to highlight several case reports and one large series of substantial thrombocytopenia in patients dialyzed with nominally biocompatible dialyzers.

PLATELET NUMBER, SURVIVAL, AND FUNCTION IN CHRONIC KIDNEY DISEASE

In predialysis patients, as well as in hemodialysis patients, platelet number tends to be reduced,[1] in the range of 175–180,000/mm^3 compared with 250,000/mm^3 in healthy controls. In continuous ambulatory peritoneal dialysis patients, platelet counts have been reported to be closer to the normal range.[2] Platelet survival in hemodialysis patients is thought to be of normal duration, although the only paper examining this was published in 1967.[3] The megakaryocyte number in bone marrow is normal,[1] but the reticulated platelet count, a measure of thrombopoiesis, is reduced, despite elevated thrombopoietin levels.[2,4]

Effect of hemodialysis on the platelet count and function

The majority of the studies published in English between 1981 and 2011 examining the effects of dialysis on platelet count and/or function were reviewed and are listed in the table under Supplemental Materials online.

Platelet count

In studies of dialysis effects on platelets, platelet counts typically have been measured before dialysis, 15 or 30 min into dialysis, and at intervals up to the end of treatment. In most studies, the platelet count falls slightly (typically 5–15%) during the first 15–30 min of dialysis. Almost invariably, the platelet count then either returns to the predialysis level or overshoots it slightly by the end of the dialysis session.[5–14]

Cuprammonium cellulose (CU) or so-called Cuprophan membranes (no longer widely used) markedly activate the complement system as measured by intradialytic release of complement fragments such as C3a or C5a des-Arg, and by this criterion have been judged to be relatively bioincompatible. Reuse of Cuprophan membranes without bleach can potentially reduce their degree of complement activation due to protein coating after initial use. The early intradialytic fall

in platelet count is attenuated when cellulosic membranes are reused without bleach.[14–16] A large number of studies have been conducted comparing the early intradialytic fall in platelet count with cellulosic membranes to membranes known to cause lower amounts of complement activation such as cellulose acetate (CA), polymethylmethacrylate (PMMA), polysulfone (PS), or polyacrylonitrile (PAN and AN69). Amato *et al.*[13] found a 7% drop in platelet count at 30 min when dialyzing with CU versus 2.5% using PS, but this was not statistically significant. Kuwahara *et al.*[15] found a 3.7% platelet drop at 15 min with CA versus an 8.6% decrease with CU. Hoenich *et al.*[17] found a similar fall in platelet count with PS (Fresenius) as with CA, and these were lower than that with CU. In a study comparing CA, cellulose triacetate (CTA), and Fresenius Hemoflow PS, Hoenich *et al.* found that the intradialytic platelet fall was comparable between CTA and PS, but increased with CA.[18] With PAN, a reduced degree of intradialytic fall in platelets was measured in some,[5,7,19] but not all,[20–22] studies. Mujais *et al.*[23] compared PS, PAN, and a modified form of PAN membrane called 'SPAN' designed to reduce generation of bradykinin. The intradialytic platelet count profile was identical for PS and PAN, but a significantly greater platelet drop was seen with SPAN. Most of the above studies focused on a single dialysis session, and only a few examined the effects of using different dialysis membranes for an extended period and the impact on the predialysis platelet count. One such extended study was by Hakim *et al.*,[11] which found that the predialysis platelet count using CU membranes was substantially lower than that when PMMA was used.

PS membranes are available from several different manufacturers and differ substantially in their composition. They can be manufactured in either a low-flux or a high-flux configuration. Many (but not all) PS membranes contain polyvinylpyrrolidone (PVP), which is incorporated into the membrane in various amounts both to increase its hydrophilic nature and to affect the membrane's flux characteristics.[24] The biocompatibility of PS membranes varies according to the degree of added PVP, as well as other factors.[24] Polyethersulfone (PES) membranes are a variant of PS, designed to have better clearance of high-molecular-weight toxins while restricting passage of albumin. Depending on how the modification is done, PES membranes may be more biocompatible than PS membranes based on the degree of leukopenia[25] and platelet adhesion.[26] The Gambro 'Polyflux' membrane is a blend of polyarylethersulfone, PS, and PVP.[27] In one study, platelet counts were compared using three brands of PS dialyzers,[28] one of which contained no PVP. The fall in platelet count with the PVP-free PS was substantially greater than that with either of the PS membranes containing PVP. Hoenich and Katopodis[27] found superimposable intradialytic platelet count curves for PS and PES. Stefoni *et al.*[29] found no changes in intradialytic platelet count with the Fresenius PS dialyzer versus the Fresenius X-series Helixone dialyzer. Opatrny *et al.* compared the same two membranes and also found no difference.[30] In another paper, Stefoni *et al.*[31] looked at platelet numbers with a PES membrane. No intradialytic drop in platelets could be identified.

Dialyzers continue to be sterilized by a variety of methods, including ethylene oxide, γ-ray, steam, and electron beam. The method of sterilization may affect biocompatibility, even in cases where the same basic membrane material is used.[32] In one study,[33] the biocompatibility of Fresenius low-flux PS sterilized using ethylene oxide was compared with high-flux PS sterilized with either ethylene oxide or steam. The platelet counts were not different among these three groups, although intradialytic leukopenia was reduced with the steam-sterilized dialyzer.

Measures of platelet degranulation and aggregation

The platelet count is a blunt instrument as far as platelet activation is concerned, and various measures used to study platelet activation during dialysis are reviewed in Table 1. Platelet factor 4 (PF4) and β-thromboglobulin are substances contained within platelet α-granules, and changes in platelet PF4 or β-thromboglobulin levels are useful indicators of platelet degranulation and activation during dialysis. The appearance of specific markers on the surface of platelets corresponds to activated receptor binding to fibrinogen and von Willebrand factor, or to degranulation of α-granules and lysozomes. These surface markers can be quantified by flow cytometry monoclonal antibody–based measurements. Surface markers of particular interest are listed in Table 1 and include CD41 (GPIIb/IIIa), PAC-1, CD42b (GPIb), CD62P (selectin), CD63 (glycoprotein 53), and annexin-V.[34] Flow cytometry is believed to be one of the best ways to detect platelet activation. CD41 is a marker for the activation-dependent receptor GPIIb/IIIa and therefore for fibrinogen binding capacity. PAC-1 will detect only the activated form of this receptor, which has opened up for fibrinogen binding. CD42b or GP1b functions as a receptor for von Willebrand factor. CD62P detects P-selectin, which is a component of the α-granule membrane, which only becomes incorporated into the surface platelet membrane with degranulation. Hence, CD62P is a marker for a degranulated platelet. CD63 detects degranulation of platelet-dense granules in a manner similar to P-selectin (Figure 1).

Circulating platelet aggregates have been associated with cardiovascular risk factors such as myocardial infarction, transient ischemic attack, angina, and peripheral arterial insufficiency.[35] Platelets form aggregates not only with themselves but with other formed elements of the blood, and a variety of techniques using flow cytometry have been developed to detect these; basically, one looks for platelet-specific antigens in the leukocyte (neutrophil, monocyte, and lymphocyte) traces of the flow cytometry output.

One good study of platelet surface activation markers that looked at multiple membranes was by Cases *et al.*[36] They measured CD62 (P-selectin marker) in platelets exiting versus those entering the dialyzer, and found increases in CD62 in blood exiting the dialyzer 5 min after beginning dialysis.

Table 1 | Commonly measured biomarkers of platelet activation in dialyzer studies

Measure	Significance	Examples where this was used (reference no.)
Platelet count	Clinically significant thrombocytopenia (heightened risk of bleeding) occurs when count approximately $<20{,}000/mm^3$	5, 11, 10
MPV	A drop may reflect degranulated platelets; an increase may reflect new platelets release	31
^{111}Indium-labeled platelets	Location of platelet deposition	49
PF4	Protein proteoglycan complex, MW=358 kDa, released from α-granules when platelets aggregate. A high serum level indicative of acute platelet activation, but it also is stored in endothelial cells and released by heparin	42
βTG	Amino-acid complex, MW=36 kDa, released from α-granules when platelets aggregate. A high serum level indicative of acute platelet activation	42
CD41 (GPIIb/IIIa)	Platelet activation promotes a structural change in the GPIIb/IIIa receptor. Total and bound are measured. Increases, particularly of bound GPIIb/IIIa, indicate platelet activation. Overexpression of GPIIb/IIIa may be a sign of chronic platelet activation and may be a cardiovascular risk factor	42
CD42b (GPIb, glycoprotein Ib)	Detects von Willebrand factor receptor	43
CD62P	Detects P-selectin, platelet α-granule degranulation	43
CD63	Detects glycoprotein 53, platelet lysosomal degranulation	43
PAC-1	Detects conformational change in fibrinogen receptor GPIIb/IIIa	43
Annexin-V-positive platelets	A measure of phosphatidylserine externalization; may correlate with P-selectin positivity	54
Platelet aggregometry	Platelet aggregation in response to ADP, collagen, or epinephrine	102
Platelet aggregates	Platelet aggregates	35
Platelet-erythrocyte/ neutrophil/ monocyte aggregates	Activated platelets tend to stick to other white blood cells	41
Lactase dehydrogenase activity	Measured after lysis of adhered platelets: a measure of degree of platelet adhesion to a membrane	47

Abbreviations: βTG, β-thromboglobulin; MPV, mean platelet volume; MW, molecular weight; PF4, platelet factor 4.

There were clear differences among membranes, with CU > CA > PMMA > PS > PAN (AN69). Komarnicki *et al*,[37] using a CU membrane, did not find evidence of platelet activation in terms of surface receptors for fibrinogen or von Willebrand factor (CD41a and CD42b). He measured platelet levels of these markers 15 min into dialysis, but dialyzer inlet blood only was sampled; dialyzer outlet samples were not reported. In a follow-up paper in 1997, Cases *et al.*[36] showed that it was important whether dialyzer inlet or outlet blood was studied. Using the CD62 P-selectin surface marker, and comparing CU, CA, CTA, hemophan, and PS, as well as studying levels 5 min into dialysis, CD62 marker levels remained unchanged in the blood inlet line, but outlet levels were threefold higher with CU and twofold higher with the CA, CTA, hemophan, and PS membranes. Aggarwal *et al.*[38] used a slightly different method to assess CD61 (GPIIIa), CD42, and CD62 platelet surface markers in inlet and outlet blood during dialysis. These marker levels were increased in the outlet blood early in dialysis. However, at the end of dialysis, the ability of platelets to express CD62 in response to ADP was reduced, which was evidence for impaired platelet function at the end of dialysis or consumption of activated platelets. This was accompanied by a greater nonspecific binding of fibrinogen to the platelet surface at the end of dialysis.

Gawaz *et al.*[39] used flow cytometry to identify platelet–leukocyte aggregates by looking for the CD41 platelet surface marker on the leukocyte trace. No difference in blood inlet level of platelet–leukocyte aggregates was found in the course of a dialysis session, but the outlet levels were increased throughout dialysis, to an approximately equal extent with PS, PAN, and SPAN membranes. An indirect functional study of platelet–leukocyte aggregates was conducted by Bonomini *et al.*[40] They measured the expression of a CD62P tag on leukocytes as an indication of platelet–leukocyte aggregation, and also measured neutrophil reactive oxygen species, finding that reactive oxygen species generation was higher in neutrophils tagged with CD62P compared with those without this tag. Their conclusion was that platelet–neutrophil aggregation was leading to reactive oxygen species generation.

In another study, Bonomini *et al.*[41] measured CD62 and CD63 platelet surface marker levels and markers of leukocyte-platelet aggregation in the inlet blood periodically during dialysis, and used these measurements to compare platelet activation among low- and high-flux PES dialyzers and PS dialyzers, all manufactured by Bellco. There was considerably less neutrophil–platelet aggregate formation with the PES dialyzers versus PS, and the prevalence of CD62P and CD63 platelet activation markers were lower with the high-flux PES membrane compared with either the PS membrane or the low-flux PES membrane.

Kuragano and co-workers[42] measured CD41 platelet surface markers indicative of both resting and activated (bound state) GPIIb/IIIa receptor during dialysis with either a PS or CTA membrane. Total GPIIb/IIIa activity was not different during dialysis or between the two membranes, but bound GPIIb/IIIa increased progressively during dialysis with

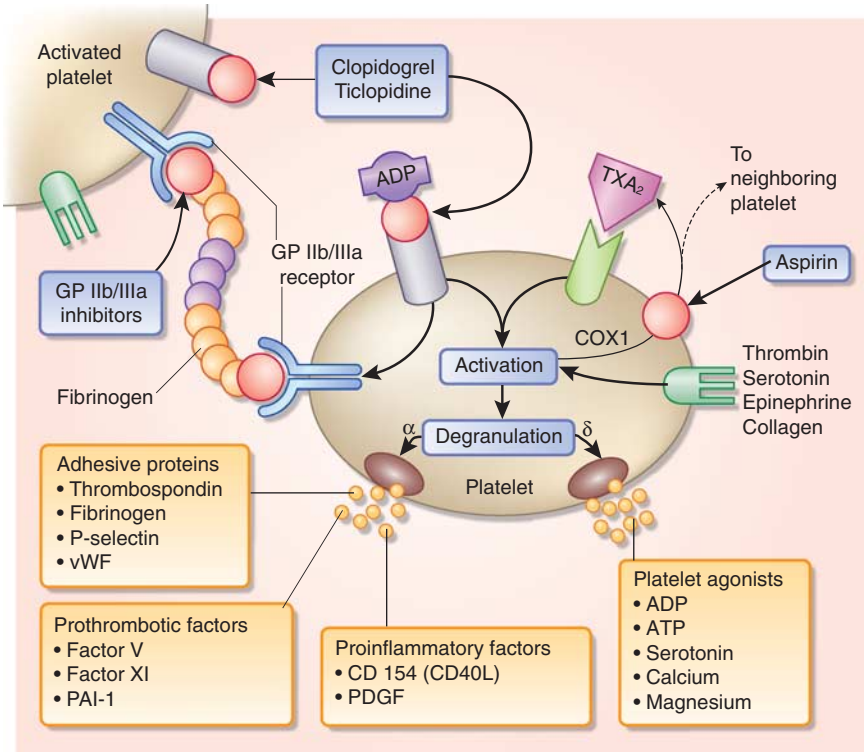

Figure 1 | Mechanisms of platelet activation.[104]

the PS membrane and was unchanged with the CTA membrane. In contrast, PF4 levels with CTA were considerably higher than with the PS membrane.

Thijs *et al.*[43] compared several platelet surface markers during dialysis with CU membranes versus PS. CD26P and CD63 were measured as degranulation markers for P-selectin and lysozyme, and CD42b and PAC-1 antibodies were used to detect total and activated GPIIb/IIIa receptor expression. When studying outlet versus inlet levels of platelet surface markers during dialysis, the only increase detected was with the CD42b marker with the PS membrane. Platelet degranulation markers and markers of activated GPIIb/IIIa (PAC-1) were not increased in the outlet bloodlines with either membrane. However, after 2 weeks of dialysis with the PS membranes, the predialysis level of platelet surface markers for CD62P, CD63, and PAC-1, but not of CD41 or CD42b, were all increased compared with the levels after 2 weeks of dialysis using the CA membrane. These data suggested that dialysis with low-flux PS membranes may be inducing a chronically activated platelet state.

Gritters *et al.*[44] compared levels of PF4 and CD62P, both indicators of platelet degranulation, in patients dialyzed with PS membranes. The study was unique in that blood was drawn from various points in the dialysis circuit, including at two points upstream to the dialyzer, one upstream to the blood pump, and one downstream to the blood pump. PF4 levels increased after injection of low-molecular-weight heparin before starting extracorporeal circulation. CD62P levels did not increase until after dialysis was started. Platelet serotonin levels before dialysis were found to be reduced, which was evidence for platelet granule depletion. The fact that, at 5 min, there was a marked step-up in the CD62P percentage in the samples taken upstream and downstream to the blood pump, was taken to be evidence that platelet degranulation surface markers are increased not only because of exposure to the dialyzer but also because of the shear stress encountered in the blood roller pump. In a follow-up paper,[45] Gritters *et al.* compared CD62P expression with two different PS membranes when used for hemodialysis or hemodiafiltration. Different membranes (Helixone for HDF and F8 PS for HD) were used for the two procedures; CD62P expression was more pronounced and more protracted during hemodiafiltration compared with hemodialysis. The concept here is that the increased blood shear stress associated with hemofiltration might augment platelet activation. Yet another possible mechanism for platelet activation has to do with the formation of microbubbles, which are formed commonly in most extracorporeal circuits.[46]

Studies measuring platelet aggregates using methods other than flow cytometry have generally found evidence of increased aggregation in the early part of dialysis,[6] and aggregation was less with the use of more biocompatible membranes.[10,44] Gritters *et al.*, in the same study described above with regard to platelet degranulation,[44] also measured platelet aggregates by manually counting them under a light microscope and found a substantial increase in platelet aggregates at 30 min when measured at the dialyzer blood inlet downstream to the blood pump and still upstream to the dialyzer, suggesting that exposure to the roller pump alone was sufficient to create platelet aggregates.

Platelet adhesion and deposition

Release of lactic dehydrogenase (LDH) from platelets adhered to a membrane can be used to measure the degree of membrane-specific platelet adhesion during dialysis. Fushimi *et al.*[47] used this method to study the effect of incorporation of differing amounts of alkylether carbolic acid into a cellulose-based dialyzer membrane. Increasing amounts of this substance resulted in progressively lesser amounts of adhered platelets as determined by the LDH assay, and this correlated roughly with C5a generation but not with contact-phase coagulation activity. Hayama *et al.*[24] used the LDH method to compare the platelet adhesion properties of various PS membranes containing different amounts of PVP, including two PS membranes from Fresenius, one from Terumo, and one made by Asahi. The Asahi PS membrane, which had the most PVP, had markedly less platelet adherence using the LDH assay compared with the other membranes.

Dewanjee *et al.*[48] studied, in anesthetized pigs, the fate of ^{111}indium-labeled platelets during dialysis using a cellulosic membrane. They found increased radioactivity in the lungs and kidneys of animals killed after dialysis, suggesting some degree of embolization of platelet thrombi to these organs as a result of dialysis. They also found radioactivity in the dialyzer, but to a much lesser extent during *in vivo* versus *in vitro* studies. Windus *et al.*[49] used a similar technique in patients, looking for radiolabeled platelet deposition in polytetrafluoroethylene grafts. Reused regenerated cellulose membranes were used for dialysis. There was a marked enhancement of radioactivity measured at the graft site after dialysis. No significant change in radioactivity over the access was noted in two patients being dialyzed with native arteriovenous fistulas.

Platelet function tests

In vitro platelet aggregation tests can be conducted to measure platelet function. One can measure the slope of the first phase of platelet aggregation and the degree of aggregation of the second phase. Usually, responses to ADP, epinephrine, and other agents are tested. Berrettini *et al.*[5] compared aggregation tests before, during, and after dialysis with CU versus PAN membranes. CU membranes seemed to create worsening of aggregation versus PAN membranes when pre/post changes were compared. Ivanovich *et al.*[50] found no changes in platelet aggregation in nine patients undergoing heparin-free dialysis using a CA membrane. Andrassy *et al.*[51] found impaired platelet aggregation (% maximum response) stimulated by collagen, epinephrine, arachidonic acid, or ristocetin 30 min into dialysis using a PS membrane. Taylor and co-workers[52] compared spontaneous and collagen-induced platelet aggregation during dialysis with CU plate versus hollow-fiber dialyzers. Aggregation was reduced during dialysis to an equal extent with each of the two dialyzer configurations. Ivanovich *et al.*[19] compared ADP (low- and high-dose)-stimulated platelet aggregation 15 min into dialysis with several varieties of CU and CA membranes and also with PS and PAN. Results were quite variable, and with low-dose ADP changes in aggregation occurred in opposite directions with different CU dialyzers.

A paper by Sloand and Sloand[53] was interesting in that both platelet aggregation and bleeding time were measured. CU membranes were used. Platelet aggregation response to thrombin and platelet agglutination response to ristocetin were both prolonged after dialysis, along with bleeding time. A more recent study by Elshamaa *et al.*[54] found that ADP-stimulated platelet aggregation measured before dialysis in patients being dialyzed with a PS membrane was impaired.

HEPARIN EFFECT ON PLATELETS AND HEPARIN-INDUCED THROMBOCYTOPENIA

Heparin is known to promote platelet agglutination and activation by a nonimmunogenic mechanism that has not been completely characterized. One explanation is that heparin interacts with integrins on the platelet surface, and specifically with integrins that are part of the GPIIb/IIIa receptor.[55] Functionally, heparin exposure tends to make platelets self-aggregate, and there appears to be a certain 'memory' to this action.[56] For example, when blood is drawn into heparinized tubes in nonuremic patients who have been exposed to heparin and compared with blood from patients who have not been previously exposed, the platelet count in the heparin-exposed subjects is factitiously decreased because of spontaneous aggregate formation; this does not occur when blood is drawn into EDTA-containing tubes. Heparin also can make platelets degranulate, releasing PF4 as discussed above,[44] even without exposure of blood to the extracorporeal circuit.

Almost all of the studies of platelet activation during dialysis cited in Table 1 were conducted using heparin. The few studies in which effects of dialysis on platelet function have been assessed in the absence of heparin are of great interest. Casati *et al.*[57] compared platelet counts during heparin-free versus tight-heparin dialysis in patients at high risk of bleeding. Only predialysis and postdialysis platelet counts were measured, and there were no differences between the two groups, with a tendency for platelet counts to increase slightly with either treatment. In another study comparing heparin-free and conventional dialysis using a CU membrane, there was no fall in platelet count 30 min into conventional dialysis using heparin, and the results were not different from heparin-free dialysis.[58] Leitienne *et al.*[59] compared the effects of dialysis using a CT membrane with four different heparin protocols, using either unfractionated heparin or low-molecular-weight heparin, studying each of the compounds at two different doses. Although the degree of leucopenia and leukocyte elastase were increased with unfractionated heparin versus low-molecular-weight heparin, the decreases in platelet counts with the four anticoagulation regimens were similar.

A clinically more important problem with heparin is the fact that it binds to PF4, and the heparin-PF4 molecule is

antigenic, giving rise to IgG, as well as IgA and IgM antibodies. The IgG antibodies to the heparin–PF4 complex can then activate platelets via their surface Fc receptor. To make matters worse, endothelial-bound heparan sulfates also bind PF4, and this complex is apparently antigenic as well, attracting binding by these same IgG antibodies, followed by platelet binding to these endothelial areas with platelet activation and tissue thrombosis. For an excellent, recent review of heparin-induced thrombocytopenia (HIT), please see Syed and Reilly.[60] The clinical manifestations of HIT in dialysis patients include thrombocytopenia, often to below 100,000/mm^3, or a drop in platelet count by more than 50% from baseline, typically occurring 5–10 days after the start of heparin therapy. Only rarely is HIT associated with bleeding; rather, feared complications are thromboembolic manifestations, including deep venous thrombosis, pulmonary embolism, myocardial infarction, and limb ischemia. In dialysis patients, HIT has been linked to clotting of the extracorporeal circuit and also to clotting of the vascular access.[60] Recently, an allergic type of reaction to heparin, similar to 'first use syndrome' of dialyzers, has been described in patients with HIT,[61,62] although anaphylactoid reactions to heparin can occur even in the absence of HIT, and have been reported with exposure to adulterated heparin.[61] A number of studies have examined the effect of HIT on mortality in dialysis patients, and the results are varied, with some studies showing an adverse effect, whereas others show little risk.[60] The diagnosis of HIT is not always straightforward. ELISA antibody kits to detect IgG, IgA, and IgM PF4-heparin antibodies exist, but sometimes the antibodies detected are not associated with manifestations of HIT. A functional test involving the release of radiolabeled serotonin by heparin is available and is considered a 'gold standard'.[60] The prevalence of HIT antibodies in dialysis patients ranges from 0 to 17%, although functional HIT antibody assays are positive in only about 3% of patients.[60]

Because heparin seems to increase platelet stickiness in most if not all patients, and also can degranulate platelets, as discussed above, one would expect that, in the general population, the use of heparin for dialysis might be expected to 'amplify' dialyzer-associated effects on platelets such as activation and thrombocytopenia. In a substantial minority of patients with HIT, one might expect a 'high volume amplification' of any dialyzer-associated platelet effects. A study by Luzzatto *et al.*,[63] however, argues against this. In 50 chronic dialysis patients, six of whom had HIT antibodies, they examined the decrease in platelet count during dialysis. In the six patients with HIT antibodies, the mean decrease in platelet count (pre versus postdialysis) was ~12%, which was not markedly different from the 8% decrease in platelets in the entire group of 50 patients. Interestingly, in 10 out of 50 patients in this particular study, the postdialysis platelet count decreased by more than 35%, which was a greater drop than in most of the other studies where platelet count during dialysis was measured; the reasons for this were not examined.

Effect of non-heparin anticoagulants on platelet activation

Although heparin activates platelets, a number of other anticoagulants inhibit the platelet activation and degranulation normally seen during dialysis. For example, Gritters *et al.*[64] measured the levels of the leukocyte degranulation marker (and platelet degranulating factor) myeloperoxidase, as well as PF4, during dialysis using heparin, dalteparin, or citrate as anticoagulant. The observed increase in myeloperoxidase and PF4 observed during heparin or dalteparin-anticoagulated dialysis was markedly reduced when citrate was used. Similarly, nafamostat mesilate, a protease inhibitor that can be used as an alternative anticoagulant for dialysis, has been shown to markedly attenuate the platelet aggregation normally seen during dialysis.[65] The effects of other alternative anticoagulants and of various anti-platelet drugs on dialysis-associated platelet activation is a topic that has not been well studied and merits further investigation.

Thrombocytopenia in dialysis patients

In addition to HIT, dialysis patients, especially those in the intensive care unit, but even those treated as outpatients in dialysis units, often can be affected by other medical conditions associated with thrombocytopenia. In acutely ill patients with sepsis, thrombocytopenia is common, with or without disseminated intravascular coagulation.[66] Platelet consumption due to thrombotic thrombocytopenic purpura or idiopathic thrombocytopenic purpura, due either to immunologic diseases, especially various forms of vasculitis, or drugs, is not uncommon in renal patients. Physical destruction of platelets may occur because of intravascular catheters.[67] Thrombocytopenia and platelet dysfunction are commonly seen in liver disease, paraproteinemia, myeloproliferative disorders, and myelodysplastic syndrome.[66,67] Thrombocytopenia may be found in patients receiving nicotinamide for treatment of cholesterol abnormalities or hyperphosphatemia,[68] and many drugs sometimes taken by hemodialysis patients, including clopidogrel and other antiplatelet agents, as well as quinine, for example, can cause drug-induced thrombocytopenia.[69] Finally, in actively bleeding patients, dilutional thrombocytopenia can occur when transfusing packed red blood cells (RBCs) only, as functional platelets are not present in packed RBC transfusions.

CASE REPORTS OF CLINICALLY REMARKABLE DIALYSIS-ASSOCIATED THROMBOCYTOPENIA

Despite the many possible causes for thrombocytopenia in dialysis patients, a number of reports have been published, in which there is no obvious explanation other than the dialysis procedure. At least eight such cases have appeared (Table 2): five published as full articles,[70–74] one as an abstract presented to the National Kidney Foundation Spring Clinical Meetings in 2009,[75] and two in the US Food and Drug Administration Manufacturer and User Facility Device Experience (FDA MAUDE) database.[76] In addition, there is one Canadian province-wide analysis of thrombocytopenia with different dialyzers that was presented to the British Columbia Provincial Renal Agency in 2011.[77] The case reports all

Table 2 | Reports of clinically important, nonheparin-related thrombocytopenia believed to be associated with hemodialysis

Year	Chief author	Reference	Dialyzer(s) associated with thrombocytopenia/sterilization mode	Symptoms	Dialyzer(s) with which thrombocytopenia resolved/ sterilization mode
1983	Vicks *et al.*	70	Cordis Dow Artifical Kidney, CU/ ethylene oxide	None	PAN
2005	Yang and Lindsay	71	Fresenius Optiflux F160NR/ethylene oxide F70NRe/electron beam	Chest pain 45 min into dialysis, intradialytic hypoxemia (with Optiflux); sudden dyspnea and burning 10 min into dialysis (with F70 NR3)	Hospal Nephral ST400(AN69ST)/γ-ray
2009	ID=844712	76	Gambro Polyflux H (polyarylethersulfone, polyvinylpyrrolidone and polyamide)/steam	Dyspnea 30–45 min into dialysis	No details given
2011	ID=1713747-2009-00003	76	Fresenius Optiflux 160NRe/electron beam	None	No details given
2009	Nasika	75	Polysulfone: no details given	None	Cellulose triacetate/(probably γ-ray)
2010	Post	72	Fresenius Optiflux 160/ethylene oxide	None	Asahi REXEED AR-25S/γ-ray
2011	Olafiranye *et al.*	73	Fresenius Optiflux 200NR/electron beam	None	Baxter CT190G (cellulose triacetate)/γ-ray
2011	Posadas *et al.*	74	Fresenius Optiflux 180NR/electron beam	None	Fresenius Hemoflow (F80A)/ETO Baxter CT-190G (cellulose triacetate)/γ-ray ASAHI-AM-BIO-100/γ-ray
2011	Kiaii *et al.*	77	Electron beam–sterilized polysulfone dialyzers from two different manufacturers	None	ASAHI REXEED/γ-ray; Gambro Nephral 400/γ-ray

Abbreviation: PAN, polyacrylonitrile.

described patients with some degree of predialysis thrombocytopenia, as well as a marked (~50%) reduction in platelet count post dialysis. Bleeding was only associated with one of the cases.[70] Three cases[71,74,76] were associated with respiratory and other symptoms during the first hour, consistent with a dialyzer hypersensitivity type of syndrome.

The first case report, published in 1983,[70] describes a patient with long-standing gastrointestinal bleeding who was being treated with chronic peritoneal dialysis, whose bleeding worsened after being switched to hemodialysis using a CA membrane, and may have been worsened further when he was changed to hemodialysis using a CU membrane. Postdialysis platelet counts during the CU/CA dialysis period were markedly lower than predialysis values. Predialysis platelet counts, postdialysis platelet counts, and the intradialytic drop in platelet count improved markedly after the patient was changed to hemodialysis using a PAN membrane, and bleeding markedly improved. This patient received multiple blood transfusions throughout his course, especially during the period when platelet count was the lowest. At that time, he received 8 units of packed RBC in 5 days, and only 1 unit of platelets, followed by 8 units of packed RBCs over a 10-day period. Thus, the possibility of dilutional thrombocytopenia cannot be ruled out. The patient was new to hemodialysis, and the dialysis treatments were all carried out using beef lung heparin. The possibility of HIT was never considered, although the recovery of the platelet count with the cessation of bleeding and the use of the PAN dialyzer was not consistent with HIT as an etiology for the thrombocytopenia.

A second case, reported by Yang and Lindsay in 2005,[71] describes a complicated patient with a serious abdominal infection being dialyzed with two types of Fresenius PS dialyzers (Optiflux160 and the electron beam–sterilized F70NRe). The patient had somewhat atypical dialyzer reaction symptoms (early finger cyanosis, drop in O_2 saturation), and also chest pain at about 30 min into dialysis. The patient was changed to hemodialysis using a PAN membrane (Nephral ST), with equivocal improvement in symptoms. Pre/postplatelet count drops were marked with each of the two PS dialyzers used, but were much less prominent with the PAN dialyzer. The predialysis platelet counts were in the range of 133–148,000/mm^3 with the dialyzers which caused the most symptoms, and the platelet count before dialysis was 91,000/mm^3 on the AN69 membrane, which caused the fewest symptoms. As complicating features, the patient's intra-abdominal infection was treated with linezolid, with which thrombocytopenia is not uncommon in ESRD,[78] and thrombocytopenia occurs commonly with sepsis, which the patient had. The patient was dialyzed using heparin, and HIT antibody levels were not measured.

A third case is from the FDA MAUDE database (Table 2). This was a patient who was experiencing shortness of breath about 45 min into dialysis and who was believed to be having a 'possible dialyzer reaction'. The dialyzer used was listed as Gambro Polyflux H. The platelet count was noted to be markedly depressed on admission to hospital. HIT was certainly not ruled out, and other reasons for

thrombocytopenia cannot be excluded given the small amount of detail available.

A fourth case, also from the FDA MAUDE database, was a patient who developed thrombocytopenia concurrent with a change of dialyzer brand to a Fresenius Optiflux 160NRe electron-beam–sterilized dialyzer. The previous brand of dialyzer is not mentioned. There was a marked intradialytic, as well as a predialysis, fall in the platelet count, which reverted to baseline when the dialyzer was changed back to the previous brand.

A fifth case reported in a National Kidney Foundation Spring Meeting abstract in 2009[75] describes an incident dialysis patient whose platelet count was noted to be 77,000/mm^3 after a few days of dialysis with a PS membrane versus 150,000/mm^3 before beginning dialysis. HIT antibodies were assayed and were negative, and there was improvement of thrombocytopenia on changing from a PS to a CTA membrane.

A sixth case was reported by Post *et al.* in 2010.[72] This was an incident dialysis patient in whom platelet count was noted to be 78,000/mm^3 post dialysis while using a Fresenius Optiflux160 membrane. Predialysis thrombocytopenia, as well as a large pre/post drop in platelet count, persisted despite withholding heparin. Change to an Asahi PS dialyzer rapidly improved the platelet count back toward normal. In this case, there was low likelihood that the thrombocytopenia was due to HIT, because no heparin was used for the initial treatments and for most of the treatments thereafter. In addition, HIT antibodies were measured and were found to be negative.

A seventh case was described by Olafiranye *et al.* in 2011.[73] This was a middle-aged woman initiating dialysis whose platelet count dropped to 16,000/mm^3 from a baseline of 90,000/mm^3 after the first hemodialysis session using a Fresenius F200NR dialyzer (electron-beam sterilized). Workup for HIT was negative. There were no symptoms and no bleeding. On the third dialysis session, the dialyzer was changed to a Baxter CTA dialyzer (Elektra 210, γ-sterilized), after which the platelet count rapidly increased to 150,000/mm^3.

An eighth case, also reported in 2011,[74] was a prevalent hemodialysis patient who was experiencing shortness of breath during her dialysis sessions using a Fresenius F180NR dialyzer. The predialysis platelet count was noted to be low at 50,000/mm^3, as compared with her usual baseline of 150,000/mm^3. The postdialysis platelet count was measured and noted to be low, $\sim$20,000/mm^3. No bleeding was present. The patient did have some atypical chest pain during dialysis. An extensive workup for thrombocytopenia was negative. Thrombocytopenia promptly resolved after the patient was changed to a Baxter CT190G CTA dialyzer.

A province-wide analysis of thrombocytopenia associated with dialysis was reported in 2011 in poster form to the British Columbia Provincial Renal Agency and later published.[77] The analysis was triggered by 'a case of profound and unexplained thrombocytopenia' in a patient being dialyzed using a electron beam–sterilized PS dialyzer in whom thrombocytopenia promptly resolved on changing to a Nephral 400 (AN69, γ-ray sterilized) dialyzer. Similar dialysis unit cases were identified, and then, in two different Canadian Provinces, an increase in the incidence of thrombocytopenia was appreciated concurrent with a recent adoption of wider use of electron beam–sterilized PS dialyzers. These dialyzers were supplied by two different manufacturers. The risk of thrombocytopenia province-wide associated with the use of an electron beam–sterilized PS dialyzer was found to be 2.52 (confidence interval = 1.20–5.29, $P = 0.02$).

One possible explanation for the thrombocytopenic effect of the PS dialyzers listed in Table 2 is the use of electron beam sterilization. Electron beam sterilization alters the surface property of PS-PVP membranes, increasing their hydrophilicity.[79] Different sterilization properties of membranes can affect their biocompatibility.[32] Against such an interpretation is the results of a study that to date has been reported only in abstract form by Ryder *et al.*;[80] predialysis platelet counts in 2043 prevalent dialysis patients were compared before and after changing from a non-electron-beam–sterilized PS dialyzer to one sterilized by electron beam irradiation. This change was made between November 2005 and March 2006. There was a high variability in the predialysis platelet count, but the change in dialyzer sterilization method apparently had no effect. It remains possible that subsequent to 2005/2006 some change in the dialyzer manufacturing process was made in PS electron beam–sterilized dialyzers that may be responsible for the increased incidence of thrombocytopenia seen with such dialyzers in 2009/2010 as reported by Kiaii *et al.*[77]

CONCLUSIONS

Platelet surface markers measured in dialyzer outlet blood give evidence for both platelet activation and degranulation in the course of dialysis. In addition, there is evidence for formation of platelet–platelet and platelet–leukocyte aggregates. The clinical consequences of this effect of dialysis on platelets are unknown. Some of the activation and aggregation of platelets during dialysis may be due to exposure of blood to the roller pump segment of the dialysis tubing or to microbubbles and does not depend on exposure to the dialyzer membrane *per se*. With regard to dialyzer membranes, platelet activation seems to be reduced with reused dialyzers and with synthetic versus cellulosic membranes. However, platelet activation can be demonstrated with some PS membranes, and the amount of activation may differ depending on the manufacturer and the PVP content. The platelet count decreases during dialysis, but this decrease is usually small, tends to be maximal at 15–30 min into dialysis, and mostly resolves by the end of dialysis. Recent reports of substantial thrombocytopenia associated with some brands of electron beam–sterilized PS dialyzers have yet to be explained. More attention needs to be paid to potential synergistic effects between dialyzer membrane manufacturing processes and methods of sterilization, and especially to

determine whether and how the use of electron beam sterilization may impact the interaction between certain PS membranes and platelets. Finally, the results suggest that platelet activation occurs almost universally when blood is subjected to dialysis and even to extracorporeal circulation only when using a blood roller pump. This platelet activation may be accompanied by formation of platelet–platelet and platelet–leukocyte aggregates, which might then lodge in and initiate harmful changes in the microcirculation. Renal replacement therapies that require prolonged exposure times to extracorporeal blood circuits, such as frequent long nocturnal hemodialysis, various forms of continuous renal replacement therapy, and wearable kidneys, may engender as yet unrecognized harmful effects related to chronic platelet activation, and such effects should be anticipated and looked for with the goal of their prevention or minimization.

NOTE REGARDING SUPPLEMENTAL MATERIALS

A detailed table reviewing the studies examined between 1980 and 2011 dealing with the effects of dialysis on platelets is presented in the Supplemental Materials online. The additional references to that table[81–103] are listed below.

DISCLOSURE

JTD declares no competing interests. AAB is an employee of Baxter Healthcare Corporation, a company that manufactures dialysis devices, solutions, and medical products.

SUPPLEMENTARY MATERIAL

Table S1. Selected studies on the effects of dialyzers on measures of platelet function.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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