

# EFFECT OF HEMODIALYSIS ON HEMOSTATIC PLATELET FUNCTION IN UREMIC PATIENTS

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## Introduction

Anemia and platelet dysfunction are major contributors to the hemorrhagic diathesis seen in patients with end-stage renal disease (ESRD). Following hemodialysis, there is frequently a clinical improvement in bleeding. However, such clinical improvement is not typically accompanied by any consistent change using standard clotting assays. Evaluation of platelet function has shown a decrease in platelet membrane glycoprotein (GP) Ib and a functional improvement in GPIIb/IIIa following hemodialysis. Currently used laboratory tests separate the elements of primary hemostasis (platelet responses) from secondary hemostasis (coagulation cascade) and therefore are not sensitive to the effect of platelet procoagulant activity and platelet microparticle formation. We evaluated the effect of hemodialysis on coagulation using a new laboratory instrument, the Clot Signature Analyzer (CSA) (Xylum Corporation, Scarsdale, NY).

## Methods

Blood was collected before and immediately following hemodialysis in ten patients with ESRD. Platelet GPIb function was analyzed by platelet aggregation in response to incremental concentrations of ristocetin. Hemostatic function was further evaluated by the CSA instrument. This instrument uses a continuous flow system to determine the time to platelet plug formation and platelet-dependent fibrin clot formation for non-anticoagulated whole blood. Whole blood flows through a tube that is punctured by a needle to cause a sudden increase in shear stress and activation of hemostatic pathways (figure 1). Because it uses non-anticoagulated whole blood, this system is sensitive to platelet procoagulant function and platelet microparticle formation. Statistical significance was assessed using a two-tailed student's t-test for paired data. Differences in pre-dialysis and post-dialysis values were considered significant at  $p < 0.05$ .

## Results

Screening tests of secondary hemostasis (prothrombin time, partial thromboplastin time, and thrombin time) were not significantly different following dialysis. Post-dialysis values of factor VIII and von Willebrand factor were significantly higher than pre-dialysis values (figure 2).

GPIb activity following hemodialysis was consistently decreased, as shown by diminished aggregation in response to incremental concentrations of ristocetin (figure 3). The EC50 for ristocetin following hemodialysis was 0.93 mg/ml, a statistically significant increase of 0.07 mg/ml over the pre-dialysis value ( $p=0.01$ ) (figure 4).

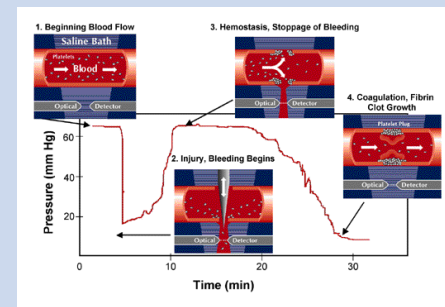


Fig. 1  
 Clot Signature Analyzer design. Blood flow through a channel (1) is disrupted by a needle puncture (2), which causes a sudden increase in shear stress and activates hemostasis. Formation of a platelet plug (3) restores flow. Finally, a platelet-dependent fibrin clot is formed, which terminates blood flow (4). The instrument measures the pressure in the channel to monitor blood flow and reports the time to platelet plug formation and platelet-dependent fibrin clot formation.

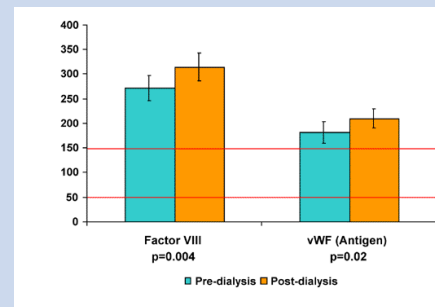


Fig. 2  
 Levels of factor VIII and von Willebrand factor are significantly elevated following hemodialysis. The red lines indicate the normal range.

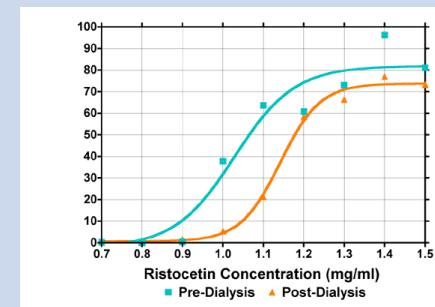


Fig. 3  
 Platelet aggregation in response to ristocetin is diminished following hemodialysis. The graph shows the rate of change of transmittance (measure of aggregation) on the y-axis against the concentration of ristocetin on the x-axis. This graph of the data from a single patient is representative of results from 8 of the 9 patients studied.

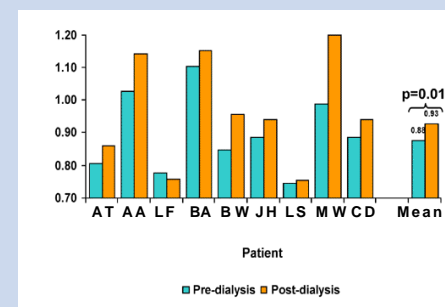


Fig. 4  
 The EC50 for ristocetin was calculated for each specimen as the concentration of ristocetin required to produce half-maximal aggregation. Platelet aggregation was measured in response to incremental concentrations of ristocetin (see figure 3). The mean EC50 is significantly increased following hemodialysis, which correlates with loss of functional GPIb.

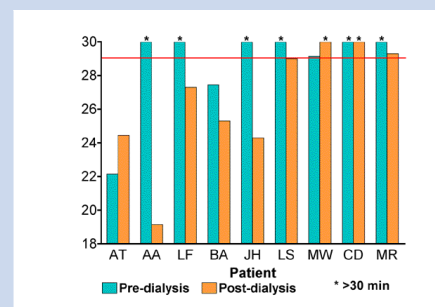


Fig. 5  
 Time to platelet-dependent fibrin clot formation measured by the CSA instrument is shortened after hemodialysis in 6 out of the 8 patients for whom data could be analyzed. The red line indicates the upper limit of normal.

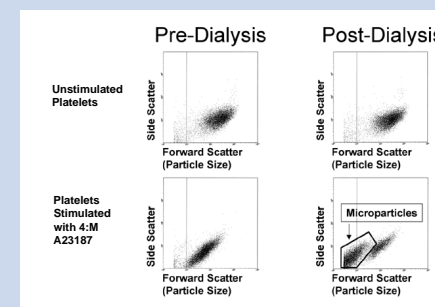


Fig. 6  
 Platelet microparticle formation was assessed before (left panel) and after (right panel) dialysis using platelet rich plasma (unstimulated and after stimulation with 4- $\mu$ M calcium ionophore A23187). A dramatic increase in microparticle formation after stimulation with A23187 is seen following hemodialysis (right panel).

Baseline CSA platelet plug formation was abnormally prolonged in 6 patients and platelet-dependent fibrin clot formation was abnormally prolonged in 7 patients. In 6 patients, a clot had still not formed after the 30 minute observation period. Following dialysis, the time to platelet-dependent clot formation was shortened for 6 of the 8 patients for whom data could be analyzed (figure 5).

Platelet microparticle formation was evaluated using flow cytometry in two patients. In response to 4  $\mu$ M calcium ionophore A23187, there was a dramatic increase in platelet microparticles following hemodialysis (figure 6).

## Discussion

The bleeding diathesis of ESRD and the effect of hemodialysis on it are incompletely understood. Traditional measures of platelet function show a decrease in platelet GPIb function following hemodialysis. Current laboratory tests are limited by the fact that they separate primary hemostasis from secondary hemostasis and therefore do not evaluate the role played by platelets in the coagulation cascade through their procoagulant activity and microparticle formation. The CSA instrument shows a strong trend toward shortening of the time to platelet-dependent fibrin clot formation for whole blood. This assay is sensitive to defects in platelet procoagulant function and platelet microparticle formation. Improvement in these parameters is, therefore, a possible mechanism by which hemodialysis produces an improvement in the bleeding diathesis of ESRD. Preliminary data on platelet microparticle formation from two patients supports this hypothesis. Studies are ongoing to assess platelet procoagulant function following hemodialysis.

## Conclusions

- Shear-dependent platelet plug formation is defective in ESRD patients.
- Hemodialysis results in decreased GPIb function manifested by decreased GPIb mediated platelet aggregation in response to ristocetin even in the presence of increased von Willebrand factor levels.
- Platelet-dependent fibrin clot formation is defective in ESRD patients despite normal screening studies of secondary hemostasis (prothrombin time, partial thromboplastin time)
- Platelet-dependent fibrin clot formation is improved by hemodialysis, which may be related to improvement in platelet procoagulant activity or platelet microparticle formation.