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 GP Ib/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, Scandia, NY).

Methods
Blood was collected before and immediately following hemodialysis in ten patients with ESRD. Platelet GP Ib 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.

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 0.03 mg/ml, a statistically significant increase of 0.07 mg/ml over the pre-dialysis value (p=0.01) (Figure 4).

Platelet microparticle formation was evaluated using flow cytometry in two patients. In response to 4 mM 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 GP Ib 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.
- Hemostasis results in decreased GPIb activity 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.