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Platelet Adhesion Testing May Predict Early Hemodialysis Arteriovenous Graft and Fistula Failure in End-Stage Renal Disease Patients

Samer E. ElChoufani, PhD, MT(ASCP), Paul Bolin, MD, Saiqua Waien, MD, Cynthia R. Christiano, MD, Donald Holbert, PhD, and Arthur P. Bode, PhD

Vascular access thrombosis (VAT) is the most morbid and costly complication in end-stage renal disease (ESRD) patients. Although hypercoagulability is a major risk factor for VAT, in most patients, the cause of hypercoagulability cannot be identified despite clinical suspicion. In this study, platelet hyperreactivity was investigated for a possible role in the hypercoagulability of ESRD and VAT in 42 patients with arteriovenous (AV) grafts or fistulas. Platelet adhesion, platelet aggregation, and the history of VAT were assessed. The statistics included a nonparametric 2-factor ANOVA, a Mann-Whitney analysis, and a Kaplan-Meier analysis of hemodialysis angioaccess survival to examine platelet hyperadhesiveness as a predictor of access survival. The study showed a significant correlation between increased platelet adhesiveness and shortened survival of the primary hemodialysis angioaccess. Collagen-induced platelet aggregation reflected a significantly higher response in those with shortened access survival. These findings may have significant clinical implications for risk assessment and prevention of VAT.

Keywords: platelet adhesion; platelet aggregation; AV fistula; AV graft; vascular access thrombosis

Introduction

According to the US Renal Data System (USRDS) 2005 annual report, in 2003, the number of end-stage renal disease (ESRD) patients who were receiving hemodialysis as the primary renal replacement therapy was 298 101 in the United States (66% of total ESRD patients). Successful hemodialysis requires access to large blood vessels capable of sustaining rapid extracorporeal blood flow. Arteriovenous (AV) Gore-Tex grafts remain the most common form of permanent hemodialysis vascular access, followed by AV fistulas. However, hemodialysis vascular access shunts (referring to both fistulas and grafts) are not free of complications, affecting the health and clinical management of ESRD patients. Vascular access failure is the most frequent cause of hospitalization of patients with ESRD. In addition, the economic impact of this problem on society is large. In the United States, the associated annual costs incurred by the Medicare system resulting from hemodialysis vascular access failure are estimated at $1 billion to $2 billion.[1]

Of all the other vascular access complications, vascular access thrombosis (VAT) remains the leading cause of fistula and graft loss.[2] The majority of cases of Gore-Tex graft thrombosis are associated with a phenomenon of stenosis of the venous outflow tract. These "anatomic" lesions are characterized by

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intimal and fibromuscular hyperplasia. In a smaller number of cases, however, graft thrombosis occurs in the absence of an identifiable anatomic lesion and is referred to as "spontaneous" thrombosis. In such cases, hypercoagulability traits have been found to be a major risk factor for hemodialysis graft thrombosis in multiple studies.[3,4] Adhesion of platelets to the thrombogenic site may be a contributor to the acute or chronic process of thrombosis.

Thrombosis in uremic patients might be considered a paradox in the face of the more common uremia-associated bleeding diathesis. Abnormal bleeding in patients with ESRD has long been recognized. However, any evidence of accelerated intravascular coagulation might indicate the presence of a prothrombotic state instead. The bleeding diathesis and prothrombotic state in uremia are both multifactorial and attributed to diverse hemostatic derangements. Uremic bleeding has been associated with a platelet function deficit, vascular abnormalities, anemia, and azotemia.[5] In contrast, extracorporeal activation of platelets in the hemodialysis circuit, despite the use of low-molecular weight heparins, appears to induce a temporary thrombophilic state, promoting acute thrombosis in some patients.[6] However, why certain patients on chronic hemodialysis appear particularly prone to recurrent thrombosis is unknown. Although the incidence of VAT may be reduced with the use of antiplatelet agents, such as low-dose aspirin, sulfinpyrazone, or ticlopidine, such agents are not routinely used in dialysis centers because of limited efficacy and concern for increasing bleeding complications in nonthrombophilic patients. Identification of individuals at high risk for thrombosis is the key to improving the efficacy of anticoagulant therapy in the ESRD patient population by solely targeting prothrombotic patients, in lieu of nonspecific, universal anticoagulation, which would be potentially harmful to the majority of ESRD patients.

Platelets play a central role in the initiation and propagation of the hemostatic response. Circulating platelets interact with proteins of the vascular subendothelial matrix that are exposed following vascular injury that disrupts the endothelial layer. Once damage is detected, platelets respond rapidly through a cascade of events or steps leading to the formation of a hemostatic plug stabilized with a fibrin meshwork, thus preventing hemorrhage. Platelet adhesion to the subendothelium represents the most crucial initial event in hemostasis, thrombus formation, and ultimately, blood vessel repair.[7] A defect in platelet adhesion to the subendothelium is the most common cause of spontaneous bleeding, as is the case in von Willebrand disease.

In some scenarios, platelet hyperreactivity is associated with a thrombophilic state that places the individual at a high risk for acute hemostatic events. Identification of platelet hyperreactivity is problematic. Platelet function analysis, such as aggregation studies, has been used as a diagnostic tool in the clinical laboratory for quite a long time but primarily as a means to assess the risk for bleeding. Although not adopted in the clinical laboratory, platelet adhesion testing has found application in the detailed analysis of platelet function and the action of various antiplatelet drugs.[8] The Baumgartner perfusion chamber has been used in some laboratories to investigate platelet hyperresponsiveness or hyperadhesiveness.[9-11] In our investigation of hemodialysis AV shunt occlusion in ESRD, we hypothesized that the platelet adhesion response might be related to accelerated loss of AV shunt patency. We used the Baumgartner perfusion test to segregate ESRD patients into high responders and low responders to investigate correlations with actual AV fistula and graft patency rates taken from a comprehensive clinical chart review.

Platelet hyperreactivity per se in uremic patients may not be obvious when compared with normal controls. However, given the fact that the hemodialysis population has a tendency toward platelet function inhibition, those patients who approach or retain near-normal platelet function, despite the presence of uremia, could be perceived as being in a thrombophilic state, especially with the impetus of abnormal vascular surfaces presented by AV grafts. This heightened platelet function might, in part, explain why some ESRD patients lose their hemodialysis access earlier or more frequently than the rest of the patient population with suppressed platelet function. Assessment of platelet adhesion capacity would seem to be an appropriate test to identify those patients at risk for vascular access occlusion.

Materials and Methods

Study Participants

A total of 42 ESRD patients with Gore-Tex AV grafts or natural AV fistulas were enrolled after giving formal consent for blood sampling at the ECU Dialysis Center between 2002 and 2005. There were 22 female and 20 male participants in the study. The

mean age was 53.5 ± 14.3 years. The youngest patient was 23 years old, whereas the oldest was 81 years old. The number of patients with a native AV fistula was the same ($n = 21$) as the number of those with a Gore-Tex AV graft at the time of enrollment.

The etiologies of ESRD were hypertension ($n = 21$), diabetes mellitus ($n = 9$), SLE ($n = 3$), focal segmental glomerulosclerosis ($n = 3$), membranous nephropathy ($n = 2$), AIDS nephropathy ($n = 2$), glomerulonephritis ($n = 1$), and pauci-immune rapidly progressive glomerulonephritis ($n = 1$). The mean length of ESRD replacement therapy by hemodialysis for the patients at the time of sampling was 34.5 ± 25.2 months, with the shortest being approximately 4 months and the longest being 10.75 years.

The Baumgartner Adhesion Assay

The Baumgartner perfusion system was originally described by Hans Baumgartner in 1973.[12,13] This system is used to assess platelet adhesion, under moderate arterial shear conditions, to a mechanically and chemically damaged (de-endothelialized) blood vessel in a quantitative fashion. Our experimental procedure is presented in detail in what follows.

Blood vessel harvest and preparation. Mongrel dogs were used as the source of blood vessel tissue. The carotid arteries were exposed by dissection, and a balloon catheter (Edward Lifesciences; Irvine, California) was inserted into the reachable superior end. The catheter was inflated and moved back and forth toward the heart in a linear motion, 5 to 6 times, at a constant speed to mechanically loosen the endothelial layer in situ. The recoverable artery portions were then excised and transported to the lab in a vessel storage buffer (VSB; 0.2 mol/L Tris, pH 7.4, 0.01% penicillin G, 0.02% streptomycin). Later, on the same day, the recovered arteries were stripped of any loose connective tissue and cut into 1-cm long segments. Each segment was inverted inside out on an acrylic rod fitted specifically for the Baumgartner annular chamber. A medium of bovine chymotrypsin (Sigma Alrich; St Louis, Missouri) at a concentration of 0.4 mg/mL in 0.1 M Tris–calcic buffer (1 mmol/L $CaCl_2$, 0.2 mol/L Tris, pH 7.4) was prepared. The rods were then incubated in the chymotrypsin medium at 37°C for 16 to 18 hours with continuous vertical agitation. This enzymatic peptide digestion provided a means of removing the amorphous basement membrane–like debris and microfibrils from the subendothelial layer, thus exposing collagen fibrils in the vessel wall matrix. The combined mechanical and chemical injury ensures a proper "denuding" of the blood vessel to provide a uniform thrombogenic surface.

Blood sample collection. From each ESRD patient, a 60-mL syringe of blood was drawn into 6 mL 3.8% trisodium citrate (1:10), generally, after 2 hours from the start of dialysis. The blood samples were transported to the laboratory as soon as they were collected. A normal donor blood sample was also collected as an experiment control for every set of Baumgartner experiments. No blood processing was required for these experiments. The whole-blood samples were used within 2 hours of collection to avoid pH changes that would alter platelet activity.

Platelet adhesion assay. The first perfusion step was performed using VSB (60 mL) into the assembled annular perfusion chamber for a duration of 2 minutes of circulation time (all perfusion steps were performed at 120 mL/min). This step was considered to be a system check to ensure the absence of leaks and the stability of the canine artery segment(s) on the rod in the face of the fast shear rate. The second perfusion step was most critical, comprising the exposure of the blood sample (60 mL) to the thrombogenic surface for 5 minutes at 120 mL/min. The third perfusion was a second recirculation of VSB for 2 minutes and served as a flushing step that removed loosely trapped platelets from the surface of the blood vessel. The fourth and final perfusion step was done with 2% paraformaldehyde (50 mL) for a duration of 2 minutes to initiate the fixation process, where platelets were permanently attached to the blood vessel via cross linkage of surface proteins. At the end of each experiment, the rod was carefully removed from the annular chamber and placed in a solution of 2% paraformaldehyde for at least 2 more hours to complete the fixation process.

Adhesion assay analysis. At the end of the fixation step (above), the rod with the platelet-exposed blood vessel segment was immersed in VSB and stored at 4°C overnight. On the next day, the artery segment was cut off the plastic rod longitudinally, and a small quarter was cut out and stained with a cocktail of fluorescent monoclonal antibodies to platelet glycoproteins. In the first staining step, the blood vessel quarter was incubated with a solution of mouse anti-human FITC-labeled anti-GPIb (CD42b) and anti-GPIIIa (CD61) antibodies (Dako; Carpinteria,

California) for at least 30 minutes and then washed. Fluorescence enhancement was accomplished using a rabbit anti-mouse RPE-labeled immunoglobulin (rabbit $F(ab')^2$; Dako) for an additional 30 minutes. After a second washing step, the stained artery quarter was examined under a Microphot FX fluorescent microscope (Nikon; Melville, New York), using a 20× water immersion objective. Digital pictures of at least 10 fields were taken randomly using a CCD100s digital camera (DAGE-MTI Inc; Michigan City, Indiana) and patched into a Scion frame grabber board (Scion Corporation; Frederick, Maryland) in a Pentium III computer. Platelet coverage of each field was estimated as a percentage of the total area of the blood vessel background and calculated using the Scion imaging software. Finally, the arithmetic mean of the platelet coverage of the 10 fields was calculated, which served as the result reported for the assay.

Patient Medical Chart Review

The medical charts of the patients participating in this research project were reviewed by a group of nephrologists who were blinded to the adhesion assay results or other in vitro data generated from the blood sample collections. All patients had an outpatient dialysis paper chart from which a preliminary review was conducted, according to local approval under HIPPA (Health Insurance Portability and Accountability Act) guidelines and regulations. The information obtained included date of initiation of dialysis, medical history, and access history. These data were complemented with a review of 2 electronic charts: WebSMS® and Logician®. The data collected from these 2 sources included medications, laboratory results, and an in-depth access history. The in-depth access history included a review of the surgical interventions and any radiological studies performed. The parameter that was derived from this chart review for each patient was the number of days of hemodialysis access patency from creation of the AV shunt until the first documented surgical intervention to restore vascular access, which represented the first access loss event in each patient. This parameter was presumptively used as the true "thrombosis index" for each patient.

Collagen-Induced Platelet Aggregation

A 20-mL syringe of blood was obtained from each ESRD patient at the end of dialysis in 3.8% trisodium citrate (1:10). In the laboratory, the blood was centrifuged at 96*g* for 15 minutes to obtain platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained by centrifuging the remainder of the blood sample at 700*g* for 10 minutes. When necessary, the platelet count in the PRP was adjusted to 200×10^3 to 300×10^3 per microliter by mixing with the native PPP. Platelet counts were measured on a Coulter counter (Beckman Coulter; Fullerton, California). The PRP was kept in lightly capped plastic tubes to avoid pH changes. In addition, aggregation studies were always completed within 4 hours of blood collection. A PAP4 platelet aggregometer (Bio/Data Corporation; Horsham, Pennsylvania) was used in these studies at 37°C. Baseline or 100% light transmittance was set using native PPP.

After aggregometer prewarming and adjustment, 450 µL of platelet count–adjusted patient PRP was added to a glass cuvette with stirring, followed by 50 µL of agonist, such as collagen (Bio/Data Corporation; Horsham, Pennsylvania) or others. The aggregation response was generally allowed to proceed for 10 minutes to reach a maximum. Collagen aggregation was repeated with 2-fold serially decreasing concentrations until no significant slope was detected. The results were recorded as a percentage of maximal light transmittance and maximum rate of change (slope).

Statistical Analyses

In this study, the clinical outcome of interest was hemodialysis access survival. Therefore a Kaplan-Meier graphical analysis was applied for patients subgrouped by in vitro platelet response. Comparison of the primary hemodialysis access survival times across subgroups was tested for significance using the Breslow's generalized Wilcoxon test. A complementary statistical analysis was performed with a nonparametric Mann-Whitney test (also called the Wilcoxon test) to compare the ranked collagen-induced aggregation data of the study participants, who were grouped according to access loss status. A secondary statistical analysis was performed using the Mantel-Cox test to compare the median hemodialysis access survival times for patients grouped according to AV fistula and AV Gore-Tex grafts.

Results

Platelet Adhesion Values of Controls

Whole blood from 29 normal donors was tested in the Baumgartner adhesion assay during the collection of

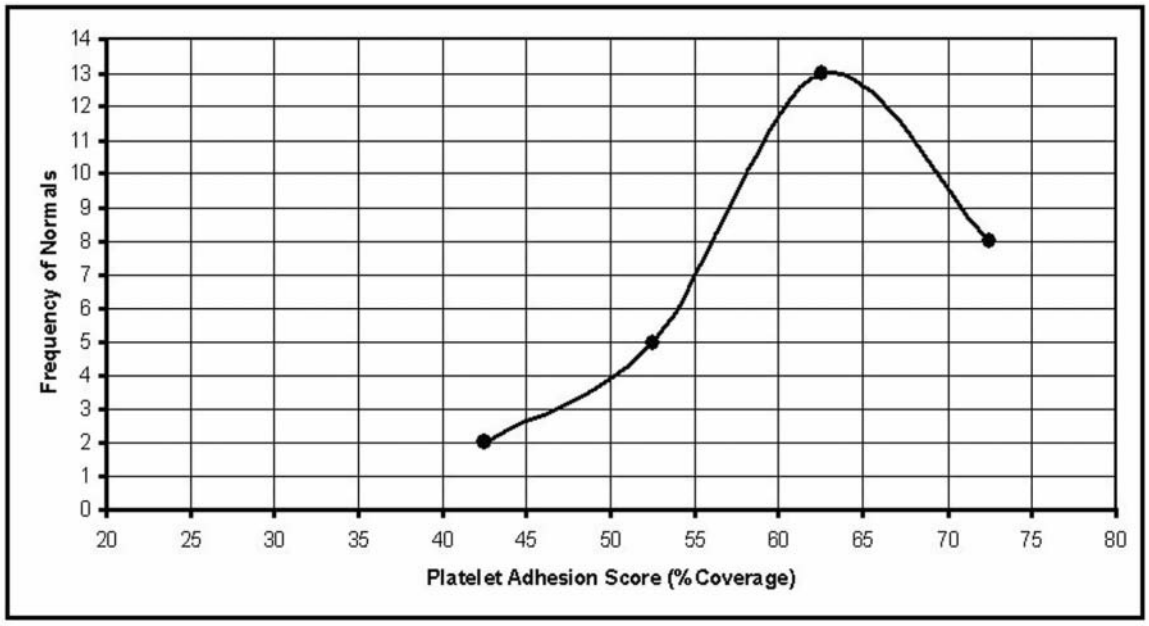

Figure 1. Frequency plot of the normal control platelet adhesion data.

data from patients. The mean value for percent platelet coverage for the normal samples was 66% ± 9% and showed a general Gaussian distribution, reflecting technical variability and the biodiversity of platelet function in the general population (Figure 1). The day-to-day results for normal whole blood in the Baumgartner adhesion assay were subject mainly to uncontrolled variability in the quality of the target blood vessel tissue obtained from canine necropsies. To provide a paired control point for marking the reactivity of ESRD patient platelets as being above expected levels, a normal blood sample was collected and run with each set of harvested blood vessel tissues.

The data obtained from the platelet adhesion assessment of the 42 ESRD patients in the study were in the range between 27% and 74% for the platelet adhesion curve, with a mean of 59% ± 10%. Likewise, the data were plotted on a frequency plot (Figure 2). The plot contains additional platelet adhesion values obtained from a few patients who were sampled multiple times.

From the frequency plot in figure 2, no obvious bimodality was apparent in the patient distribution of platelet adhesion scores. Therefore, we could not set a platelet adhesion score cutoff that would segregate the patients into 2 subpopulations of high and low responders based on their platelet adhesion raw scores. The patients were instead categorized as having "enhanced platelet adhesion" or "reduced platelet adhesion" after a comparison of raw scores with the platelet adhesion value of the normal control run on the same day. From the platelet adhesion score of the normal sample, a cutoff point for enhanced adhesion was set as within 1 SD of the normal platelet adhesion value (equivalent to 9 percentage points). Any patient platelet coverage values that fell below this cutoff were characterized as showing reduced adhesion. The data are shown in Figure 3.

Hemodialysis Access Patency

The parameter used to measure the degree of vascular access survival was the number of days of access patency until the first documented surgical intervention, which represented the first access loss event in each patient. This parameter was derived from an extensive medical chart review performed by a nephrologist blinded to the in vitro results. The review focused exclusively on VAT of grafts and fistulas only. With the use of a cutoff of 12 months for nominal life span of these 2 types of hemodialysis access (supported in the literature), the patient population was divided into 2 subpopulations: thrombosis history-positive versus thrombosis history-negative. These 2 subpopulations were compared on the basis of spontaneous or collagen-induced platelet

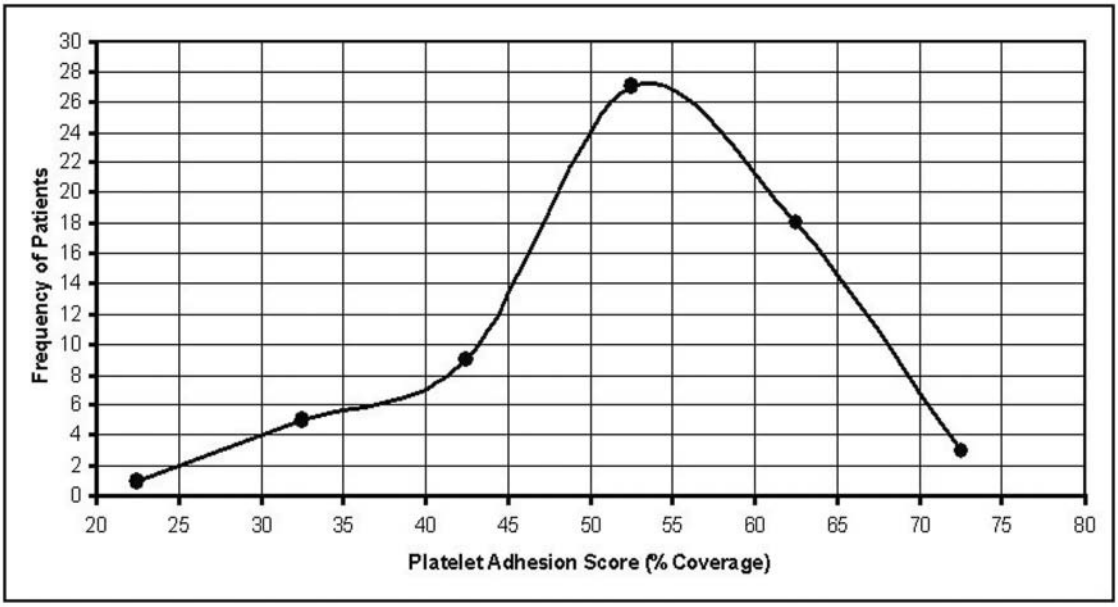

Figure 2. Frequency plot of the patients' platelet adhesion data.

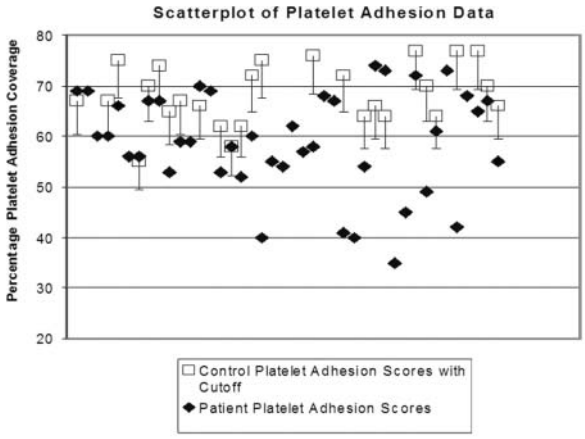

Figure 3. Scatterplot of control versus patient platelet adhesion data.

aggregation (Table 1). Eight patients had no history of vascular access loss in the chart review.

From the primary hemodialysis vascular access patency data, the overall median primary access survival for the whole population was 239 ± 35 days. To better examine the access survival data, the study participants were segregated on the basis of platelet adhesion status (enhanced vs reduced) or the type of AV hemodialysis access (fistulas vs grafts). The median access survival for the enhanced platelet adhesion subpopulation (n = 21) was 203 ± 44 days,

Table 1. Average Ranking for Each Group Based on Access Patency for Aggregation Responses

First Access Patency	Spontaneous PLT AGG	Collagen PLT AGG
<365 Days	18	13*
>365 Days	10	21

NOTE: PLT AGG = platelet aggregation.
2-tailed P; *P = .022.

compared with a median of 286 ± 55 days for the reduced platelet adhesion subpopulation (n = 21), and the difference was statistically significant using the Breslow test (P = .027; P < .05). The median access patency for patients with an AV graft as the primary access was 165 ± 62 days (n = 15). The rest of the patients were fitted with an AV fistula as the primary access (n = 27) and had a median access patency of 238 ± 37 days, in keeping with the values in the literature. The difference between these 2 groups was also statistically significant using the Mantel-Cox test (P = .032). Strangely, the median access survival time for the entire population (n = 42) and the median for the AV fistula subpopulation (n = 27) are almost the same. This finding was the result of the process of censoring the data for 8 of the 27 patients fitted with an AV fistula (explained further in the next section).

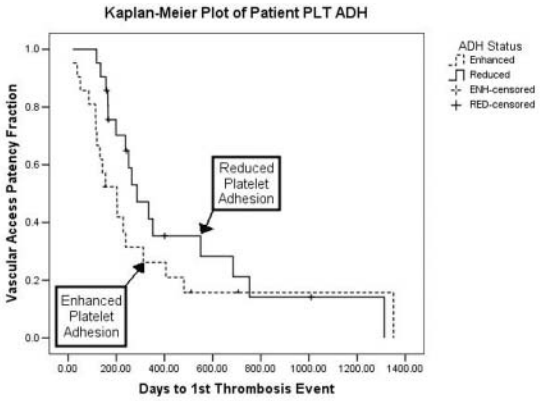

Figure 4. Kaplan-Meier plot of access survival according to platelet adhesion status. PLT, platelet; ADH, adhesion; RED, reduced; ENH, enhanced.

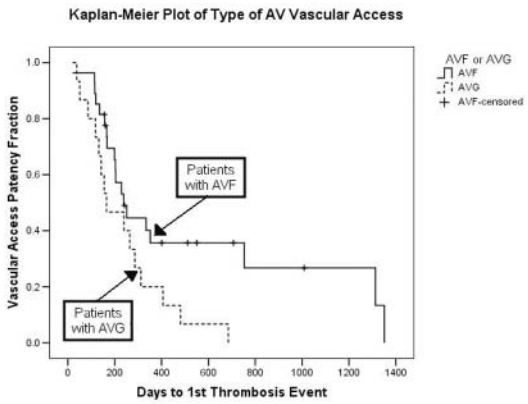

Figure 5. Kaplan-Meier plot of access survival according to type of vascular access. AVF, arteriovenous fistula; AVG, arteriovenous graft.

Censorship

For the 8 patients who had no history of VAT, the data had to be censored to be incorporated in the statistical analysis correctly because these data did not represent an "actual" terminating event. Typically, for purposes of analysis, a dichotomous (or indicator) variable is used to distinguish *true* survival times, for those patients who experienced the event of interest, from *censored* survival times, for those who did not experience the event. In general, censored observations arise whenever the dependent variable of interest represents the time to a terminal event, and the duration of the study is limited in time. In this study project, hemodialysis access failure was the dependent variable of interest. For the 8 patients whose data had to be censored, the access was still patent throughout the study, and the terminating event did not occur.

Kaplan-Meier Plots

A "time-to-event" analysis was used to examine survival rates of the hemodialysis vascular access in the patient population segregated according to enhanced platelet adhesion status and reduced platelet adhesion status. This procedure involves the successive multiplication of individual estimated survival probabilities or, in this case, hemodialysis access survival. Figure 4 shows the Kaplan-Meier plot for access survival with respect to platelet adhesion status.

As can be seen from the plot, the reduced platelet adhesion group significantly outlasted the enhanced platelet adhesion group with respect to vascular access patency duration. The statistical comparison showed clearly that the group of patients identified in the Baumgartner as having enhanced platelet adhesion had significantly reduced AV hemodialysis access patency rates as compared with those identified as having reduced platelet adhesion ($P = .027$ using the Breslow [generalized Wilcoxon] test). The 2 curves intersect around day 700, where both patient groups have only an ≈20% patency rate. Up until that point, the patient group with reduced platelet adhesion had a 1.5-to 2.0-fold better access patency rate than the patient group with enhanced platelet adhesion.

Figure 5 is a Kaplan-Meier plot showing the access survival functions of the patients with respect to the type of vascular access (AV fistula or AV graft). As can be seen in the graphical presentation, the access loss curve for the AV fistula group plateaued around day 300, whereas the access failure rate for the AV graft group apparently continued in a downward trend. In fact, by day 300, 55% of all primary fistulas (a total of 27) had failed, compared with 75% of primary Gore-Tex grafts (total of 15), which reflects the higher failure rate of synthetic AV grafts reported in the literature. As mentioned earlier, the difference in median access survival times between the AV fistula and AV graft survival curves was statistically significant ($P = .032$ using the Log Rank [Mantel-Cox] test).

Platelet Aggregation Studies

Platelet aggregation studies served to corroborate the findings from platelet adhesion testing. The

aggregation assays that were used to assess heightened platelet reactivity (collagen-induced aggregation and spontaneous platelet aggregation) were compared with the hemodialysis access patency data.

Figures 6A and 6B show the aggregation curves for 2 patients with differing thrombosis histories. The patient corresponding to Figure 6A responded well to minimal concentrations of collagen, when it was serially diluted by 50% and 25% from the initial level of 1.9 mg/mL. This patient, therefore, showed a hyperresponse that actually exceeded the responses of several normal donor controls (data not shown). The patient corresponding to Figure 6B, however, responded poorly to the 50% dilution with an exceedingly long lag phase and failed to respond at all to the 25% dilution. Therefore, this patient displayed a much lower level of response overall to collagen. Interestingly, the patient with the hyperresponse, corresponding to Figure 6A, had a very rapid primary hemodialysis access failure (38 days) compared with the patient with the minimal response (550 days) corresponding to Figure 6B. Because collagen-induced aggregation is similar in principle to platelet adhesion to denuded blood vessel strips in the Baumgartner adhesion chamber, this observation is certainly consistent with our finding of an association between enhanced platelet adhesion and rapid primary AV shunt failure, shown in the Kaplan-Meier plot in Figure 4.

The collagen aggregation response for each patient was ranked ordinally according to overall measurement of slope and lag time for the series of collagen concentrations. Table 1 shows the ranking averages (in aggregation response) for the thrombosis history-positive and history-negative patients (using the 12-month cutoff as a threshold) for response to collagen. Response in the spontaneous platelet aggregation test was similarly ranked and is also presented in Table 1.

A nonparametric Mann-Whitney statistical analysis was performed to compare the thrombosis history-positive versus history-negative groups with respect to collagen aggregation and spontaneous aggregation. The 2-tailed *P* value for the collagen aggregation parameter was .022, indicating statistical significance for the greater collagen response (lower rank) obtained in the group with lesser AV hemodialysis access patency rates. In contrast, the spontaneous platelet aggregation parameter appeared to show an opposite trend, suggesting that the results obtained with collagen response were not prompted by a spontaneous aggregation phenomenon.

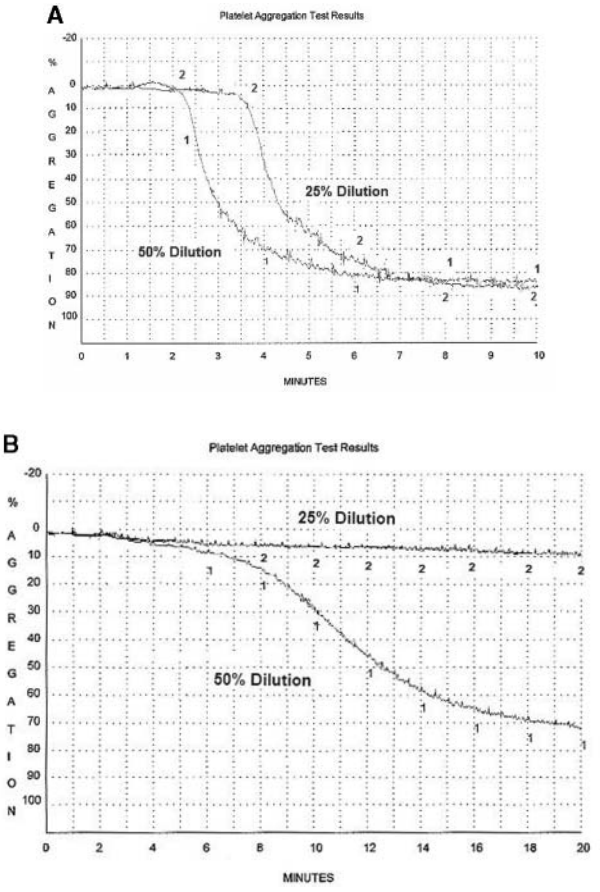

Figure 6. (A) An end-stage renal disease (ESRD) patient who is hyperresponsive to minimal collagen concentrations. (B) An ESRD patient with a minimal (1) or absent (2) response to collagen.

Discussion

This study has shown that platelet adhesion capability is an important correlate in AV graft and fistula patency in ESRD patients. Our initial conclusion was drawn by direct assaying of platelet adhesion in the Baumgartner annular perfusion chamber and was supported by measurement of platelet aggregation in response to collagen, both of which significantly correlated with early failure of the first created AV shunt in the enrolled ESRD patients. We are not sure of the physiological mechanism behind this correlation; however, our results highlight the importance of platelet function in VAT. Most likely, vascular access occlusion is an accumulative process, and in the presence of a demonstrable platelet hyperreactivity, patency declines at a much higher rate. We postulate that in other patients predisposed to losing their vascular access because of

other thrombotic tendencies, actual loss of patency is somewhat slower in those with reduced platelet adhesion.

Most VAT events have an underlying anatomical lesion characterized by neointimal fibromuscular hyperplasia, especially at the venous anastomosis site. In the presence of a concomitant enhanced platelet function despite the uremic state, overlying thrombosis might occur at a more rapid pace. In other cases of VAT where no anatomic lesion could be demonstrated by radiological studies, platelet hyperadhesiveness may play a primary role in loss of AV graft and fistula patency. Future studies are needed to elucidate the driving forces behind the platelet hyperreactivity contributing to VAT in certain ESRD patients. In addition, the association of accelerated VAT with enhanced platelet adhesion should be confirmed in a prospective study of the general ESRD population to demonstrate the potential utility of the in vitro information in determining those patients at risk of early AV access patency loss.

The Baumgartner perfusion apparatus is a system that is designed to mimic the physiology of blood circulation and the events that occur in vivo in response to vascular injury, in which the von Willebrand factor (vWF), collagen, and other adhesive matrix proteins are exposed. This system is used to assess platelet adhesion under high-shear conditions to a mechanically and chemically damaged (de-endothelialized) blood vessel in a quantitative fashion. Hence, the Baumgartner adhesion system enables the analysis of primary hemostasis (platelet–subendothelium interaction).

In a correlating study of primary hemostasis, we investigated collagen aggregation, which typically occurs in a single wave following a lag phase. During the lag phase, no aggregation is observed, but platelets start adhering to the soluble collagen. Therefore, to some extent, collagen aggregation reflects the platelet adhesion function parameter that is assessed in the Baumgartner perfusion system. In like manner to the Baumgartner adhesion data, collagen aggregation also significantly correlated with the clinical history of vascular access loss in the study population.

During the medical chart review of these study patients, we tried several approaches to the analysis of the history of AV fistula and graft failure in the study population to come up with a quantitation of actual thrombosis. The parameter that we felt best reflected the impact or the extent of the VAT process was the number of days of access patency, from first surgical shunt implantation until the first documented surgical intervention to declot and/or revise the occluded shunt, which we termed primary (first) vascular access loss. Although this clinical survey of hemodialysis vascular access loss was performed retrospectively, the significant association between these clinical events and the in vitro platelet function parameters of adhesion and collagen aggregation remain quite important. If this association is confirmed in another cohort of patients on a prospective basis, these platelet function parameters may become the focus of more attention in the dialysis center to identify particular patients at high risk for VAT. Those patients with an enhanced platelet function profile may then be therapeutically targeted with specific antiplatelet therapy. Because the majority of ESRD patients are often hemorrhagic, antiplatelet therapy poses a greater risk for excessive or spontaneous bleeding if given on a universal basis in all dialysis patients.[14-17]

Kaplan-Meier plots are used to examine studies where a primary outcome of interest is "time to event," where the event could be death, recurrence of a disease, failure of a system component, and so on.[18(pp625-640)] This procedure involves the successive multiplication of individual estimated survival probabilities or, in this case, hemodialysis access survival. As mentioned earlier, VAT could be thought of as an accumulative process in certain ESRD patients. The rate of clinical thrombosis might be based on the status of platelet function. In Figure 4, we have shown that enhanced platelet adhesion as revealed in the Baumgartner adhesion apparatus is associated with accelerated loss of patency of AV fistulas and grafts in ESRD patients. In particular, at day 100, 95% of AV shunts were still patent in the reduced platelet adhesion group, compared with 80% in the enhanced platelet adhesion group. At day 200, 70% of AV shunts were still patent in the reduced platelet adhesion group, compared with just 40% in the enhanced platelet adhesion group. At day 300, 45% of shunts were still usable in the reduced platelet adhesion group, compared with 25% in the enhanced adhesion group. At day 400, 35% of shunts were still patent in the reduced adhesion group, compared with 20% in the enhanced adhesion group. The pattern of loss for the enhanced adhesion group plateaued around day 450 at 15%, whereas in the reduced adhesion group, the patency remained above 15% until the 2 groups equalized around day 750.

In Figure 5, we showed that natural AV fistulas tend to do better than synthetic AV Gore-Tex grafts with respect to duration of access patency and usability. For example, at day 100, 85% of all primary AV fistulas were still patent, compared with 70% of all primary AV grafts. At day 200, 55% of all AV fistulas were still patent, compared with 45% of all AV grafts. As can be seen, the difference between AV fistula and graft survival fractions was relatively small up to this point. However, interestingly, the fraction of failing AV grafts increased steeply past day 225, whereas AV fistula failure contrastingly increased less remarkably. By day 700, all AV grafts had failed, whereas 35% of all AV fistulas still remained patent and usable. However, all AV fistulas had failed around day 1300 in our study, which is almost twice the life span of AV grafts. These observations are in keeping with the literature on the expected life span of natural fistulas as compared with synthetic Gore-Tex grafts.[19,20]

In conclusion, we have demonstrated a clinically important relationship between the loss of hemodialysis AV shunts and platelet adhesion function in ESRD patients. If this relationship is confirmed in further prospective studies, then platelet adhesion testing may become an important tool for the risk stratification of ESRD patients. Adoption of new therapeutic modalities that target patients with proven enhanced platelet function should be beneficial for the maintenance of AV fistula and graft patency.

In addition, this finding of a possible role of enhanced platelet adhesion in accelerating the loss of primary hemodialysis AV shunts may promote the adoption of platelet adhesion assays into clinical laboratory medicine as a measure of thrombotic risk. Indeed, with a few modifications, the Baumgartner adhesion apparatus could be integrated into clinical platelet function testing, along with the more familiar platelet aggregation, thromboelastography, flow cytometry, and so on. Ideally, a platelet adhesion profile could be established for every newly admitted ESRD patient to establish a baseline platelet adhesion status before AV shunts are placed and dialysis therapy is initiated. If baseline or later platelet adhesion testing reveals enhanced platelet adhesion status relative to an established norm, this would carry an implication of an elevated risk for VAT and trigger a more specific therapeutic intervention, perhaps retarding or circumventing a terminal occlusion event. The significant association between enhanced platelet adhesion and shortened survival of hemodialysis AV fistulas and grafts revealed in our retrospective study may provide a means of predicting VAT before the actual event and thus reduce a common morbidity in ESRD patients that requires costly surgical intervention.

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