Plasma Levels of Plasminogen Activator Inhibitor Type 1, Factor VIII, Prothrombin Activation Fragment 1+2, Anticardiolipin, and Antiprothrombin Antibodies are Risk Factors for Thrombosis in Hemodialysis Patients

Daniela Molino,*†‡ Natale G. De Santo,* Rosa Marotta,*†§ Pietro Anastasio,* Mahrokh Mosavat,† and Domenico De Lucia†

Patients with end-stage renal disease are prone to hemorrhagic complications and simultaneously are at risk for a variety of thrombotic complications such as thrombosis of dialysis blood access, the subclavian vein, coronary arteries, cerebral vessel, and retinal veins, as well as priapism. The study was devised for the following purposes: (1) to identify the markers of thrombophilia in hemodialyzed patients, (2) to establish a role for antiphospholipid antibodies in thrombosis of the vascular access, (3) to characterize phospholipid antibodies in hemodialysis patients, and (4) to study the effects of dialysis on coagulation cascade. A group of 20 hemodialysis patients with no thrombotic complications (NTC) and 20 hemodialysis patients with thrombotic complications (TC) were studied along with 400 volunteer blood donors. Patients with systemic lupus erythematosus and those with nephrotic syndrome were excluded. All patients underwent a screening prothrombin time, activated partial thromboplastin time, fibrinogen (Fg), coagulation factors of the intrinsic and extrinsic pathways, antithrombin III (AT-III), protein C (PC), protein S (PS), resistance to activated protein C, prothrombin activation fragment 1+2 (F1+2), plasminogen, tissue type plasminogen activator (t-PA), plasminogen tissue activator inhibitor type-1 (PAI-1), anticardiolipin antibodies type M and G (ACA-IgM and ACA-IgG), lupus anticoagulant antibodies, and antiprothrombin antibodies type M and G (aPT-IgM and aPT-IgG). The study showed that PAI-1, F 1+2, factor VIII, ACA-IgM, and aPT-IgM levels were increased significantly over controls both in TC and NTC, however, they could distinguish patients with thrombotic complications from those without, being increased maximally in the former group. The novelty of the study is represented by the significant aPT increase that was observed in non–systemic lupus erythematosus hemodialysis patients, and particularly in those with thrombotic events. In addition, there was a reduction of factor XII during the treatment. It is possible to assume in the TC group and, to a lesser extent, also in the NTC group that endothelial cells liberate PAI-1 in the vascular lumen, which causes hypofibrinolysis. In addition, an excess of factor VIII is activated by endothelial dysfunction with subsequent activation of the coagulation cascade as shown by increased F1+2 and fibrinogen. ACA-IgM, in turn, is capable of interfering with the system of protein C, a potent anticoagulant factor that inactivates cofactors Va and VIIIa. They also induce the expression of procoagulant factors on the surface of the endothelial cells. In conclusion, the hypercoagulable state caused by alterations of coagulation and fibrinolytic factors is a

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The hemostatic abnormalities associated with uremia are complex and can result in both hemorrhagic and thrombotic complications. Many studies explain the bleeding in renal failure, although less is known about hypercoagulability in patients receiving maintenance hemodialysis. Bleeding in uremic patients is the consequence of abnormal platelet function. Bleeding traditionally has been considered one of the clinical manifestations of uremia. The hemorrhagic events are usually of a mild degree and ecchymoses or purpura dominates the picture (Table 1). An example of bleeding is that associated with hematoma of the rectus abdominis, which occurs at the site of anastomosis of the superior and inferior epigastric arteries and is associated with severe abdominal pain of sudden or gradual onset, nausea, and vomiting. A tender, fixed, abdominal mass is felt and the abdominal wall is guarded or rigid. The skin over the mass is warm as well as discolored. Other vascular complications include subclavian vein, coronary artery, cerebral vessels, and retinal vein, as well as priapism and thrombosis of dialysis blood access.

Hypercoagulability in uremia has been associated with increased plasminogen tissue activator inhibitor type-1 (PAI-1) concentrations, especially in those with thrombosis of the vascular access. Also, an increase of factor VII has been found in thrombosis of the fistula and in uremic patients with coronary stenosis. Factor VIII, in turn, has been found to be increased or normal and in no case specific for arteriovenous fistula dysfunction.

An intriguing finding emerges from studies on anticardiolipin antibodies (ACAs), which block the protein C system and induce expression of procoagulant factors. ACA–immunoglobulin M (IgM) levels have been found to be increased in dialyzed patients, independently from thrombotic events.

Because thrombosis of vascular access is a leading cause of hemodialysis morbidity, the present study was devised to study the coagulation pathways and fibrinolysis factors potentially capable of inducing fistula dysfunction. Data are provided indicating that PAI-1, factor VIII, prothrombin activation fragments 1+2 (F1+2), fibrinogen (Fg), ACA-IgG, and antiprothrombin antibodies (aPT) specifically are increased in patients with thrombosis of vascular access. ACA-IgM, although increased, did not distinguish the patients with thrombotic complications from those without.

To ascertain the factors that distinguish patients with dysfunction/thrombosis of the arteriovenous fistula from those who do not undergo such complicating events, a study was devised specifically (1) to identify the markers of thrombophilia in hemodialyzed patients, (2) to establish a role for antiphospholipid antibodies in thrombosis of the vascular access, (3) to characterize phospholipid antibodies in hemodialysis patients, and (4) to study the effects of dialysis on coagulation cascade.

### Materials and Methods

A total of 40 uremic patients treated with hemodialysis for 24 to 36 months were enrolled in the study, along with 400 healthy age- and sex-matched blood donor volunteers (control group) recruited in the Campania Region at our University Hospital (Table 2). Twenty patients presented with thrombocytic complications (TC group) of the vascular access and in other vascular districts such as coronaries, retina, and inferior limbs. The remaining patients did not experience thrombotic complications (NTC group).

Causes of uremia in patients in the TC group were: polycystic kidney (2 patients), chronic pyelonephritis (4 patients), nephroangiosclerosis (7 patients), diabetic nephropathy (4 patients), and glomerulonephritis of unknown origin (3 patients). In the NTC group causes for uremia were nephroangiosclerosis (6 patients), diabetic nephropathy (3 patients), focal and segmental glomerulosclerosis (1 patient), polycystic kidney (1 patient), chronic pyelonephritis (5 patients), and glomerulonephritis of unknown origin (4 patients).

All patients underwent a screening prothrombin time, activated partial thromboplastin time, fibrinogen (Fg), coagu-
fuged for 20 minutes at 1,700 ratio was added as anticoagulant. Citrated blood was centri-
puncture. Trisodium citrate (0.1 mol/L) in one-tenth volume mL) was collected in silicone-treated glass tubes by veni-
dialysis session, immediately before the session. Blood (4.5 type plasminogen activator (t-PA), PAI-1, ACA-IgM and IgG,
was aliquoted and stored at

Results

Table 2 indicates that no significant difference was found
between the TC and NTC groups for Fg, thrombin, factor V, factor VII, factor X, factor XII, von Willebrand factor (vWF),
PC, PS, AT-III, plasminogen, activated protein C resistance.
The t-PA value was equal in TC and NTC patients but signif-
ically reduced in comparison with controls (P < .001).

As depicted in Figure 1, PAI-1 mean plasma levels were 48 ±
3.5 ng/mL in TC patients (P < .001 versus NTC and versus
controls, respectively). 35 ± 3.4 ng/mL in NTC patients (P <
.001 versus controls), and 22 ± 11.5 ng/mL in healthy controls.

Figure 2 reports on F1+2 plasma levels, which averaged
5.42 ± 1.25 ng/mL in TC patients, 4.58 ± .99 ng/mL in NTC
patients, and .35 ± .35 ng/mL in healthy controls. Statistical
analysis disclosed a significant difference (P < .001) for TC
versus NTC and controls as well as for NTC versus controls.

Figure 3 shows factor VIII plasma levels, which averaged
177.3% ± 26.3% in TC patients, 122.1% ± 8.17% in NTC,
and 65% ± 10% in healthy subjects for TC versus NTC, for
TC versus controls, and NTC versus controls (P < .001).

Figure 4 shows that ACA-IgM levels are significantly
higher in the TC group than in the NTC group and controls
(P < .001). A statistical difference (P < .05) also was found
for the TC group versus controls. In 2 TC patients lupus
anticoagulant antibodies were present.

Figure 5 depicts aPT-IgM levels and shows a significant
increase in the TC group (P < .001) versus the NTC group
and controls. Also, in the NTC group the level was higher
(P < .05) than in controls. The aPT-IgG levels were signifi-

Table 2 Study Population

<table>
<thead>
<tr>
<th></th>
<th>TC Patients</th>
<th>NTC Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>M/F</td>
<td>12/8</td>
<td>10/10</td>
</tr>
<tr>
<td>Age, y</td>
<td>53 ± 6</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>Dialytic age, mo (range)</td>
<td>24-36</td>
<td>24-36</td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>12.1 ± 0.6</td>
<td>12 ± 0.7</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42 ± 3.4</td>
<td>43 ± 3.1</td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Systemic lupus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amputation of a limb, n</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Infection of at least 2 arteriovenous fistulas, n</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3 Fg, Factor VII, Factor V, Factor II, vWF, PC, AT-III, t-PA, PLG, and APCr in the Various Experimental Groups

<table>
<thead>
<tr>
<th></th>
<th>TC Patients</th>
<th>NTC Patients</th>
<th>Healthy Control Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fg (mg/dL)</td>
<td>382 ± 17.7</td>
<td>400 ± 32.5</td>
<td>265 ± 40</td>
</tr>
<tr>
<td>Factor VII act (%)</td>
<td>119.2 ± 8.2</td>
<td>116.6 ± 8.1</td>
<td>100 ± 25</td>
</tr>
<tr>
<td>Factor X act (%)</td>
<td>103.5 ± 15</td>
<td>95 ± 14</td>
<td>100 ± 25</td>
</tr>
<tr>
<td>Factor V act (%)</td>
<td>76.3 ± 11</td>
<td>83 ± 7</td>
<td>80 ± 20</td>
</tr>
<tr>
<td>Factor II act (%)</td>
<td>90.2 ± 5.1</td>
<td>85.5 ± 13</td>
<td>100 ± 25</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>223 ± 14</td>
<td>225 ± 13</td>
<td>110 ± 10*</td>
</tr>
<tr>
<td>PC concentration (%)</td>
<td>87 ± 4.65</td>
<td>92 ± 3</td>
<td>98 ± 15</td>
</tr>
<tr>
<td>AT-III Act (%)</td>
<td>102 ± 4.59</td>
<td>108 ± 3.2</td>
<td>98.4 ± 15.4</td>
</tr>
<tr>
<td>t-PA (ng/mL)</td>
<td>2.6 ± 0.77</td>
<td>1.7 ± 1.3</td>
<td>7.5 ± 4.8*</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>90 ± 13</td>
<td>83 ± 4.9</td>
<td>135 ± 12</td>
</tr>
<tr>
<td>APCr (n ratio)</td>
<td>1 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>&gt;0.75</td>
</tr>
</tbody>
</table>

*P < .001 versus TC and NTC groups.
significantly lower in controls than in the TC and NTC groups (P < .05).

Dialysis had no effect on thrombin, factor V, factor VII, factor VIII, vWF, PC, PS, AT-III, plasminogen, F1+2, PAI-1, and t-PA (results not shown). At the end of the dialytic session, factor XII (data not shown) was decreased significantly versus baseline values (P < .001).

Discussion

In uremia, many abnormalities of coagulation and fibrinolysis have been reported, although PT and activated partial thromboplastin time are normal, Fg, factor VIII, PAI-1, and α2antiplasmin are increased. Factor VII and vWF are increased inconstantly. A decrease occurred for AT-III and t-PA. PC and PS are increased inconstantly.

The present study showed that PAI-1, F1+2, factor VIII, ACA-IgM, and aPT-IgM levels could distinguish patients with thrombotic complications from those without, being increased maximally in the former group. In addition, there was a decrease of factor XII during the treatment.

However, it also should be stressed that in the NTC group, PAI-1, F1+2, Fg, factor VIII, ACA-IgG, and aPT-IgG levels were increased significantly in comparison with healthy people (Table 4). The data show that a significant aPT level increase occurs in non–systemic lupus erythematosus hemodialysis patients, and particularly in those with thrombotic events. Dialysis caused a further increase of the aPT (IgG) level. These results suggest that aPT might have a role for hypercoagulability in uremic non–systemic lupus erythematosus patients, however, they do not prove a direct correlation of aPT with thrombotic events. Therefore, aPT might contribute to blood hypercoagulability by increasing endothelial damage in association with other factors yet undefined.

To understand the meaning of the differences between data in patients with and without thrombosis, it might be of help to recall the coagulation cascade (Fig. 6).

It is possible to assume in the TC group, and to a lesser extent in the NTC group, that endothelial cells liberate PAI-1

![Figure 1](image1.png)

Figure 1  Factor VIII in TC, NTC, and control groups. *P < .001 for the TC group versus the NTC and control groups. **P < .05 for the NTC versus control groups.

![Figure 2](image2.png)

Figure 2  PAI-1 in TC, NTC, and control groups. *P < .001 for the TC group versus the NTC and control groups. **P < .05 for the NTC versus control groups.

![Figure 3](image3.png)

Figure 3  F1+2 in TC, NTC, and control groups. *P < .001 pre-TC and NTC versus control groups. **P < .05 for the TC versus NTC groups.

![Figure 4](image4.png)

Figure 4  (A) ACA-IgG and (B) ACA-IgM in TC, NTC, and control groups. (A) *P < .001 per TC versus NTC and control groups. (B) *P < .001 for TC versus NTC and control groups, **P < .05 for NTC versus control groups.
in the vascular lumen, which causes hypofibrinolysis (Fig. 7). An excess of factor VIII is activated by endothelial dysfunction with subsequent activation of the coagulation cascade as shown by increased F1 + 2 and Fg levels.

ACA-IgM, in turn, is capable of interfering with the system of PC, a potent anticoagulant factor that inactivates cofactors Va and VIIIa. They also induce the expression of procoagulant factors on the surface of the endothelial cells. Thus, the hypercoagulable state caused by alterations of coagulation and fibrinolytic factors is a cause of vascular access dysfunction and thrombosis of various districts. Thrombosis of the vascular accesses is a major problem in hemodialysis patients. Thrombosis of arteriovenous fistulas leads to increased costs in the public health system and further deterioration of the patient’s quality of life. During the past years, dialysis methods have increased the survival rate of the patient on hemodialytic treatment. However, the exhaustion of vascular accesses caused by thrombotic complications limits further improvements. It is therefore mandatory to charac-

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**Table 4 Differences Between TC and NTC Groups**

<table>
<thead>
<tr>
<th>TC Patients</th>
<th>NTC Patients</th>
</tr>
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<tbody>
<tr>
<td>FII</td>
<td>=</td>
</tr>
<tr>
<td>FV</td>
<td>=</td>
</tr>
<tr>
<td>FVII</td>
<td>=</td>
</tr>
<tr>
<td>FX</td>
<td>=</td>
</tr>
<tr>
<td>FXII</td>
<td>=</td>
</tr>
<tr>
<td>AT-III</td>
<td>=</td>
</tr>
<tr>
<td>vWF</td>
<td>↑</td>
</tr>
<tr>
<td>FVIII</td>
<td>↑</td>
</tr>
<tr>
<td>Fg</td>
<td>↑</td>
</tr>
<tr>
<td>t-PA</td>
<td>↓</td>
</tr>
<tr>
<td>PAI-1</td>
<td>↑</td>
</tr>
<tr>
<td>F1 + 2</td>
<td>↑</td>
</tr>
<tr>
<td>ACA-IgG</td>
<td>↑</td>
</tr>
<tr>
<td>ACA-IgM</td>
<td>↑</td>
</tr>
<tr>
<td>aPT-IgG</td>
<td>↑</td>
</tr>
<tr>
<td>aPT-IgM</td>
<td>↑</td>
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</tbody>
</table>

Abbreviations: FII, Factor II; FV, Factor V; FVII, Factor VII; FX, Factor X; FXII, Factor XII, ↑, =, unchanged; Increased; ↓, Depressed.

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**Figure 5** (A) Antiprothrombin antibodies IgG and (B) antiprothrombin antibodies IgM. (A) *P < .05 for TC and NTC groups versus control group. (B) *P < .001 for TC versus control groups. **P < .05 for NTC versus TC and control groups.

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**Figure 6** Secondary hemostasis. □, procoagulant factor; ○, anticoagulant factor; →, stimuli; ----, inhibition.
term the risk factors for thrombotic complications in these patients to avoid thrombotic events.

There were no obese patients in the study, therefore, we cannot include in this discussion the fact that obesity is associated with disturbances in Fg, factor VIII, and vWF, whereas less consistent results have been found for factor VII. Recently, it has been shown that the adipocyte itself is able to produce PAI-1, possibly explaining the high levels found in obesity.23

Available data show that in uremic hemodialysis patients, hypercoagulability is caused by modifications in the coagulation pathway and in fibrinolysis. These modifications may result from endothelial cell activation and damage. Endothelium must be considered not only as a simple container where all the important biochemical reactions take place, but as a primer of the hemostatic balance. In uremia, oxidative stress and inflammation may damage endothelial cells,24,25 as suggested by increased plasma levels of PAI-1, which is produced for the most important part by endothelial cells, which in the average person has a total weight of 1 kg and can cover a surface equal to 6 tennis courts. The continuous endothelial damage is expressed by the increase of PAI-1 and factor VIII plasma levels. These factors should be considered as direct and indirect markers of endothelium dysfunction, similar to others such as vWF and thrombomodulin. The endothelial damage activates an excess of factor VIII. This may lead to a greater production of thrombin and fibrin deposition, which is not sufficiently removed from the vasculature because the fibrinolytic system is inhibited by increased PAI-1 levels (Fig. 5). Therefore, uremic patients treated with hemodialysis show a state of hypercoagulability when compared with healthy subjects. The data allow speculation that patients with thrombotic complications have a greater state of blood hypercoagulability and endothelium damage in comparison with patients without thrombotic complications, owing to important abnormalities of coagulation cascade, such as increased levels of PAI-1 and factor VIIIa, F1 + 2, ACA IgM, and aPT IgM.

References

Figure 7 Coagulation and fibrinolysis in uremia. □, procoagulant factor; □, increased factor; ○, anticoagulant factor; ○, reduced factor; →, stimuli; ---, inhibition.