High Rate of Acute Rejections in Renal Allograft Recipients with Thrombophilic Risk Factors

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Abstract. Inherited and acquired thrombophilic disorders predispose patients for thromboembolic and probably other occlusive vascular events that occur when additional risk factors play in concert. Because acute rejections in renal transplant recipients may reflect vascular events, and an impairment of the fibrinolytic system in immunosuppressed patients has been previously described, the implications of genetic or acquired risk factors of thrombophilia for the occurrence of early acute rejections after kidney transplantation were evaluated. The following risk factors of thrombophilia were determined in 97 patients after cadaveric kidney transplantation: factor V Leiden mutation, protein S, protein C, and antithrombin deficiency. In a retrospective analysis, the prevalence of acute rejections, the histologic classification when rejection episodes had been confirmed by biopsy, and other vascular complications were evaluated. In 21 of the 97 patients, an inherited or acquired risk factor of thrombophilia was detected. Prevalence of acute rejections was 71% in the first 6 mo after transplantation in patients with a thrombophilic disorder and significantly higher compared with patients without thrombophilia (41%; $P = 0.017$). The distribution of classic risk factors associated with acute rejections, such as number of human leukocyte antigen mismatches or percentage of panel-reactive antibodies, was similar in patients with and without thrombophilia. In the eight patients with thrombophilia and historically proven acute rejection, four patients had an acute vascular rejection, and in two patients a vascular involvement was suspected. Furthermore, prevalence of cerebral or coronary vascular disease, or venous thromboembolic complications, was significantly higher in patients with a thrombophilic clotting defect (67%) compared with patients with normal hemostasis parameters (28%; $P < 0.002$). It is concluded that renal allograft recipients with thrombophilia are at risk of developing an acute rejection or other vascular event. Although the determination of thrombotic risk factors was performed at least 3 mo after an acute rejection episode, it can be presumed that acute rejection episodes are associated with subsequent coagulatory abnormalities with further consequences for transplant survival. Thus, pretransplant evaluation of genetic and acquired risk factors of thrombophilia is recommended.

Numerous histologic studies have shown that during acute renal allograft rejection, formation of intravascular microthrombi, capillary platelet-fibrin aggregation, and arterial or venous thromboembolism occurred (1,2). These alterations have been explained mainly as the vascular manifestation of acute rejection per se, but some investigators have also related typical pathologic features to coagulatory abnormalities caused by immunosuppressive therapy, uremia, or recurrence of hemolytic-uremic syndrome as the primary kidney disease (3,4). In pediatric renal transplantation, vascular thrombosis of the allograft is a major complication, accounting for approximately 20% of failed transplants (5). Reversal by effective anticoagulatory treatment is extremely rare. Major risk factors for vascular thrombosis are repeated transplantation, young recipient and donor age, and cold ischemia time (5,6). However, also in adolescent or adult renal transplant recipients, vascular thrombosis with the possibility of recurrence has been described (7). It is not clear whether capillary microthrombus formation is an incomplete or abortive manifestation of vascular thrombosis or whether both alterations reflect distinct diseases. To elucidate a possible link between coagulation abnormalities and well known vascular complications, we studied the prevalence of major genetic and acquired risk factors of thrombophilia in renal transplant recipients, and the implications for the occurrence of an early rejection and other vascular or thromboembolic events.

Materials and Methods

Patients

Ninety-seven patients who received a cadaveric kidney graft between September 1988 and December 1996 in the Transplant Center of the University of Münster were recruited for the study by a random selection process. Recipient and donor characteristics of the studied patients with comparison of the classic risk factors associated with acute rejection in patients without and with a prothrombotic state are summarized in Table 1. A total of 58 patients had a functioning graft; 39 patients were again on regular hemodialysis when thrombotic risk factors were evaluated. Patients with functioning grafts were all on double-immunosuppressive therapy composed of cyclosporin A (plasma levels, 80 to 200 ng/ml) and low doses of prednisolone.

Primary renal diseases were glomerulonephritis in 36 patients,
Table 1. Recipient and donor characteristics of studied transplant patients without and with thrombophilic risk factors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Without Thrombophilia (n = 76)</th>
<th>With Thrombophilia (n = 21)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>49/27</td>
<td>12/9</td>
<td>NS</td>
</tr>
<tr>
<td>Age at transplant (yr)</td>
<td>41 ± 13</td>
<td>45 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>Mismatches</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-A: 0/1/2</td>
<td>30/28/18</td>
<td>9/8/4</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-B: 0/1/2</td>
<td>25/37/14</td>
<td>10/9/2</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-DR: 0/1/2</td>
<td>36/37/3</td>
<td>12/9/0</td>
<td>NS</td>
</tr>
<tr>
<td>Highest pane-reactive antibodies (%)</td>
<td>6 ± 16</td>
<td>4 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>45 ± 19</td>
<td>44 ± 18</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Means ± SD are given. CI, confidence interval.

chronic interstitial nephritis in 21 patients, polycystic renal disease in 14 patients, reflux nephropathy in nine patients, diabetic nephropathy in four patients, and unknown kidney diseases in 13 patients. Patients with hemolytic-uremic syndrome or systemic lupus erythematosus as the cause of end-stage renal disease, or with a proteinuria >3 g/d, were not included in the study. In addition, 240 healthy control subjects and 160 patients on chronic hemodialysis therapy without thrombotic complications were also studied for thrombophilic risk factors and served as control groups.

Acute graft rejection was assessed by typical clinical and laboratory findings, including duplex sonography and magnetic resonance imaging, and confirmed by biopsy in 17 subjects. All patients were initially treated with steroid pulses of 500 mg/d prednisolone for 3 d. Patients who did not respond to this initial treatment with a marked decrease of serum creatinine and an improvement of clinical parameters were biopsied and treated with antilymphocytic antibodies (OKT3 or antithymocyte globulin) when acute rejection was confirmed.

Cerebral vascular disease was diagnosed by computed tomography or magnetic resonance imaging, and coronary vascular disease was diagnosed by documented history of myocardial infarction or by coronary angiography performed because of new-onset angina. Deep-vein thrombosis was confirmed by duplex sonography or venography, and pulmonary embolism was confirmed by perfusion scintigraphy. Only vascular events after transplantation were included in the statistical evaluation.

Methods

Factor V Leiden mutations, the response to activated protein C, protein C, protein S, and antithrombin were measured as described previously (8,9). The following cutoff values were used to assume a thrombotic risk factor: protein C <60%; protein S <60%; antithrombin <70% of normal.

Statistical Analyses

The time to rejection was calculated from date of transplantation to rejection. Patients who survived more than 6 mo without rejection were censored at 6 mo. The Kaplan–Meier method was used to estimate rejection rates, with comparisons based on the two-sided log-rank test. Standard errors were calculated using Greenwoods formula. For other statistical comparisons, the χ² test was used when frequencies were sufficiently large. For small sample size, the Fisher exact test was performed.

Results

In 21 of the 97 renal transplant patients (22%) studied for risk factors of thrombophilia, one of the following major thrombophilic clotting abnormalities could be detected: heterozygous factor V Leiden mutation (n = 10), protein C deficiency (n = 4), protein S deficiency (n = 2), and antithrombin deficiency (n = 5). In healthy control subjects (n = 240) and patients on chronic hemodialysis therapy without thromboembolic complications (n = 160), the medians and ranges for protein C, protein S, and antithrombin were similar compared with transplant patients without thrombophilia (Figure 1). Figure 1 also shows that allograft recipients with thrombophilic risk had prothrombotic parameters, depicted by individual symbols, clearly below the normal range and below the above-mentioned and accepted cutoff levels (10). Prevalence of factor V Leiden mutation was 6%, both for healthy control subjects and for chronic hemodialysis patients and similar as determined for transplant recipients.

Of the 97 transplant recipients, 46 patients (47%) had an acute allograft rejection within the first 6 mo after transplantation: 15 patients with acute graft rejection had a thrombophilic risk factor (33%), and in 31 patients (67%), a clotting abnormality could not be found. In 17 cases, acute rejection was confirmed by biopsy. Patients with a risk factor of thrombophilia had a high prevalence of an early acute rejection episode: eight of 10 subjects (80%) with factor V Leiden mutation, one of four patients (25%) with protein C deficiency,
and two of two patients (100%) with protein S deficiency; four of five patients (80%) with antithrombin deficiency had an acute rejection. Figure 2 shows the Kaplan–Meier estimates of the time of acute rejection in the first 6 mo after transplantation. The prevalence of an acute rejection was significantly higher in thrombophilic patients compared with patients without a clotting abnormality (71% versus 41%, \( P = 0.017 \)). The classic risk factors for acute rejection, such as human leukocyte antigen mismatches or percentage of panel-reactive antibodies, were similarly distributed in transplant patients with and without prothrombotic states (Table 1).

Thromboembolic, cerebral, or cardiovascular events occurred in 36% of the 97 studied transplant recipients, with 13 cases of thrombosis or pulmonary embolism, 12 cases of confirmed coronary artery disease or myocardial infarction, and 10 cases of ischemic stroke. Figure 3 shows that in 14 of 21 patients (67%) with genetic or acquired risk factors of thrombophilia, vascular events other than acute rejections occurred, with five cases of deep-vein thrombosis or pulmonary embolism, four cases of stroke, and five cases of coronary artery disease or myocardial infarction. Occlusive vascular disease was significantly more frequent in this patient group compared with allograft recipients without a thrombophilic risk factor (28% vascular diseases, \( P < 0.002 \)). Only one of 21 patients (5%) with thrombophilia was free of acute rejection and other vascular complications compared with 30 of 76 patients (39%) without thrombophilia (\( P < 0.003 \)).

In eight of the 15 patients with an acute rejection and a genetic or acquired risk factor of thrombophilia, rejection was confirmed by biopsy. The main histologic alterations are summarized in Table 2, together with histologic grading according to the Banff working classification (2). In four of the six patients with factor V Leiden mutation and histologically proven acute rejection, transplant biopsies showed an acute vascular rejection. Renal allograft survival ranged between 1 and 119 mo. The biopsy specimen of patient 1 is depicted in Figure 4A, which shows transmural arteritis with lymphocytic penetration of the vessel wall and fibrinoid thrombus formation indicative of acute vascular rejection. Figure 4, B and C, shows the light microscopy of specimens from the explanted allograft of the same patient presenting severe vasculopathy with arterial obliteration and ischemic glomerulopathy with chronic alterations (Figure 4B), as well as an organized thrombus of an artery (Figure 4C). In two other patients (patients 2 and 4, Table 2), histologic evaluation of removed renal transplants showed marked arterial occlusion as well as venous thrombosis.

Two of the six patients with factor V Leiden mutation and the two patients with protein S and antithrombin deficiency, respectively (Table 2), had signs of acute tubulointerstitial or interstitial rejection in transplant biopsies. All of the patients summarized in Table 2 were treated with antilymphocyte antibodies for 6 to 10 d after histologic evaluation. In patients 1 and 6, effective anticoagulation with warfarin was started after transplant biopsy.

**Discussion**

The present study focuses on the prevalence of some important genetic and acquired risk factors of thrombophilia in renal allograft recipients and the implications for the occurrence of acute rejection episodes. Furthermore, the principal lesions of acute rejection in thrombophilic transplant recipients, as well as other vascular complications, were determined in a retrospective analysis. As the key finding, we demonstrated a significantly increased occurrence of early acute rejection episodes in patients with coagulatory abnormalities. Adhesion and chemotaxis of lymphocytes in the vascular bed of the allograft in response to vascular clotting as a trigger for acute rejection or aggravation of an incipient rejection by a primary hemosta-
sis defect are hypotheses to explain the high prevalence of acute rejections.

The prevalence of inherited defects of the protein C pathway in transplant patients was similar compared with healthy control subjects or chronic hemodialysis patients without thromboembolic complications. Prothrombotic defects of the protein C pathway are caused mainly by the Arg506 to Gln point mutation on the factor V gene, leading to resistance to activated protein C, or by protein C and protein S deficiencies. To rule out that acute phase reactions related to rejections or antirejection therapy caused the prothrombotic alterations, coagulatory evaluation was performed at least 3 mo after a rejection episode or a vascular event. However, due to the retrospective design of our study, we cannot exclude that previous episodes of acute rejection had some effects on the measured coagulatory parameters. Patients with antithrombin deficiency, which has been shown to be a heterogenous disorder (11), were found more frequently in transplant and hemodialysis patients compared with healthy control subjects, which might be explained with a hypoproteinemic state of renal patients due to malnutrition or nephrotic syndrome. For that reason, transplant patients with a proteinuria >3 g/d were not included in the study, as stated under the exclusion criteria. The five transplant recipients with antithrombin deficiency analyzed for rejection occurrence had markedly reduced antithrombin levels in association with a moderate proteinuria, so that an inherited or an acquired antithrombin deficiency might be discussed.

Previously, the high frequency of thromboembolic complications in renal transplant recipients was attributed to immunosuppressive therapy, such as cyclosporin A (3,12) or OKT3 treatment (13). The major coagulatory alterations of allograft recipients treated with cyclosporin A were attributed to an impairment of fibrinolysis and endothelial damage. Steroid therapy has not been attributed to an aggravation of thrombophilic alterations. On the contrary, in pediatric patients with leukemia, high-dose treatment with prednisolone led to an increase but not a decline of antithrombin and protein C levels (14). The combination of inherited thrombophilia and secondary alterations of the coagulatory system may be the rationale for the described high prevalence of rejections and other vascular events. It is important to stress that other acquired or genetic cardiovascular risk factors frequently seen in patients with renal diseases, i.e., hyperlipidemia, hyperhomocystinemia, or hypertension, may further evoke vascular events (15-17). Our statistical analyses, however, excluded a direct correlation between the occurrence of acute rejections or other occlusive vascular events and atherogenic risk factors such as hypertension or cigarette smoking (data not shown).

An association between thrombophilic risk factors and acute rejection in transplanted patients is suggested in the four patients with histologically proven acute vascular rejection. Of the four remaining patients with predominant tubulointerstitial rejection, in only two cases (occlusive vasculopathy, occlusive glomerulitis) was a vascular involvement suspected. Thus, a prothrombotic abnormality cannot be anticipated by the histologic diagnosis, but should be determined before transplantation by laboratory tests in all patients awaiting organ transplantation.

In conclusion, the present retrospective evaluation shows a high prevalence of acute rejection episodes in renal transplant recipients with hereditary or acquired prothrombotic states. From this study one may also suppose that acute rejection episodes are associated with subsequent coagulation abnormalities, which might have further implications on transplant survival. These preliminary results need to be confirmed in a prospective multicenter survey. Whether prophylactic oral an-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Gender</th>
<th>Thrombophilic Clotting Abnormality</th>
<th>Major Histologic Finding</th>
<th>Banff Category</th>
<th>Transplant Survival</th>
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<tbody>
<tr>
<td>1</td>
<td>52/M</td>
<td>APC-R</td>
<td>Acute vascular rej.; arterial and venous thrombosis</td>
<td>Grade II</td>
<td>1 mo</td>
</tr>
<tr>
<td>2</td>
<td>66/M</td>
<td>APC-R</td>
<td>Acute vascular rej.; venous thrombosis of removed graft</td>
<td>Grade II</td>
<td>12 mo</td>
</tr>
<tr>
<td>3</td>
<td>13/M</td>
<td>APC-R</td>
<td>Acute vascular rej.; occlusive vasculopathy</td>
<td>Grade I</td>
<td>119 mo</td>
</tr>
<tr>
<td>4</td>
<td>20/F</td>
<td>APC-R</td>
<td>Acute vascular rej.; thrombotic microangiopathy; venous thrombosis of removed graft</td>
<td>Grade II</td>
<td>33 mo</td>
</tr>
<tr>
<td>5</td>
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<td>APC-R</td>
<td>Acute tubular and interstitial rej.; interstitial hemorrhage</td>
<td>Grade III</td>
<td>Funct. graft</td>
</tr>
<tr>
<td>6</td>
<td>54/F</td>
<td>APC-R</td>
<td>Acute tubular and interstitial rej.; occlusive vasculopathy</td>
<td>Grade I</td>
<td>Funct. graft</td>
</tr>
<tr>
<td>7</td>
<td>32/F</td>
<td>AT deficiency</td>
<td>Acute interstitial rej.; severe occlusive glomerulitis</td>
<td>Grade III</td>
<td>3 mo</td>
</tr>
<tr>
<td>8</td>
<td>23/F</td>
<td>PS deficiency</td>
<td>Acute interstitial rej.</td>
<td>Grade I</td>
<td>43 mo</td>
</tr>
</tbody>
</table>

* APC-R, activated protein C resistance determined as factor V Leiden mutation; rej., rejection; Funct., functioning; AT, antithrombin; PS, protein S.
Figure 4. Allograft biopsy sample (A) and specimens of the removed graft (B and C) of a patient with heterozygous factor V Leiden mutation (patient 1, Table 2). Acute vascular rejection is indicated by transmural arteritis with lymphocytic infiltration of the intima and media. A fibrinoid thrombus obliterates the lumen. The removed allograft shows obliterative vasculopathy and severe chronic glomerulopathy and arterial thrombosis in organization. A through C, periodic acid-Schiff stain. Magnification: ×180 in A and C; ×90 in B.

ticoagulation or administration of low molecular weight heparin in thrombophilic allograft recipients can increase the transplant survival rate is the subject of an ongoing clinical trial.

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References