Altered flow properties of blood and increased plasma fibrinogen in cyclosporin-treated renal allograft recipients

Torbjörn Linde, Bo Sandhagen, Ulla Backman and Bengt Fellström

Department of Medical Sciences, University Hospital, Uppsala, Sweden

Abstract

Background. Abnormalities in blood rheology may be factors contributing to cardiovascular complications and the progression of renal failure in kidney allograft recipients. The haemorheological variables haematocrit, fibrinogen, whole blood viscosity, plasma viscosity, erythrocyte aggregation tendency and fluidity were measured in 27 cyclosporin A (CyA)-treated patients who had received a renal graft at least 6 months previously. Their creatinine clearance was in the range of 12–92 ml/min/1.73 m² (mean 55 ± 19). The values were compared with those obtained from a control group comprising 20 healthy subjects matched according to age, sex and smoking habits.

Results. The haematocrit, plasma fibrinogen, whole blood viscosity, plasma viscosity, erythrocyte aggregation tendency, body mass index (BMI), mean arterial pressure (MAP) and serum triglycerides were increased in the transplanted patients, and the serum high density lipoprotein (HDL)-cholesterol and erythrocyte fluidity decreased. The haemorheological variables were used as dependent variables in a stepwise regression analysis with age, MAP, BMI, urinary albumin excretion rate, blood CyA concentration, creatinine clearance, and serum triglycerides, cholesterol and HDL-cholesterol as independent variables. Plasma fibrinogen was positively correlated with BMI and blood CyA. The whole blood viscosity was positively correlated with blood CyA and negatively with serum HDL-cholesterol. Only serum triglycerides remained correlated with erythrocyte aggregation tendency.

Conclusions. All variables with a known impact on blood viscosity were altered in the present group of renal transplant recipients. Inappropriate regulation of erythrocyte formation, overweight, the use of CyA, high triglycerides and low HDL-cholesterol levels may be factors contributing to this. The importance of impaired flow properties of blood for the development of cardiovascular diseases and transplant glomerulosclerosis needs to be examined.

Key words: cyclosporin; erythrocyte deformability; fibrinogen; haemorheology; plasma viscosity; renal transplantation

Introduction

Cardiovascular complications are very common in renal transplant patients. Several cardiovascular risk factors, such as hyperlipidaemia, hypertension and insulin resistance, have also been identified in these patients. There are some reports indicating that certain changes in the flow properties of blood are associated with an increased risk for the development of cardiovascular diseases. Blood viscosity is more strongly related to left ventricular hypertrophy than is any other haemodynamic factor [1], and is associated both with the degree of peripheral atherosclerosis [2] and coronary artery disease [3]. In addition, both patients with acute brain infarction and subjects with risk factors for stroke show an increased blood viscosity [4], indicating its role as an important cardiovascular risk factor.

The viscosity of blood is determined by the haematocrit, plasma viscosity, erythrocyte aggregation tendency and deformability [5]. Mainly as a result of increased fibrinogen levels, the plasma viscosity [6] and erythrocyte aggregation tendency [7] are increased in patients with end-stage renal disease. Erythrocyte deformability, a variable reflecting the ability of the erythrocytes to change shape in response to a deforming force, has been found to decrease with renal function and to be low in haemodialysis patients [8]. Theoretically, the impaired flow properties of blood may have an impact on the progression of chronic renal failure by increasing the glomerular blood flow resistance. Beneficial effects of pentoxifylline, a drug known to improve haemorheology, in patients with diabetic nephropathy [9] and other renal diseases [10], may support this hypothesis.

Thus, in patients with decreased renal function, the flow properties of blood are impaired, which may be a cardiovascular risk factor and contribute to the
progression of renal failure. The aim of the present study was to elucidate whether haemorheological abnormalities also exist in patients with a functioning renal graft.

Patients and methods

Study population

A total of 27 renal transplant recipients were investigated at the time-point for the inclusion in a trial aimed to examine the effects of a lipid-lowering drug (ALERT Study). The patients were renal or combined renal and pancreas transplant subjects >6 months previously and at an age of between 30 and 75 years. The total cholesterol level had to be in the range 4.0–9.0 mmol/l and, in the case of a former myocardial infarction, in the range 4.0–7.0 mmol/l. All patients were maintained with cyclosporin A (CyA) (Sandimmun Neoral®, Novartis) combined with steroids in all but one case. No patient had been treated due to an acute rejection during the last 3 months. Liver enzymes and total bilirubin had to be less than twice the normal upper limit. No patient was on erythropoietin therapy.

Patients included had been on renal replacement therapy for 95 ± 43 months. The primary renal diseases were chronic glomerulonephritis in 16 patients, polycystic kidney disease for 95 ± 43 months. The primary renal diseases were chronic glomerulonephritis in 16 patients, polycystic kidney disease in four, diabetic nephropathy in four, other in two cases and unknown in one. Twenty of the patients were recipients of cadaveric transplants, and the remaining seven had received transplants from living donors. Three of the patients with diabetes also had a pancreas graft.

Physical and laboratory characteristics of the patients were compared with data obtained from a group of 20 healthy volunteers matched according to age, sex and smoking habits. All these subjects were employees at the medical centre or their relatives. Baseline data of transplant recipients and the normal subjects are given in Table 1.

Laboratory methods

Body mass index (BMI), as a measurement of obesity, was defined as body weight in kilograms divided by squared length in metres. Blood pressure was measured after the patient had been in a supine position for 10 min. Mean arterial pressure (MAP) was calculated from diastolic pressure plus one-third of the pulse pressure.

Blood samples were obtained from an antecubital vein. The haematocrit was analysed by micro-haematocrit centrifugation at 11 000 g for 5 min without correction for trapped plasma.

Within 20 min after blood collection, the haemorheological variables were assessed at 37 °C in a Low Shear 30 rotational viscometer (Contraves AG, Zürich, Switzerland). Plasma viscosity was assessed at a shear rate of 38 s⁻¹ and apparent whole blood viscosity at 100 s⁻¹ at native haematocrit.

As proposed by the International Committee for Standardization in Haematology [11], erythrocyte aggregation tendency was assessed as whole blood viscosity at native haematocrit and plasma viscosity. The unit for erythrocyte aggregation is dimensionless.

Erythrocyte deformability was assessed as the fluidity (inverse viscosity) of red blood cells separated from plasma and buffy coat and resuspended in isotonic phosphate-buffered saline (pH 7.4) to a haematocrit of 55%. A shear rate of 1 s⁻¹ was used.

Plasma fibrinogen was determined by rate nephelometry. Other biochemical variables were analysed using standard laboratory techniques for hospital use.

Statistical evaluation

Values are presented as means ± SD. The statistical package StatView®SE + Graphics (Abacus Concepts, Inc, USA) was used for all statistical calculations. Unpaired two-tailed t-test, simple, stepwise multiple and multiple regression analyses were used. The level of statistical significance was set at P < 0.05.

Results

Compared with the control group, the kidney transplant recipients had an increased BMI and MAP. Serum triglycerides were increased and high density lipoprotein (HDL)-cholesterol decreased, whereas no statistically significant difference was detected in total

<p>| Table 1. Characteristics of the transplanted patients and healthy subjects |
|--------------------------|--------------------------|--------------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th>Transplanted patients</th>
<th>Normal subjects</th>
<th>P (unpaired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 12</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>Females/males</td>
<td>9/18</td>
<td>7/13</td>
</tr>
<tr>
<td>Smokers</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 5</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>105 ± 10</td>
<td>91 ± 7</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>142 ± 44</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>2.0 ± 0.9</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>6.3 ± 1.1</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.3 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²)</td>
<td>55 ± 19</td>
<td></td>
</tr>
<tr>
<td>Urinary albumin excretion (g/24h)</td>
<td>0.15 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>Blood cyclosporine (ng/ml)</td>
<td>117 ± 29</td>
<td></td>
</tr>
</tbody>
</table>
serum cholesterol. The creatinine clearance varied between 12 and 92 ml/min/1.73 m² and serum creatinine was increased in the transplant recipients. The mean CyA concentration in blood, 12 h after intake, was within the therapeutic range chosen in our unit. Albuminuria in the range 0–30 mg/day was found in 14 patients and in the range 31–300 mg/day in 12 patients. One patient had a marked albuminuria of 2 g/day (Table 1).

Apart from a slight increase in white blood cell count, no significant differences in the haematological variables measured were detected (Table 2).

All haemorheological variables differed between the two groups. The haematocrit, plasma fibrinogen, whole blood viscosity, plasma viscosity and erythrocyte aggregation tendency were increased and erythrocyte fluidity decreased in renal transplant recipients compared with healthy subjects (Table 3).

In the group of kidney recipients, the haemorheological variables (haematocrit, fibrinogen, whole blood viscosity, plasma viscosity, aggregation tendency and fluidity) were used as dependent variables in a stepwise regression analysis with age, MAP, BMI, urinary albumin excretion rate, blood CyA, creatinine clearance, serum triglycerides, serum cholesterol and serum HDL-cholesterol as independent variables. BMI (Figure 1) and blood CyA remained positively correlated with plasma fibrinogen. Blood CyA remained positively and serum HDL-cholesterol negatively correlated with the whole blood viscosity. Erythrocyte aggregation tendency was positively correlated with serum triglycerides (Table 4). None of the independent variables were significantly correlated with the haematocrit, plasma viscosity or erythrocyte fluidity.

In simple linear regression analysis, plasma fibrinogen was positively correlated with BMI, erythrocyte aggregation tendency and the plasma viscosity (Figure 1).

### Discussion

In the present investigation, all variables measured with a known impact on blood viscosity were altered. The whole blood viscosity in vitro is determined mainly by the haematocrit [5]. In our patients, the mean haematocrit exceeded that in normal subjects matched according to age, sex and smoking habits, factors with an impact on the haematocrit. Post-transplant erythrocytosis is relatively common in patients with a renal graft, and a prevalence of 6.5–35.8% has been found [12]. If a haematocrit exceeding 51% in males and 47% in females was taken as definition of post-transplant erythrocytosis [13], the condition was found in four (15%) of our patients. The mechanisms underlying the condition are poorly understood. In patients with chronic renal failure, the haematocrit generally decreases with decreasing renal function [14]. In the present group of renal transplant recipients, no correlation between graft function and haematocrit was found, however. This finding may support propositions that inappropriate erythropoietin production by the native kidneys or increased sensitivity of erythroid precursors explain an increased haematocrit in renal transplant recipients [13].

In contrast to the findings of Koppensteiner and collaborators [15], who recently reported haemorheological findings after renal transplantation, the plasma viscosity was increased in our patients compared with the healthy subjects. The plasma concentration of

---

**Table 2. Haematological variables**

<table>
<thead>
<tr>
<th></th>
<th>Transplanted patients</th>
<th>Normal subjects</th>
<th>P (unpaired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/l)</td>
<td>140 ± 16</td>
<td>134 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>91 ± 5</td>
<td>90 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>MCHC (g/l)</td>
<td>343 ± 20</td>
<td>341 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cells (10⁹/l)</td>
<td>6.8 ± 1.2</td>
<td>5.2 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets (10⁹/l)</td>
<td>247 ± 55</td>
<td>232 ± 52</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are given as means ± SD.

**Table 3. Haemorheological variables**

<table>
<thead>
<tr>
<th></th>
<th>Transplanted patients</th>
<th>Normal subjects</th>
<th>P (unpaired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td>44 ± 4</td>
<td>42 ± 3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td>4.2 ± 0.8</td>
<td>3.2 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apparent whole blood viscosity (mPa·s)</td>
<td>4.5 ± 0.6</td>
<td>4.0 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma viscosity (mPa·s)</td>
<td>1.36 ± 0.10</td>
<td>1.25 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erythrocyte aggregation tendency</td>
<td>1.13 ± 0.09</td>
<td>1.01 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erythrocyte fluidity (l/Pa·s)</td>
<td>92 ± 10</td>
<td>101 ± 6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are given as means ± SD.
Fibrinogen is an important determinant of plasma viscosity [5]. In the present study, plasma fibrinogen was increased and correlated with the plasma viscosity, indicating that increased fibrinogen levels could be one factor explaining the increased plasma viscosity in our patients.

Increased plasma levels of fibrinogen are common in patients on regular dialysis [15, 16]. After renal transplantation, plasma fibrinogen remains elevated [15], or increases further [16]. Plasma fibrinogen is also known to be increased in subjects with obesity [17]. In the present investigation, the patient BMI exceeded that of healthy subjects and was correlated with plasma fibrinogen. Obesity may, consequently, be a factor contributing to the increased plasma fibrinogen in renal transplant recipients.

Patients on steroids and azathioprine alone [16] or FK 506 [18] have been shown to have significantly lower fibrinogen levels than those receiving cyclosporin. Moreover, CyA-treated patients with aplastic anaemia experience higher fibrinogen levels than control subjects or patients with anaemia not treated with CyA [19]. In the present study, all patients were maintained on CyA, and the CyA concentration in blood remained significantly correlated with plasma fibrinogen in the stepwise multiple analysis. The $R^2$ value obtained when blood CyA and BMI were used as independent variables indicates that 40% of the variation in plasma fibrinogen is explained by variations in these two variables.

Fibrinogen acts as a bridging molecule between red blood cells [5], and high fibrinogen levels may, therefore, contribute to the increased erythrocyte aggregation tendency found in the present and previous studies. Such a proposition is supported by the finding of a correlation between the two variables. As in patients with type I diabetes [20], our regression analysis indicates that elevated concentrations of triglycerides may be another factor of importance to erythrocyte aggregation tendency in kidney transplant recipients.

The diameter of an erythrocyte at rest is $\sim 8 \mu m$ while the smallest nutritive capillaries have a diameter of 2–3 $\mu m$ [21]. This implies that the ability of the erythrocyte to undergo deformation is of great significance for the microcirculation. Erythrocyte deformability deteriorates with decreasing renal function, and is decreased in dialysis patients [8]. In the present study, erythrocyte deformability was measured as erythrocyte fluidity. This variable was decreased in the patients compared with the healthy subjects and was at approximately the same level as was demonstrated in a previously examined group comprising pre-dialysis and haemodialysis patients [22]. Erythrocyte deformability was not correlated with graft function, and factors other than the decreased renal function consequently seem to be the cause of the reduced deformability in the present study. The use of CyA may be one such factor, since erythrocyte deformability has been shown to be lower in renal transplant recipients using CyA compared with those using azathioprine and prednisolone [23]. Changes in the cell membrane lipid composition [23] and calcium homeostasis [24] may
be mechanisms by which CyA could cause a decreased erythrocyte deformability.

As in healthy subjects [25], low levels of HDL-cholesterol were associated with an increased whole blood viscosity in the present group of kidney transplant recipients. HDL has been proposed to compete with low-density lipoprotein (LDL) for erythrocyte binding and thereby antagonizes LDL-induced erythrocyte aggregation [25]. However, in the present investigation, no correlation between erythrocyte aggregation tendency and HDL-cholesterol was found. Neither was any correlation between HDL-cholesterol or any other components of blood viscosity found, and the mechanisms explaining the interrelationship between the whole blood viscosity and HDL-cholesterol therefore cannot be explained by data available from the present investigation.

In summary, all variables measured with a known impact on blood rheology were altered and contributed to an increased whole blood viscosity in the present group of renal transplant recipients. We propose that an inappropriate regulation of erythrocyte formation, obesity, the use of CyA, high levels of triglycerides and immunosuppressive drug regime on cardiovascular risk profile or any other components of blood viscosity found, deformability.

Investigation, no correlation between erythrocyte aggregation [25]. However, in the present 10. Sharma AK, Gupta R, Chablani P, Sharma P. Progression of erythropoietin. Blood Coag Fibrinolysis 1997; 8: 449–453


Sloop GD, Garber DW. The effects of low-density lipoprotein and high-density lipoprotein on blood viscosity correlate with their association with risk of atherosclerosis in humans. Clin Sci (Colch) 1997; 92: 473–479

Received for publication: 14.10.98
Accepted: 30.1.99

Acknowledgements. The skilful technical assistance of R. N. Gitte Johansson is gratefully acknowledged. Dr Ulla Backman died on August 17, 1998.

References

1. Devereux RB, Drayer JIM, Chien S et al. Whole blood viscosity as a determinant of cardiac hypertrophy in systemic hyperten- sion. Am J Cardiol 1984; 54: 592–595

2. Lowe GD, Fowkes FG, Dawes J, Donnan PT, Lennie SE, Housley E. Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. Circulation 1993; 87: 1915–1920


