Assessment of plasminogen activator inhibitor-1 and von Willebrand factor as a marers of endothelial function in patients with end-stage kidney disease after allotransplantation during a one-year follow-up

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Abstract: Objectives. The aim of our study was to determine the endothelial function in patients with chronic kidney disease (CKD), stage V (end-stage renal disease – ESRD), before and during a one-year observation after kidney allotransplantation. **Patients and methods.** We studied 40 patients with stabile graft function after their first kidney transplantation, including 21 females (mean age 41 ±12.5 yrs) and 19 males (mean age 43.6 ±13.3 yrs), treated already with hemodialysis because of ESRD. **Results.** After transplantation we observed significant decrease in serum creatinine concentrations (Cr) (7.32 ±2.07 mg/dl to 1.50 ±0.45 mg/dl at 6 months and 1.61 ±0.58 mg/dl after 12 months after transplantation). During 12 months we found changes only in plasminogen activator inhibitor-1 (PAI-1) levels. Von Willebrandt factor (vWF) levels remained unaltered during follow-up and total homocysteine (tHcy) concentration decreased, but this change was not statistically significant. There was no correlation between vWF and PAI-1 concentrations and other clinical and laboratory findings. **Conclusions.** In our study we did not find any improvement of endothelial function during the first year after kidney allotransplantation. The mean Cr concentration at the end of study was 1.6 mg/dl, which indicates the chronic kidney graft insufficiency. We conclude that despite kidney allotransplantation endothelial function is impaired like in CKD, but with more advanced abnormalities in the cardiovascular system.

Key words: chronic kidney disease, endothelium, kidney allotransplantation

INTRODUCTION

Endothelial dysfunction occurs in numerous morbidities increasing the risk of development of cardiovascular system diseases, e.g. in hypertension or dislypidemia, and also in smokers or persons under stress [1-3]. It is emphasized that endothelial dysfunction is one of the first potentially reversible manifestations of cardiovascular system pathologies [4,5]. Atherosclerosis and its thrombotic complications are a quite serious problem in chronic kidney disease (CKD) patients and in persons with renal transplant. According to some authors, death risk in CKD patients during renal replacement therapy is increased 10 to 30-times in comparison with general population. It is, however, lower in patients with renal transplant than in dialyzed patients [6]. Therefore, currently the researchers' attention focuses on assessment of endothelium and its involvement in pathogenesis of numerous diseases, including chronic kidney disease. Endothelial dysfunction leads to disruption of intercellular communication; it results in increased expression of adhesion molecules, increased chemokine secretion and adhesion and activation of granulocytes and thrombocytes. Therefore, in diagnostic procedure behavior of individual markers of endothelial function, e.g. plasminogen activator inhibitor type 1 (PAI-1), von Willebrand factor (vWF), tissue plasminogen activator (t-PA) [7], endothelin 1, asymmetric dimethylarginine, and also total homocysteine (tHcy) [8] is analyzed.

The aim of this study was assessment of changes in PAI-1 and vWF levels as markers of endothelial function within the first 12 months after kidney allotransplantation and determination of relation between the indices of endothelial function and the assessed clinical parameters and additional tests within a six-month or one-year period after renal transplantation.

PATIENTS AND METHODS

Assent to carry out the study has been obtained from Local Committee of Ethics of Scientific Research (L.dz. KB/87/2001 of 2001.04.19) at Ludwik Rydygier Medical College in Bydgoszcz of Nicolaus Copernicus University in Toruń (CM UMK).

The study involved 40 patients after the first renal transplant, including 21 females (mean age 41.1 ±12.5 years) and 19 males (mean age 43.6 ±13.3 years), who have previously undergone renal replacement therapy with hemodialysis due to end-stage renal disease. The patients were hospitalized in the Department of Transplantology and General Surgery of CM UMK. The patients were Polish citizens and all had kidneys transplanted from a deceased donors. The recipients were registered in the National Register of Recipients of Vascular Organs. They underwent required qualification tests and they were selected according to human leukocyte antigen (HLA). The selection has been made by HLA Laboratory of Immunology Unit of the Transplantology Institute in Warsaw. Patient histories were taken and medical examinations were performed; systolic (SBP) and diastolic (DBP) blood pressure were recorded and mean arterial pressure (MAP) was calculated.

Causes of chronic kidney disease in the organ recipients were: chronic glomerulonephritis – 24 patients (60%), obstructive nephropathy – 4 (10%), hypertensive nephropathy– 3 (7.5%), acute tubular necrosis – 3 (7.5%), polycystic kidney disease – 3 (7.5%), diabetic nephropathy – 1 (2.5%), lupus nephropathy – 1 (2.5%) and tubulointerstitial nephropathy – 1 (2.5%).

Directly after operation the patients were staying in the Department of Transplantology and General Surgery of CM UMK. During the stay, their clinical state was assessed, diuresis was measured and laboratory tests were carried out according to the determined schedule. At the same time triple drug immunosuppressive therapy was implemented, including cyclosporine (22 persons) or tacrolimus (18 persons) combined with azathioprine or mycophenolate mofetil and prednisone.

In order to obtain correct values of the laboratory parameters and their mutual relations, a control group consisting of 14 volunteers (mean age 40.1 ±11.2), including 7 females and 7 males aged 22–58 was selected. They underwent single determination of the studied laboratory and clinical parameters.

Blood samples for tests of fibrinolytic and coagulation system and for other laboratory tests were taken on the day of transplantation (day 0), upon written consent of the patient, immediately upon their arrival to the Department of Transplantology and General Surgery of CM UMK. Prior to renal transplantation each patient was subjected to hemodialysis without anticoagulant as a routine preparation for renal transplantation. The aim of hemodialysis was achieving an optimum state of dehydration, comparable to so-called "dry mass", enabling a surgery.

Blood samples were taken by puncture of the bend of the elbow by using minimum stasis, to plastic syringes containing 3.2% sodium citrate, maintaining citrate to blood rate of 1:9. Syringes with blood were immediately transported in a cold box to the Department and Chair of Laboratory Diagnostic CM UMK, where they were spun 3000 rpm in 4°C for 20 minutes. Poor-platelet plasma obtained in this way was portioned and frozen in –70°C until tHcy was determined in the Department and Chair of Laboratory Diagnostic CM UMK, and PAI-1 and vWF in the Department and Chair of Pathophysiology. Complete blood count, blood urea nitrogen (BUN), creatinine (Cr), electrolytes, total cholesterol, triglycerides, total calcium, phosphorus, glucose, transaminases, uric acid (UAC) levels were determined using the remaining blood. The following fasting blood samples were taken from patients during routine control examination in 6- and 12-month intervals counting from the day of renal transplantation in the Transplant Outpatient Clinic of the Specialist Clinic Complex of the CM UMK University Hospital. In all members of the control and test group glomerular filtration rate (GFR) was assessed, calculated according to Cockroft Gault formula and determined for 1.73 m^2. The values obtained were then subjected to statistical analysis.

Statistical analysis

The results were presented as a mean ± standard deviation. Presence of normal distribution of the analyzed statistical data was assessed by means of Shapiro-Wilk test and standard Pearson χ^2 test. In order to use Student's t-test to compare two means of small samples, an analysis of normal distribution was carried out. Considering that the analyzed group has 40 samples, a hypothesis of equality of 2 means was proposed, verified by U test of standard normal distribution. Relations between the parameters were analyzed by means of Spearman rank correlation coefficient (for abnormal distribution) or Pearson correlation coefficient (for normal distribution). Moreover, multifactor relations between the selected parameters were tested. For all analyzes, $p < 0.05$ value was assumed to be statistically significant.

Determination results were drawn up using Excel for Windows 98 and STATISTICA 5.0 PL for Windows 98 by StatSoft.

RESULTS

Prior to renal transplantation the patients were undergoing renal replacement therapy for approx. 40 months, including males 35.5 ±25.3 months, and females – 44.2 ±40.1 months. The difference was not statistically significant. 72% patients in the study group were treated for arterial hypertension, mostly with two drugs. Mean cold ischemia time in transplanted kidney was 1588 minutes.

Characteristic increase in PAI-1 levels both during the first 6 months, and 12 months after renal transplantation (Fig. 1) was observed in the study group. The obtained values were not significantly different from the control group (Tab. 1).

Table 1. Clinical parameters and laboratory test results in control and study groups prior to renal transplantation, 6 and 12 months afterwards

Parameters	Study group			Control group
	prior to transplantation ("0")	6 months after transplantation ("6")	12 months after transplantation ("12")	
GFR (ml/min/1,73m²)	2.43 ±1.61[A]	64.88 ±21.48 [B,D]	64.39 ±22.07[C,E]	117.79 ±48.48
Cr (mg/dl)	7.32 ±2.07[A]	1.50 ±0.45[B,D]	1.61 ±0.58[C,E]	0.91 ±0.07
MAP (mmHg)	104.98 ±12.55[A]	102.10 ±8.84[B]	106.28 ±9.23[C,F]	84.40 ±10.51
SBP (mmHg)	142.25 ±19.83[A]	37.37 ±14.93[B]	145.12 ±17.0 [C,F]	115.35 ±14.33
DBP (mmHg)	86.125 ±10.4[A]	4.5 ±7.9[B]	86.95 ±7.86[C]	68.92 ±9.02
PAI-1 (ng/ml)	15.52 ±14.04[A]	27.80 ±14.93[D]	34.39 ±21.36[E,F]	25.26 ±16.84
tHcy (μmol/l)	20.21 ±8.57[A]	7.86 ±6.15[B]	17.10 ±6.22[C]	8.39 ±2.54
vWF (%)	121.47 ±20.28[A]	119.57 ±20.95[B]	119.17 ±25.12[C]	244.10 ±121.32
cholesterol (mg/dl)	223.72 ±49.21	244.35 ±52.44[B,D]	229.91 ±68.13	204.0 ±37.03
Triglycerides (mg/dl)	191.4 ±91.36[A]	191.5 ±114.83[B]	181.75 ±75.48[C]	96.92 ±43.95
UAC (mg/dl)	4.03 ±1.48	6.58 ±1.26[B, D]	6.62 ±1.59[C, E]	4.42 ±1.19
Glucose (mg/dl)	95.6 ±29.2	91.42 ±19.11	98.62 ±40.04	92.35 ±7.44
Hemoglobin (g/dl)	11.58 ±1.71[A]	13.73 ±2.39[D]	14.25 ±2.37[E, F]	14.31 ±1.25

Statistically significant differences ($p < 0.05$) were marked with letters, depending on follow-up period: A – "0" vs control group, B – "6" vs. control group, C – "12" vs. control group, D – "0" vs. "6", E – "0" vs. "12", F – "6" vs. "12". Abbreviations: Cr – creatinine, DBP – diastolic blood pressure, GFR – glomerular filtration rate, MAP – mean arterial pressure, PAI-1 – plasminogen activator inhibitor type 1, SBP – systolic blood pressure, tHcy – homocysteine, UAC – uric acid, vWF – von Willebrand factor

Concentration of von Willebrand factor was not showing significant changes in the study group (Fig. 2), and values obtained both prior to and after renal transplantation, were significantly lower than in the control group (tab. 1).

Correlations between the obtained values of PAI-1, vWF and tHcy after renal transplantation and the results of laboratory and clinical tests proved relation between the tHcy levels (as dependent variable) and Cr and BUN levels (as independent variables) within 0–6, 6–12 and 0–12 months after transplantation. Relation between SBP (as independent variable) and tHcy (as dependent variable) within the second half year after transplantation was also observed. No significant relations were observed between vWF and PAI-1 and the analyzed clinical and laboratory values.

Within the period prior to transplantation, the patients with chronic kidney disease (stage V) were demonstrating higher MAP (104.95 ±12.55 vs. 84.40 ±10.51 mmHg), higher Cr level and lower GFR than in the control group (table 1). The assessed markers of endothelial function (PAI-1, vWF) in the study group were significantly lower, whereas tHcy levels were higher than in the control group. Prior to renal transplantation, the study group did not differ from the control group in respect of UAC levels, cholesterol levels and plasma glucose concentration (Tab. 1).

After renal transplantation and upon it started functioning, there was observed statistically significant decrease in plasma Cr levels: 7.32 ±2.07 mg/dl prior to transplantation, to 1.50 ±0.45 mg/dl in 6 months after transplantation and 1.61 ±0.58 mg/dl after 12 months since renal transplantation (table 1). A mean value of creatinine levels was 1.61 mg/dl and corresponding value of GFR was 64.39 ±22.07 ml/kg/1.73 m² (Fig. 3).

A multiple regression analysis for creatinine levels as dependent variable was carried out only with the variables demonstrating significant linear correlations. A relation between the creatinine levels and body weight in the individual follow-up periods was only found. Multiple regressions carried out for vWF and PAI-1 (as dependent variables) showed no significant correlations with laboratory parameters and clinical data (as independent variables).

Due to proposed hypothesis of impact of changes in the assessed parameters within 0–6 months after transplantation on functions of endothelium and transplanted kidney, multifactor correlations were carried out for the tested dependent variables (vWF, PAI-1, tHcy, Cr, GFR) within the period of 6–12 months after transplantation. PAI-1 levels demonstrated relation with increase in body weight (inverse relation) and decrease in Cr levels within 0–6 months, and this relation did not recur within 6–12 months of follow-up. Multifactor correlations were also carried out for the parameters determining function of the transplanted kidney. There was found a repeating relation between changes in Cr levels (dependent variable) and Δ tHcy levels (independent variable) within follow-up periods of 0–6 and 6–12 months after renal transplantation. Other repeating correlations were not found (Tab. 2).
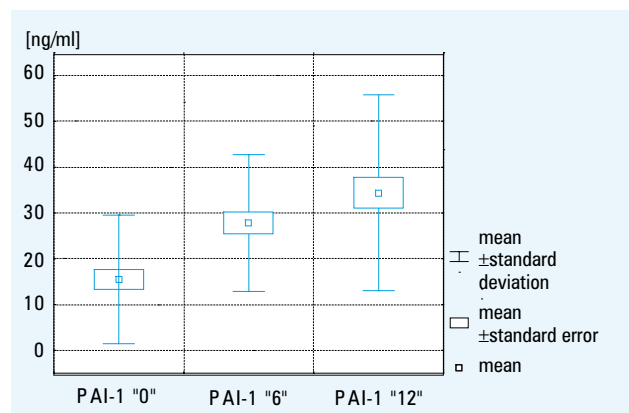
Fig. 1. Changes in concentration of plasminogen activator inhibitor type 1 (PAI-1) in kidney transplant patient during one-year follow--up
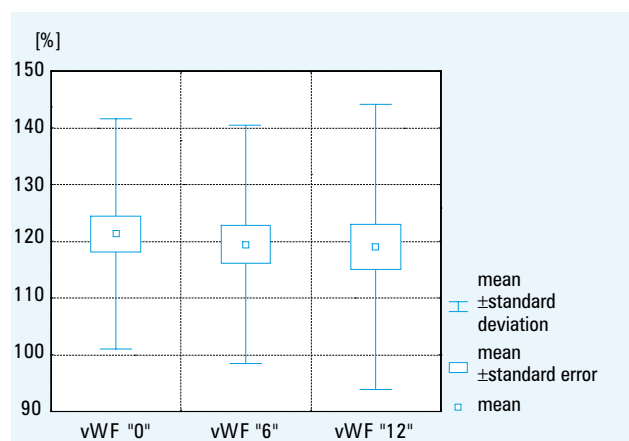
Fig. 2. Changes in concentration of von Willebrand factor antigen (vWF) in kidney transplant patient during one-year follow-up
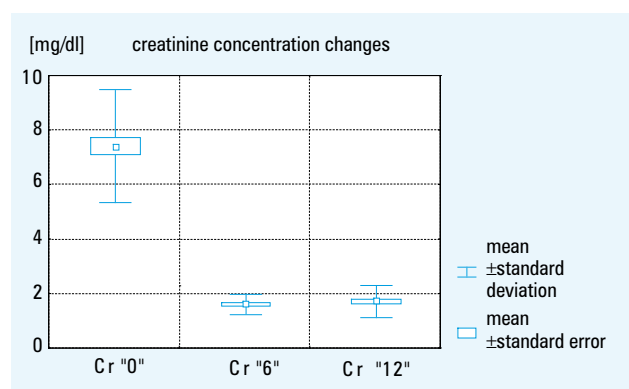
Fig. 3. Plasma creatinine concentration in study group during one-year follow-up (Cr "0" = 7.32 ±2.07 mg/dl; Cr "6" = 1.50 ±0.45 mg/dl; Cr "12" = 1.61 ±0.58 mg/dl). Cr – creatinine

Table 2. Correlation coefficients calculated for plasma creatinine levels in kidney transplant patients, related to studied clinical and laboratory parameters prior to transplantation ("0") and 6 ("6") and 12 ("12") months afterwards

Parameters		"0"	"6"	"12"
Body weight	p	0.99	0.036	0.24
	r	0.005	0.16	0.18
SBP	p	0.19	0.46	0.41
	r	−0.208	0.12	0.13
DBP	p	0.33	0.93	0.69
	r	−0.15	0.01	−0.06
MAP	p	0.22	0.64	0.77
	r	−0.19	0.07	0.04
PAI-1	p	0.059	0.2	0.01
	r	−0.3	0.86	0.98
vWF	p	0.3	0.86	0.26
	r	−0.16	−0.02	0.1
tHcy	p	0.003	0.02	0.001
	r	0.45	0.36	0.49
UAC	p	0.000	0.019	0.000
	r	0.623	0.36	0.53
Cholesterol	p	0.34	0.018	0.44
	r	−0.15	−0.371	−0.12
Triglycerides	p	0.037	0.52	0.76
	r	−0.33	−0.104	−0.04
Glycemia	p	0.63	0.38	0.6
	r	−0.07	−0.24	0.08

p and r values given respectively. Abbreviations – see table 1

DISCUSSION

Endothelial function was assessed based on changes in vWF and PAI-1 levels. In comparison with the control group, the study group demonstrated increased PAI-1 levels in 6. and 12. month of the follow-up since transplantation, as well as statistically higher PAI-1 levels prior to transplantation. The determined vWF levels in each follow-up period in the study group were also statistically lower than in the control group. The results obtained in patients after renal transplantation diverge from expectations, what may result from the size of sample and short follow-up period covering 12 months after renal transplantation. Mean plasma Cr levels decreased from 7.32 mg/dl to 1.5 mg/dl after 6 months, and after 12 months Cr was stabilized at the level of 1.61 mg/dl. GFR increased from 2.43 ml/min/1.73 m^2 to 64.88 ml/min/1.73 m^2 and finally reached a level of 64.39 ml/min/1.73 m^2. Applying binding criteria of diagnosing chronic kidney disease, according to National Kidney Foundation of 2002 [9], in the study group there was found stage II of chronic kidney disease. Applied

classification shows significant relations between the kidney function and the risk of development of cardiovascular system diseases [10]. It is considered that GFR decrease below 60 ml/min/1.73 m^2 has considerable impact on increase in risk for the development of atherosclerosis, ischemic heart disease and cardiac insufficiency [11-13]. The first 6 months after transplantation are considered a period of particular risk for development of surgical, infection and metabolic complications, e.g. lipid and carbohydrate disorders [14,15].

Some authors find no differences in concentration or activity of selected parameters of coagulation system after renal transplantation, which at the same time are the markers of endothelial function. It may prove the persistent impairment of fibrinolytic properties of plasma. It seems that endothelial dysfunction is a consequence of production of peroxide anions, which inactivate nitric oxide (NO), and oxidized molecules of low density lipoproteins, accumulated in sites of vascular injury, hamper NO release from endothelial cells or inactivate it directly. Most sources provide information on fibrinolytic defect in patients after renal transplantation. Predominant abnormality found during a long term follow-up is impaired fibrinolysis, secondary to increased PAI-1 levels.

Increased concentrations of PAI-1 antigen express the impairment of endothelial function and increased thrombotic tendencies, and thereby an increased risk of unfavorable cardiovascular events. Data from literature prove the generalized hemostatic defect in patient after transplantation, which may demonstrate a nature of increased activity of fibrinolytic system or predomination of coagulation system [6], and also the fact that already in early stage of chronic kidney disease there appear significant changes in plasma fibrinolytic activity [6].

In CKD cases we observe frequent coexistence of hyperinsulinemia, dyslipidemia and hyperhomocysteinemia [1,6]. Patients in early stage of CKD demonstrate, like the dialyzed patients, lower activity of tissue plasminogen activator (t-PA). It may result from hampering impact of lipids on t-PA release or its synthesis in endothelial cells.

The observed increased concentration of PAI-1 antigen proved some of the data from literature [6,16,17]. However, other authors [18,19] report decreased activity of PAI-1 and increased activity of t-PA after transplantation. They did not observe significant impact of use of cyclosporine (CsA) on t-PA and PAI-1. The authors judged that lower antigen concentration and lower PAI-1 activity were related to the impact of numerous other factors, like hyperlipidemia, obesity or steroid therapy, whereas increase in concentration of PAI-1 antigen was related to increased insulin concentration. It was also observed that in kidney recipients with poorly controlled hypertension, PAI-1 concentration and activity were higher than in patients after transplantation with well controlled hypertension.

Perkowska et al. [19] found increased concentration and activity of PAI-1 in patients with chronic nephropathia of transplant in comparison with recipients with stable graft function. It shall prove progressing endothelial dysfunction and decreased plasma fibrinolytic activity, which altogether

increase the risk for thromboembolic complications. In the analyzed material there were not found statistically significant differences between the patients with normal renal function and the patients with insufficient transplanted kidney. Unbiased assessment of PAI-1 action after transplantation requires considering a method applied to determine activity and concentration of PAI-1 antigen. Due to spontaneous conversion of PAI-1 to inactive form and occurrence of t-PA/PAI-1 plasma complex in a given sample of peripheral blood, active, inactive and bound to t-PA molecules of PAI-1 are found simultaneously. Available PAI-1 antigen determination sets react with each of the aforementioned PAI-1 molecule [16,18,20]. Therefore, it seems that the method based on activity assessment is more reliable, however, more complicated and laborious as well. Numerous factors have impact on the process of spontaneous conversion of PAI-1 from active to inactive form. It is thought that obesity, hyperlipidemia and used glycocorticosteroids lead to considerable fluctuations of the process.

Hemodialysis performed prior to transplantation may influence the tested parameters of coagulation and fibrinolytic system (vWF, PAI-1), as well as on biochemical parameters. During hemodialysis, due to interaction of extracorporeal circulation with vascular system of the patient and lack of entire biocompatibility, plasma fibrinolytic activity is increased [6, 21,22]. The consequence is an increased t-PA release from the vessel wall. Using of anticoagulant (heparin) during hemodialysis does not influence the aforementioned process. Observations were based on comparison between dialysis with heparin and rinsing out the drain system with 0.9% NaCl. Hemostasis is also influenced by platelet activation, tumor necrosis α (TNF-α) release and hypoxemia [6,21,22].

Action of the assessed markers of endothelial function also depends of application of immunosuppressive therapy. Cyclosporine provokes extension of euglobulin lysis time, decrease in t-PA activity and increase in activity and concentration of PAI-1 antigen [6,17,23,24]. It is suggested that a mechanism of toxic influence of CsA and tacrolimus (Tac), a calcineurin inhibitor, is related to hampering of activity of nitric oxide synthase, which is calcium/calmodulin-dependent enzyme. The effect of those actions is decrease in synthesis and availability of NO [25]. Ligtenberg et al. [26] demonstrated impaired endothelial function in vasomotor reaction in patients after renal transplantation treated with CsA. Hampering impact of CsA on endothelial function was partly eliminated by administration of L-arginine, what proves CsA impact on bioavailability of NO. Also van den Dorpel et al. [24] proved that PAI-1 levels was higher in patients after transplantation, treated with cyclosporine, than in patients from the control group and in patients after transplantation, to whom that drug was not administered. Cyclosporine was replaced with azathioprine in patients with stable transplant function [6] and decrease in PAI-1 activity was found within approx. 12 weeks from conversion. At the same time, increase in t-PA concentration and activity was observed, what was certainly secondary to decrease in PAI-1 concentration. Incidents of cyclosporine overdose, accompanied by increase in PAI-1 con-

centration, additionally had to prove impact of this drug on increase in PAI-1 levels [6].

Impaired endothelial function during administration of calcineurin inhibitors was also observed by Ovuworie et al. [25]. They did research on changes in vascular flow, which were a response to a stimulus directly broadening the vessels or to impact of agents increasing the release of vasodilatators from vascular endothelium.

Elhasade et al. [16] assessed action of t-PA and PAI-1 in patients after kidney allotransplantation. They found that in patients after renal transplantation concentration of t-PA antigen and t-PA activity were slightly higher than in healthy persons, whereas PAI-1 activity was significantly lower in recipients, and concentration of PAI-1 antigen – higher. Additional analysis of immunosuppressive therapy did not show impact of cyclosporine on t-PA and PAI-1 activity and on concentration of t-PA antigen; however, it was found relation between concentration of PAI-1 antigen and cyclosporine. The authors were of the opinion that cyclosporine might directly damage vascular endothelium, increasing plasma thrombotic activity, intensifying platelet activity and interfering in metabolism of arachidonic acid and synthesis of prostaglandins [18,27]. Similar results are presented by van den Dorpel et al. [24], who studied plasma fibrinolytic activity upon therapy conversion from cyclosporine to azathioprine. Therapy conversion led to increase in plasma fibrinolytic activity in comparison with decrease of PAI-1 antigen. No changes in concentration of t-PA antigen were found, however, t-PA activity increased. Moreover, during CsA therapy there was found a relation between concentrations of t-PA and PAI-1 antigens and prostaglandin E_2 and thromboxane B_2 levels. No links between those parameters were observed during azathioprine therapy. Based on these observations, a conclusion was drawn of multiple influence of CsA on endothelium and coagulation and fibrinolytic system.

Patients after transplantation demonstrate numerous abnormalities in respect of fibrinolysis in comparison with healthy persons. They also demonstrate extended euglobulin lysis time, significantly decreased t-PA activity level, whereas PAI-1 activity and levels are significantly higher [6,17]. On the other hand, van den Dorpel et al. [24] showed that in patients treated with CsA, PAI-1 levels were significantly higher than in healthy persons and patients after transplantation, to whom CsA was not administered. Comparing patients treated with CsA and prednisone for at least 6 months, with a group of patients, to whom CsA was changed to Aza, significant decrease in PAI-1 activity was observed within approx. 12 weeks after they stopped taking CsA. At the same time, increased t-PA activity was found, which was probably secondary to decrease in PAI-1. Mechanism of CsA action is not entirely clear. Some suggest hampering effect on prostanoid release, intensification of oxidative stress and direct impact of CsA on endothelial cells, as well as on hepatocytes [24]. CsA impact on PAI-1 is undeniable, due to the fact that PAI-1 concentration significantly increases, e.g. upon CsA overdose.

Glycocorticosteroids also have significant impact on coagulation system. The first information concerning this issue was provided by the data on patients with Cushing's syndrome, where reduced plasma fibrinolytic activity was described [6]. Comparing patients, who underwent renal transplantation 12 months earlier and patients with Cushing's syndrome with healthy population, significant increase in both activity and concentration of PAI-1 antigen, measured before and after passive hyperemia was found in groups of studied patients [6]. Upon coming off steroids, in patients after renal transplantation no considerable improvement was observed in respect of coagulation system, what highlights multifactor effect on hemostasis.

It is thought that glycocorticosteroids may influence PAI-1 synthesis gene promoter [16,18]. Observations in patients with Cushing's syndrome showed increase in both concentration and activity of PAI-1 [26]. Małyszko et al. [17] proved impact of glycocorticosteroids on development of insulin resistance, lipid disorder and obesity, and secondarily on fibrinolysis. However, the results obtained in those studies varied in respect of t-PA level – Małyszko et al. [17] found its reduction, whereas Elhasade et al. [16] and Perkowska et al. [19] increase in t-PA level. Inconsistencies seem to be the result of different Cr concentrations (i.e. different renal function) and related other glycocorticosteroid doses. Analogous data concerning patients subjected to steroid therapy for other reasons, as well as patients after transplantation taking steroids is presented by Patrasi et al. in their study [23]. Coming off steroids in patients after transplantation did not result in radical improvement of hemostasis parameters. It proves influence of numerous factors on coagulation and fibrinolytic system [8,17,18].

Immune response in recipient against the graft may also have impact on PAI-1 and vWF. Wang et al. [27] assessed expression of PAI-1-mRNA in endothelial cells of arterial and capillary vessels of transplanted kidneys. In case of both acute and chronic rejection, increased values of PAI-1-mRNA in the vessel endothelium were proved. Torry et al. [28] assessed action of fibrin deposits, t-PA, PAI-1 and antithrombin in renal bioptates, taken due to rejection in the periods of: 1 month, from 1 month to 1 year and 1 year after transplantation. They found definitely higher amount of fibrin and t-PA deposits in smooth muscle cells of vessels of bioptates in patients with rejection within 1 month after transplantation. Also t-PA deposits showed tendency to be accumulated in renal tissue in increased amounts, but there were no difference between the studied groups in this respect. No relation with other markers of endothelial function was found.

There were also attempts to relate changes in vWF concentration to homocysteine level and lipid disorders. Blann et al. [20,29] and Witztum et al. [30] found in their studies that a change of lifestyle combined with reduced cholesterol level led to reduced vWF levels. Additionally, in multiple analysis vWF levels correlated with total cholesterol level with the amount of consumed polyunsaturated fatty acids and carbohydrates. Those relations were independent of age, body weight, arte-

rial pressure, smoking and severity of ischemic heart disease. The authors suggest that cytotoxic low-density lipoproteins are responsible for damage of endothelial cells [20,30].

It was proved in experimental studies that vWF levels increase influenced by administration of steroids and cytokines (Il-1, TNF-α) produced by monocytes and macrophages. It was also observed that high tHcy concentration hampers production of vWF, mainly by hampering of intracellular transport [31,32].

The studies carried out showed no considerable improvement of endothelial function, assessed based on determination of PAI-1 and vWF within a year from transplantation in kidney transplant patients. After a year, mean plasma Cr concentration shows a value of 1.6 mg/dl, what corresponds to chronic graft insufficiency. Therefore, it may suggest that, in spite of renal transplantation, endothelium acts, like in patients with chronic kidney disease, but with more advanced lesions in cardiovascular system.

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