Risk Factors for Vascular Disease and Arteriovenous Fistula Dysfunction in Hemodialysis Patients[1]

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ABSTRACT

Vascular access dysfunction is an important cause of morbidity for dialysis patients and a major contributor to hemodialysis cost. Thrombosis is a leading cause of vascular access failure, and usually results from stenotic lesions in the venous outflow system. This study was designed to explore the impact of serum levels of various risk factors for thrombosis and accelerated fibrointimal hyperplasia on progressive stenosis, and the subsequent thrombosis of hemodialysis fistula. A cross-sectional and 2-yr prospective pilot study was performed in 30 nondiabetic hemodialysis patients with primary arteriovenous fistula. Venous dialysis pressure, urea recirculation, color Doppler sonography, and angiography were used to monitor vascular access patency. Eleven patients (37%) developed a progressive stenosis in the venous circuit, which was complicated by thrombosis in three patients. Compared with the patients without fistula dysfunction, these patients had higher serum levels of monocyte chemoattractant protein-1 and interleukin-6, two cytokines that regulate the proliferation of vascular smooth muscle cells, which is the key mechanism in the pathogenesis of fistula stenosis. In addition, they had hyperinsulinemia, hyperlipidemia, and increased plasma levels of two hemostasis-derived risk factors for thrombosis: plasminogen activator inhibitor type 1 and factor VII. Monocyte chemoattractant protein-1, interleukin-6, plasminogen activator inhibitor type 1, factor VII, triglycerides, and the ratios for cholesterol/HDL-cholesterol, apolipoprotein (apo) A-I/apo C-III, apo A-I/apo B, and glucose/insulin were independent predictors of fistula dysfunction. This study demonstrates the influence of cytokines, hemostasis-derived vascular risk factors, hyperinsulinemia, and abnormalities of lipids and apolipoproteins on primary fistula survival. The assessment of these factors might be useful for the identification of the patients at risk of fistula stenosis and thrombosis.

Key Words: *Hemodialysis vascular access, cytokines, adhesion molecules, coagulation, uremia*

Vascular access dysfunction represents the most frequent cause of hospitalization for dialysis patients, and is responsible for a large proportion of the cost of any ESRD program (1). The primary arteriovenous fistula is uniformly recommended as the best permanent vascular access in hemodialysis patients (2). Thrombosis is a leading cause of vascular access failure, and usually results from stenotic lesions in the venous outflow system (1,2). These lesions develop from progressive neointimal hyperplasia (3,4), whose pathogenesis is incompletely understood. To date, risk factors and correlates of fistula dysfunction include diabetes mellitus, hypotension, hypoalbuminemia, anticardiolipin antibodies, increased serum levels of lipoprotein (a) [Lp(a)] and fibronectin (5–8). A significant percentage of vascular access thromboses, however, remains unexplained. This suggests that there are additional predisposing factors, yet to be discovered. The increased rate of vascular access complication in diabetes mellitus has been related, at least in part, to several prothrombotic abnormalities of hemostatic factors (2). These abnormalities clearly precede the development of renal disease (9), and possible additional effects of uremia are unknown. A hypercoagulable state caused by alterations of coagulation and fibrinolytic factors frequently occurs in renal failure (10). High plasma concentrations of fibrinogen, D-dimer, thrombin-antithrombin III complex, coagulation factor VII, von Willebrand's factor, thrombomodulin, and plasminogen activator inhibitor type 1 (PAI-1) have been described in patients with end-stage renal failure (11–13), and are indicative of a thrombophilic state and endothelial injury. In addition, these patients have often low predialysis values of protein C (14), a natural coagulation inhibitor whose deficiency is associated with an increased risk of venous thrombosis. Elevated levels of circulating adhesion molecules and cytokines have been reported in hemodialysis patients (15,16). Recent studies have demonstrated that cytokines, such as interleukin-1β (IL-1β) (17), interleukin-6 (IL-6) (18) and monocyte chemoattractant protein-1 (MCP-1) (19), regulate the

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proliferation of vascular smooth muscle cells (VSMC), which is the key mechanism in the pathogenesis of fistula stenosis as well as postangioplasty restenosis of coronary arteries and atherosclerosis. Swedberg and coworkers (3) advanced the hypothesis that platelet-derived growth factor (PDGF) is released by repeated microthrombus formation near the puncture site of the arteriovenous shunt, thus leading to a stenosing neointima layer downstream of a polytetrafluoroethylene graft. It has been recognized that cytokines, growth factors, adhesion molecules, and hemostatic factors have a major role in the pathogenesis of the restenosis of coronary arteries that occurs after angioplasty (20). Recently, Hehrlein (21) claimed analogies between the mechanism responsible for the recurrent stenosis after transluminal coronary angioplasty and the process leading to a stenosing neointimal hyperplasia in the runoff vein of the hemodialysis fistula. Although the association of lipid abnormalities and vascular access thrombosis has already been studied (6,8), to date, the influence of cytokines, vascular adhesion molecules, and hemostasis-derived risk factors on fistula patency has not been widely investigated.

We undertook a pilot study in a group of nondiabetic hemodialysis patients to explore the impact of serum levels of various risk factors for thrombosis and accelerated fibrointimal hyperplasia on progressive stenosis and subsequent thrombosis of primary arteriovenous fistula.

METHODS

Population of Patients

The study subjects were selected from a group of adult patients with ESRD, who were clinically stable and who were undergoing maintenance hemodialysis. For the purpose of this study, we considered only patients with native arteriovenous fistula that had been functioning well for at least 6 months. All patients had a normal study on color Doppler sonography (22), and values of dialysis venous pressure and urea recirculation persistently lower than 150 mm Hg and 15%. Other eligibility criteria included the absence of hypoalbuminemia (serum albumin level less than 35 g per L) and circulating anticardiolipin antibodies. Patients with diabetes mellitus, liver and collagen diseases, and malignancy were excluded to avoid the possible effects of these comorbid conditions.

Thirty hemodialysis patients (18 men and 12 women) who fulfilled these criteria were enrolled. The mean duration of dialysis was 46 months (range, 8 to 108 months). All patients were receiving our conventional hemodialysis (23) with bicarbonate bath. Dialysis prescription was guided by a goal of achieving a value of ≥ 0.65 for the urea reduction ratio. This index of adequacy of dialysis was calculated by the formula [(predialysis BUN) − (postdialysis BUN)]/(predialysis BUN), in which postdialysis BUN was the blood urea nitrogen level measured 5 min after the end of dialysis. The dialyzer membranes used were cellulosic (77% of patients) and polymethylmethacrylate (23%). All patients were dialyzed using an end-to-side vein-artery anastomosis of the cephalic vein and radial artery. Cannulation of fistulas was performed identically in all patients by the same trained personnel. Special care was taken to avoid cannulating at the same site with each treatment. All patients had no residual renal function. No patient had a documented history of stroke or myocardial infarction or had undergone coronary bypass surgery or percutaneous coronary angioplasty. None of the patients had received surgical treatment of carotid, aortoiliac, or lower-extremity atherosclerosis.

Forty healthy subjects (24 men and 16 women) served as a control group and provided blood samples for laboratory investigations.

Study Design

A cross-sectional and 2-yr prospective pilot study was performed in this group of hemodialysis patients. Baseline demographic, clinical, and laboratory indexes were recorded at the time of entry into the study. The strategy for monitoring arteriovenous fistula patency included clinical assessment, serial measurements of venous dialysis pressure, urea recirculation, and fistula flow by color Doppler sonography. Fistulography was used to provide detailed visualization of the lumen. The pressure in the venous return line of the dialysis circuit was measured at extracorporeal blood flows of 225 to 250 mL per min during the first 30 min of each dialysis through a 16-gauge needle (24). Urea recirculation was performed weekly by the stop-flow method at a blood flow rate of 200 to 250 mL per min (24). Color Doppler sonographic examinations were performed, as previously described by Nonnast-Daniel and coworkers (22), at monthly intervals or, if necessary, more frequently. The diet of the patients was not modified during the study. No antiplatelet and anticoagulant therapies were administered, except that heparin was given during the dialysis procedure. No patients received drugs that might affect serum lipid levels during the 3 months preceding the study, nor during the study. Fifteen patients were on recombinant human erythropoietin therapy. The mean (± SD) duration of therapy was 8.5 (± 4.3) months and the mean (± SD) dose was 109 (± 39) U/kg body wt per week. No changes in dosage requirement were recorded throughout the study.

Laboratory Methods

Blood samples were drawn in the morning after an overnight fast, carefully avoiding venous stasis. All blood samples were collected during the midweek hemodialysis session, immediately before the session. Hemoglobin and hematocrit values, as well as blood cell counts and chemistries were determined by routine techniques as described elsewhere (25). Cholesterol and triglyceride levels were analyzed by enzymatic procedures. High-density lipoprotein (HDL) cholesterol levels were determined with a differential dextran-sulfate/magnesium-chloride precipitation technique. Apo A-I, B, and C-III were assayed using immunoturbidometric method (Eiken, Tokyo, Japan). Lp(a) was measured with an ELISA (Macra Lp(a); Strategic Diagnostic, Newark, NJ). For the determination of coagulation parameters, blood (4.5 mL) was collected in silicone-treated glass tubes by venipuncture. Trisodium citrate (0.1 M) in 1/10 volume ratio was added as anticoagulant. The citrated blood was centrifuged for 20 min at 1700 *g* at 4°C. The supernatant was aliquoted and stored at −80°C. Coagulation factors VII and XII activities were determined with a one-stage clotting assay using commercially available reagents (Instrumentation Laboratory, Lexington, MA). Fibrinogen level was determined in an automated coagulation laboratory (ACL) autoanalyzer (ACL-3000; Instrumentation Laboratory, Lexington, MA). D-dimer

value was evaluated by an ELISA method (Boehringer, Mannheim, Germany). Protein C and S levels were estimated with a functional clotting assay (Instrumentation Laboratory, Lexington, MA). Tissue-type plasminogen activator (t-PA) and PAI-1 were assayed by enzyme immunoassay (Innotest t-PA and Innotest PAI-1; Byk-Sangtec, Dietzenbach, Germany). Prothrombin activation fragment $_{1+2}$ (F_{1+2}) level was measured by an ELISA method (Boehringwerke, Marburg, Germany). The serum levels of soluble intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, IL-1β, IL-6, tumor necrosis factor (TNF)-α, and MCP-1 were measured by sandwich immunoassay (Human soluble ICAM-1, Human soluble VCAM-1, Quantikine human IL-1β, Research and Diagnostic Systems Europe, Abington, Oxon, UK; Cytoscreen human IL-6, Cytoscreen human TNFα, BioSource International, Camarillo, CA; MCP-1/MCAF [monocyte chemoattractant and activating factor], Toray Industries, Japan). PDGF was measured in the plasma by sandwich immunoassay (Quantikine human PDGF-ab; Research and Diagnostic Systems Europe, Abington, Oxon, UK).

Statistical Analysis

Results are expressed as means ± SD unless otherwise indicated. In the cross-sectional analyses, comparisons were made using the analysis of variance, the *t* test, and the chi-squared test. In the analysis of variance, *F* test or Welch test and Brown-Forsythe test were used as appropriate. Correlation was assessed with simple and multiple linear regression and Spearman's rank test as appropriate. *P* values less than 0.05 were considered to indicate statistical significance. Baseline data of clinical and laboratory variables were compared in patients with and without progressive stenosis of the fistula. These parameters were entered into the Cox proportional hazards regression model to determine their independent association with vascular access dysfunction. All possible parameters, including age, gender, smoking habits, presence or absence of hypertension and left ventricular hypertrophy, prior months on hemodialysis, erythropoietin therapy, serum lipid and apolipoprotein profile, glucose/insulin ratio, hemostatic indexes, and circulating adhesion molecules and cytokines were entered into the analysis. The variables chosen reflected our previous experience (26) as well as reports in the literature. Calculations were performed using BMDP (Statistical Software Inc., Los Angeles, CA) statistical software.

RESULTS

Eleven patients developed a progressive venous outflow stenosis, which resulted in a lesion of greater than 60%-diameter narrowing on color Doppler sonography. Fistulography confirmed the stenotic lesions in all cases. These patients had venous dialysis pressures and urea recirculation values of more than 150 mm Hg and 20% respectively. Percutaneous transluminal angioplasty successfully corrected the stenotic lesions. However, in three patients, stenosis recurred and was complicated by thrombosis. The thrombus totally occluded the vessel and appeared on color Doppler sonography as an anechoid or hypoechoic clot within the vessel with lack of visible blood flow. These patients underwent surgical fistula revision. A value greater than 60% narrowing of fistulae was chosen as the end point for two reasons: first, because it was associated with fistula dysfunction as indicated by elevated values of venous dialysis pressure and urea recirculation in all cases. The second reason was that, in our experience, the elective treatment with percutaneous transluminal angioplasty was able to successfully correct these stenotic lesions. These end points are substantially in agreement with the recommendations by Schwab (1,29), who demonstrated that the correction by angioplasty of all angiographically determined >50% stenoses improved fistula function and prolonged access survival. There was no difference in the rate of fistula dysfunction, either between patients dialyzed with a cellulosic membrane and those dialyzed with a polymethylmethacrylate membrane (37.5% *versus* 33%), or between patients who received erythropoietin therapy and those untreated (33.3% *versus* 40.0%). Nineteen patients showed a normal study or only minimal abnormalities on color Doppler sonography throughout the study. Table 1 shows the demographic, clinical, and biochemical parameters of the study population. Special attention was placed on the comparison of the data obtained at entry into the study in the patients who developed progressive fistula stenosis with those who did not experience such an event.

PDGF, Cytokines, and Circulating Adhesion Molecules

The hemodialysis patients showed a significant increase in plasma concentration of PDGF (2783 ± 613 *versus* 1306 ± 299 pg/mL, $P < 0.001$), TNF-α (39.2 ± 9.0 *versus* 12.8 ± 4.6 pg/mL, $P < 0.001$), IL-6 (8.0 ± 6.8 *versus* 2.9 ± 2.3 pg/mL, $P < 0.005$), IL-1β (1.01 ± 1.3 *versus* 0.25 ± 0.13 pg/mL, $P < 0.05$) and MCP-1 (188 ± 32 *versus* 100 ± 45 pg/mL, $P < 0.001$) compared with the control subjects. In addition, the hemodialysis group had elevated levels of soluble immunoglobulin superfamily adhesins such as VCAM-1 (1784 ± 342 *versus* 453 ± 156 ng/mL, $P < 0.001$) and ICAM-1 (273 ± 66 *versus* 189 ± 36, $P < 0.005$), which are released by activated endothelial cells (15). As reported in Table 2, the serum concentrations of MCP-1 and IL-6 ($P < 0.025$), two cytokines that are known to regulate the proliferation of VSMC (17–19), were higher in the patients who developed fistula stenosis compared with those who did not. We did not observe any difference in the predialysis levels of cytokines between patients dialyzed with a cellulosic membrane and those dialyzed with a polymethylmethacrylate membrane.

Hemostatic and Fibrinolytic Parameters

As compared with healthy subjects, the hemodialysis patients had higher values of F_{1+2} (3.12 ± 0.88 *versus* 1.18 ± 0.52 nmol/l, $P < 0.001$) and D-dimer (642 ± 222 *versus* 246 ± 125 ng/mL, $P < 0.001$) that indicated the activation of both coagulation and fibrinolytic pathways. Despite these abnormalities, the

TABLE 1. Characteristics of the hemodialysis patients, at the time of entry into the study, and according to subsequent incidence of fistula dysfunction, and healthy subjects[a]

Characteristics and Measure	Patients with Fistula Dysfunction (N = 11)	Patients without Fistula Dysfunction (N = 19)	Healthy Subjects (N = 40)
Sex (M/F)	6/5	12/7	24/16
Age (Yr)	61.6 ± 9.7	61.1 ± 14.6	60.1 ± 12.5
Body-Mass Index[b]	23.0 ± 2.9	21.5 ± 2.1	25.1 ± 1.8
Months of Dialysis	44.5 ± 43.9	47.5 ± 37.4	
Duration of Arteriovenous Fistula (Months)	18.3 ± 14.5[c]	44.1 ± 32.3	
Urea Reduction Ratio[d]	0.66 ± 0.01	0.66 ± 0.01	
Hypertension (Number of Patients)	5	7	
Left Ventricular Hypertrophy (Number of Patients)	8	11	
Smoking (Number of Patients)	3	4	
Hemoglobin (g/dL)	9.8 ± 1.7	9.7 ± 1.7	13.8 ± 1.4
Platelet Count (10^3/mm^3)	245 ± 67	220 ± 31	229 ± 52
Serum Albumin (g/L)	43.0 ± 3.4	40.8 ± 3.2	44.5 ± 2.6
Serum Glucose (mg/dL)	99.9 ± 8.4	98.3 ± 7.5	99.0 ± 7.0
Plasma Insulin (μU/mL)	21.8 ± 7.1[e]	12.0 ± 5.5	9.2 ± 3.0
Glucose/Insulin Ratio	5.0 ± 1.6[e]	9.1 ± 2.4	12.2 ± 3.1

[a] Plus-minus values are means ± SD.
[b] Body mass index was calculated by dividing the dry weight in kilograms by the square of the height in meters.
[c] $P < 0.05$ for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.
[d] Urea Reduction Ratio was calculated by the formula ((predialysis-BUN) − (postdialysis BUN))/(predialysis BUN).
[e] $P < 0.001$ for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.

TABLE 2. Hemostatic indexes, circulating adhesion molecules, and cytokines in hemodialysis patients, at the time of entry into the study, according to subsequent incidence of fistula dysfunction, and healthy subjects[a]

Index	Patients with Fistula Dysfunction (N = 11)	Patients without Fistula Dysfunction (N = 19)	Healthy Subjects (N = 40)
Factor VII (%)	152 ± 26[b]	134 ± 20	94 ± 33
Factor XII (%)	120 ± 34[b]	95 ± 32	91 ± 20
PAI-1 (ng/mL)	57.5 ± 33.6[c]	24.7 ± 21.1	31.1 ± 11.9
t-PA (ng/mL)	6.9 ± 4.2	5.7 ± 4.3	7.0 ± 3.2
F_{1+2} (nmol/L)	3.09 ± 0.96	3.14 ± 0.87	1.18 ± 0.52
D-Dimer (ng/mL)	617 ± 208	657 ± 234	246 ± 125
Fibrinogen (mg/dL)	515 ± 157	446 ± 114	344 ± 71
Protein C (%)	133 ± 35[d]	101 ± 21	130 ± 29
Protein S (%)	134 ± 17[b]	112 ± 21	107 ± 19
MCP-1 (pg/mL)	212 ± 22[c]	174 ± 28	100 ± 45
IL-1β (pg/mL)	1.15 ± 1.65	0.87 ± 0.91	0.25 ± 0.13
IL-6 (pg/mL)	9.6 ± 3.5[b]	6.4 ± 6.1	2.9 ± 2.3
TNFα (pg/mL)	36.4 ± 6.5	41.1 ± 10.2	12.8 ± 4.6
PDGF (pg/mL)	2806 ± 547	2653 ± 775	1306 ± 299
sVCAM-1 (ng/mL)	1671 ± 374	1865 ± 307	453 ± 156
sICAM-1 (ng/mL)	278 ± 76	268 ± 61	189 ± 36

[a] Values are means ± SD. PAI-1, plasminogen activator inhibitor type 1; t-PA, tissue plasminogen activator; F_{1+2}, prothrombin activation fragment F_{1+2}; MCP-1, monocyte chemoattractant protein-1; IL, interleukin; TNF_α, tumor necrosis factor-α; PDGF, platelet-derived growth factor; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1.
[b] $P < 0.025$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.
[c] $P < 0.005$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.
[d] $P < 0.01$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.

plasma levels of t-PA (6.2 ± 4.2 *versus* 7.0 ± 3.2 ng/mL), one of the major activators of fibrinolysis, and PAI-1 (36.3 ± 30.1 *versus* 31.1 ± 11.9 ng/mL), the specific inhibitor of both tissue-type and urokinase-type plasminogen activators, did not differ from healthy subjects. Uremic patients had lower values of protein C (113 ± 31 *versus* 130 ± 29%, $P < 0.05$), one of the major natural coagulation inhibitors. In hemo-

dialysis patients, we found increased values of fibrinogen (473 ± 133 *versus* 344 ± 71 mg/dL, $P < 0.001$) and factor VII (140 ± 25 *versus* 94 ± 33%, $P < 0.001$), two well-accepted risk factors for thrombotic disorders in the general population (28). Comparisons between patients with and without fistula dysfunction are shown in Table 2. Patients who developed fistula stenosis had higher values of factor VII ($P < 0.025$), factor XII ($P < 0.025$), and PAI-1 ($P < 0.005$). Forty-five percent of these patients had values of factor VII (greater than 160%) and PAI-1 (greater than 54 ng/mL) which have been reported to occur in association with thrombotic disorder (28). Conversely, of the 19 patients who did not experience fistula dysfunction, only three (16%) and two (11%) patients had increased values of factor VII and PAI-1, respectively. Interestingly, the level of protein C in patients with fistula dysfunction was significantly higher compared with patients without fistula dysfunction (133 ± 35 *versus* 101 ± 21%, $P < 0.01$), although not different from that of healthy subjects (130 ± 29%).

Lipid and Apolipoprotein Profile

As compared with healthy subjects, the hemodialysis patients had higher concentrations of triglycerides (196 ± 83 *versus* 95 ± 32 mg/dL, $P < 0.001$), Lp(a) (20.9 ± 16.8 *versus* 10.5 ± 10.1 mg/dL, $P < 0.005$), and apo C-III (20.3 ± 9.5 *versus* 9.1 ± 2.8 mg/dL, $P < 0.001$), and lower values for HDL cholesterol (34.3 ± 11.4 *versus* 48.6 ± 12.4 mg/dL, $P < 0.001$) and apo A-I (122.2 ± 19.9 *versus* 159.9 ± 31.0 mg/dL, $P < 0.001$). There was no significant difference in total cholesterol levels (207 ± 64 *versus* 196 ± 31 mg/dL). These abnormalities resulted in an increased ratio of total cholesterol to HDL cholesterol (5.8 ± 2.1 *versus* 4.3 ± 1.4, $P < 0.001$) and lower values for the ratios apo A-I/apo C-III (7.1 ± 3.1 *versus* 18.8 ± 6.4, $P < 0.001$), and apo A-I/apo B (1.24 ± 0.33 *versus* 1.38 ± 0.48, $P < 0.05$). Comparisons between patients with and without fistula dysfunction are shown in Table 3. The patients who experienced fistula dysfunction had a more severe hyperlipidemia as indicated by higher values of cholesterol, triglycerides, apo C-III, and apo B and lower values of HDL cholesterol. These abnormalities resulted in an increased ratio of cholesterol/HDL cholesterol and lower values for the ratios of apo A-I/apo C-III and apo A-I/apo B. There was no significant difference in Lp(a) concentrations between patients with and without fistula dysfunction, but the mean values in these subgroups were approximately twice as high as the mean Lp(a) concentration in the healthy subjects. The fistula dysfunction group had also a lower fasting glucose-to-insulin ratio ($P < 0.001$), which suggested a more severe degree of insulin resistance. A substantial fraction of patients who experienced progressive stenosis of the fistula had values of lipids and apolipoproteins, which have been reported to occur in association with an increased incidence of cardiovascular events in the nonuremic population (27). Low values of HDL cholesterol (less than 35 mg/dL) (82% *versus* 37%), apo A-I/apo B ratio (less than 1.0) (55% *versus* 16%), and apo A-I/apo C-III ratio (less than 6.0) (91% *versus* 37%), and high levels of triglycerides (greater than 200 mg/dL) (91% *versus* 11%), and total cholesterol-to-HDL cholesterol ratio (greater than 4.5) (91% *versus* 47%) were more common in patients with fistula dysfunction compared with those without fistula dysfunction.

Prediction of Fistula Dysfunction

Regression analyses indicated significant correlations between cytokine and lipid levels and hemostatic parameters (Table 4). Hyperinsulinemia and several indexes of uremic dyslipidemia correlated with the

TABLE 3. Serum lipid and apolipoprotein concentrations in hemodialysis patients, at the time of entry into the study, according to subsequent incidence of fistula dysfunction, and healthy subjects[a]

Index	Patients with Fistula Dysfunction ($N = 11$)	Patients without Fistula Dysfunction ($N = 19$)	Healthy Subjects ($N = 40$)
Cholesterol (mg/dL)	244 ± 81[b]	179 ± 35	196 ± 31
Triglycerides (mg/dL)	273 ± 62[c]	150 ± 55	95 ± 32
HDL cholesterol (mg/dL)	29.0 ± 8.2[b]	37.7 ± 12.1	48.6 ± 12.4
Lp(a) (mg/dL)	19.4 ± 16.5	21.7 ± 17.4	10.5 ± 10.1
Apolipoprotein A-I (mg/dL)	120.4 ± 18.4	123.4 ± 21.3	159.9 ± 31.0
Apolipoprotein B (mg/dL)	110.3 ± 26.3[d]	90.6 ± 16.6	116.6 ± 28.4
Apolipoprotein C-III (mg/dL)	27.1 ± 8.5[c]	15.9 ± 7.3	9.1 ± 2.8
Ratios			
Cholesterol/HDL-C	8.3 ± 1.6[c]	4.9 ± 1.4	4.3 ± 1.4
Apo A-I/apo B	1.03 ± 0.22[d]	1.37 ± 0.32	1.38 ± 0.48
Apo A-I/apo C-III	4.7 ± 1.3[c]	8.5 ± 2.9	18.8 ± 6.4

[a] Values are means ± SD. Lp(a), lipoprotein (a); HDL-C, HDL cholesterol; apo, apolipoprotein.
[b] $P < 0.025$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.
[c] $P < 0.001$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.
[d] $P < 0.01$, for the comparison with the values in the group of patients without fistula dysfunction by analysis of variance.

TABLE 4. Correlation coefficients (*r* values) among serum concentrations of lipids, apolipoproteins, cytokines, and hemostasis indexes in 30 hemodialysis patients[a]

	Factor VII	Apo A-I/C-III	MCP-1	Cholesterol	PAI-1	Triglycerides	Apo C-III	Apo A-I/B	IL-6
Apo A-I/C-III	−0.555[b]								
MCP-1	0.448[c]	−0.419[c]							
Cholesterol	0.153	−0.436[c]	0.376[d]						
PAI-1	0.343	−0.419[c]	0.232	0.456[e]					
Triglycerides	0.433[c]	−0.737[b]	0.446[c]	0.510[f]	0.580[b]				
Apo C-III	0.551[f]	−0.847[b]	0.420[c]	0.590[b]	0.461[e]	0.788[b]			
Apo A-I/B	0.216	0.400[d]	0.294	0.279	0.301	0.365[d]	0.313		
IL-6	0.222	0.228	0.125	0.087	0.501[f]	0.293	0.004	−0.47[e]	
Glucose/insulin	−0.418[c]	0.684[b]	0.511[f]	−0.424[c]	−0.383[d]	−0.606[b]	−0.631[b]	0.149	−0.035

[a] Laboratory measurements were performed at the time of enrollment. apo, apolipoprotein; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor type 1; IL-6, interleukin 6.
[b] $P < 0.001$.
[c] $P < 0.025$.
[d] $P < 0.05$.
[e] $P < 0.01$.
[f] $P < 0.005$.

levels of PAI-1, factor VII, and MCP-1. Interleukin-6 correlated with fibrinogen and PAI-1, suggesting the possibility that acute phase reactions contributed in increasing the plasma levels of these prothrombotic factors. Factor VII correlated with triglyceride (r = 0.43, $P < 0.025$), apo B (r = 0.42, $P < 0.025$), and factor XII (r = 0.51, $P < 0.005$) levels, indicating the possible role of hyperlipoproteinemia in enhancing factor VII levels. Independent predictors of fistula survival were derived using the Cox proportional hazards model. Monocyte chemoattractant protein-1 (chi-square, 10.3; P = 0.001), IL-6 (chi-square, 7.1; P = 0.008), PAI-1 (chi-square, 9.7; P = 0.002), factor VII (chi-square, 6.0; P = 0.014), and triglyceride (chi-square, 13.8; $P < 0.0001$) levels, and the ratios apo A-I/apo C-III (chi-square, 15.0; $P < 0.0001$), apo A-I/apo B (chi-square, 9.1; P = 0.003), total cholesterol/HDL cholesterol (chi-square 9.1; P = 0.003) and glucose/insulin (chi-square, 13.6; $P < 0.0001$) were significant independent predictors of fistula dysfunction, after having adjusted for age, sex, body mass index, and erythropoietin therapy.

DISCUSSION

Maintaining patent vascular access remains one of the most challenging problems in hemodialysis patients. The prevention of vascular access thrombosis must ultimately depend on an early detection of the risk factors. It is clear that stenoses lead to thrombosis and fistula failure, and prevention or correction of venous stenoses dramatically decreases the rate of fistula loss (29,30). However, the causes of stenoses in the venous circuit are poorly understood. In this pilot study, we demonstrated the influence of cytokines, hemostasis-derived risk factors, hyperinsulinemia, and abnormalities of lipids and apolipoproteins on the progressive stenosis and subsequent thrombosis of primary arteriovenous fistula.

VSMC proliferation and migration across the internal elastic lamina to form the neointima is the hallmark of fistula stenosis (31). It has been hypothesized that upstream release of PDGF, or other growth factors, may contribute to the proliferation of VSMC in the intima of the runoff vein (3), however, the interactions between growth factors in the development of neointimal formation have not been completely elucidated so far. Our study provides evidence that elevated levels of IL-6 and MCP-1 are powerful risk indicators for fistula dysfunction. Recent studies *in vitro* have demonstrated that IL-6 has a distinct ability in stimulating VSMC growth in a PDGF-dependent manner (18), and in enhancing the endothelial synthesis of PAI-1 (32). The patients who experienced fistula dysfunction had higher values of IL-6 and PAI-1 compared with those who did not. The potential role of IL-6 in the pathogenesis of fistula dysfunction is very intriguing. Interleukin-6 may contribute to the progressive fistula stenosis, secondary to the neointima formation, through the stimulation of VSMC proliferation. In addition, IL-6 may predispose to thrombosis by enhancing the endothelial synthesis of PAI-1, one of the major inhibitors of fibrinolysis, which acts via the inactivation of both tissue-type and urokinase-type plasminogen activators. It is interesting to note that a high value of PAI-1 is a well-established risk factor for vascular thrombosis in the nonuremic population (28). Other factors may enhance the synthesis of PAI-1 in hemodialysis patients. We found a positive correlation between insulin and PAI-1, and in our study, the patients who experienced progressive stenosis of the fistula had higher plasma insulin levels and lower fasting glucose/insulin ratio. Hyperinsulinemia increases the synthesis and secretion of PAI-1 by hepatocytes and endothelial cells, either by a direct mechanism or via its effects on lipid metabolism (32,33). It has been suggested that hyperinsulinemia,

in the presence of elevated levels of PDGF, enhances VSMC replication, thus contributing to the development of neointimal hyperplasia (31).

In hemodialysis patients, the increased concentrations of IL-1β and TNFα may induce endothelial cells to express prothrombotic activities (11), including production of IL-6 and PAI-1, and expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). The enhanced circulating levels of VCAM-1 and ICAM-1 probably are the result of endothelial activation and increased leukocyte/endothelial cell interactions (34). However, we could not find any significant difference in these parameters between patients with and without fistula dysfunction. Although a role for these adhesion molecules has recently been proposed in atherosclerosis (34), further studies are required to clarify whether they are also involved in the pathogenesis of fistula stenosis.

The association between elevated levels of MCP-1 and fistula dysfunction is intriguing. Monocyte chemoattractant protein-1 is a cytokine produced by endothelial cells in response to IL-1β, TNFα and oxidised lipoproteins (35), whose participation in the atherogenesis has recently been demonstrated (36). Monocyte chemoattractant protein-1 inhibits the proliferation of VSMC *in vitro*, and its effect is independent of prostaglandin or nitric oxide generation (19). One of the functions of endothelial cells is to maintain the mitogenic quiescence of the underlying VSMC in the media. In the process of neointimal formation, leading to fistula stenosis, the excessive proliferation of VSMC is considered a response to endothelial injury (21). The enhanced levels of MCP-1 may result from the activation of endothelial cells. We speculate that MCP-1 secreted from endothelial cells may represent an attempt to counteract neointimal formation via an inhibition of VSMC proliferation. This hypothesis is coincident with the findings by Ikeda and coworkers (19), who suggested that the enhanced secretion of MCP-1 in atherosclerotic lesions prevents the development of atherosclerosis through this growth-inhibiting effect. We are also tempted to speculate that in uremic patients, MCP-1 production may be elicited by alterations in lipid metabolism. This hypothesis is supported by the correlations of MCP-1 with levels of apo C-III, triglycerides, and cholesterol. Moreover, in a primate model of atherosclerosis, dietary-induced hypercholesterolemia resulted in strong expression of MCP-1 by VSMC (37). The association of lipid abnormalities and vascular access thrombosis has already been studied (6,8). Goldwasser and coworkers analyzed the influence of serum Lp(a) on vascular access occlusion, and reported that values $\geq$ 57 mg/dL were associated with an increased risk (8). Our 2-yr prospective study provides evidence that high total cholesterol/HDL-cholesterol ratio, high triglyceride levels, and low values of apoA-I/apo C-III and apo A-I/apo B ratios are powerful risk indicators for fistula dysfunction. Compared with the group without fistula dysfunction, the patients who experienced progressive fistula stenosis showed a more severe hyperlipoproteinemia as indicated by lower values of HDL cholesterol and apo A-I/apo B ratio, and higher values of triglycerides, apolipoprotein B, and total cholesterol/HDL-cholesterol ratio. In addition, these patients had a lower apo A-I/apo C-III ratio that is considered the hallmark of uremic dyslipidemia (38). There was no significant difference in serum Lp(a) between patients with and without fistula dysfunction, but the mean values in both of these groups were approximately twice as high as the mean Lp(a) concentration in the healthy subjects. Serum Lp(a) was not a correlate of vascular access dysfunction but, unlike Goldwasser and colleagues (8), none of our patients had values greater than 57 mg/dL.

Another point that deserves consideration is whether the abnormalities of hemostasis may have a role in the pathogenesis of fistula dysfunction. Recently Hehrlein (21) claimed analogies between the mechanism leading to fistula failure and the process responsible for the restenosis of coronary arteries that occurs after angioplasty. Analyses of the possible involvement of coagulation in restenotic lesions suggest a critical role for thrombin (39,40). Thrombin may be important not only in terms of coagulation but also via its effects on VSMC migration and growth (41). Moreover, activation by thrombin induces the synthesis of PDGF, MCP-1, EDRF by endothelial cells (42,43). In hemodialysis patients, the high values of F_{1+2} indicated an increased generation of thrombin and provided a measure of the enhanced rate of cleavage of prothrombin to thrombin by factor Xa. However, we did not find any significant difference in F_{1+2} between patients with and without fistula dysfunction. Further studies are, therefore, required to clarify the possible role of enhanced thrombin generation in the pathogenesis of fistula stenosis and thrombosis. Compared with healthy subjects, the whole group of hemodialysis patients had a lower value of protein C, a natural coagulation inhibitor whose deficiency is associated with an increased risk of venous thrombosis (44). It is interesting to note that protein C in patients with fistula dysfunction was significantly higher compared with patients without fistula dysfunction, although not different from healthy subjects. In patients with fistula stenosis, this finding might reflect an attempt to counteract the increased levels of PAI-1. Indeed, protein C acts as a profibrinolytic and anticoagulant agent via proteolytic inactivation of PAI-1, factor Va, and VIIIa (44). The activation of the fibrinolytic pathway was an almost universal feature in our patients as indicated by the high levels of D-dimer, a stable end product of crosslinked fibrin degradation.

In our study, elevated levels of factor VII were associated with an increased risk of fistula dysfunction. This finding is very intriguing because factor VII plays an important role in the initiation of tissue factor pathway-induced coagulation, and an increase in fac-

tor VII coagulant activity has been recognized as a cardiovascular risk factor in the general population (28). Activation of factor VII is generally achieved by tissue factor with formation of a factor VIIa/tissue factor complex, which is necessary for rapid proteolytic activation of coagulation factors IX and X (45). Tissue factor is produced by stimulated endothelium. The expression of tissue factor on endothelial cells may be induced *in vitro* by cytokines or by adhesion molecule-dependent interaction with lymphocytes (46). It is conceivable that the marked increase of factor VII in hemodialysis patients may be related to endothelial cell injury. As a matter of fact, recent studies reported an increased plasma tissue factor level in these patients (47,48). Uremic dyslipidemia might also be implicated in the mechanism leading to the increase of factor VII. Apolipoprotein B containing triglyceride-rich lipoproteins may increase the factor VII (49). One mechanism is the hydrophobic adsorption of coagulation protein onto triglyceride-rich lipoprotein particles and subsequent decrease in its fractional catabolic rate (50). Another possibility is that triglyceride-rich lipoproteins first activate factors XII, XI, and IX, which in turn convert the single chain factor VII to the fully activated two-chain form (51). The correlations of factor VII to factor XII, triglycerides and apolipoprotein B may support these mechanisms in our patients.

To our knowledge, a comprehensive approach that explores the impact of serum levels of cytokines, adhesion molecules, insulin, lipids, and hemostatic factors on progressive stenosis, and the subsequent thrombosis of primary arteriovenous fistula, has not been previously described. Clearly, we should be cautious in extending to all dialysis patients the conclusions drawn from a limited population, like that reported in the study presented here. We are now planning to focus on the parameters relevant to the outcome in a more extensive study including patients with arteriovenous fistulas constructed with synthetic material, such as polytetrafluoroethylene, and diabetic patients. Prevention of venous stenosis is an important goal in reducing the risk of fistula thrombosis, but as the underlying pathophysiology is poorly understood, the pharmacologic prevention is relegated primarily to future research (24,52). In light of the results of this study, we are planning a trial to investigate whether drugs that affect cytokines, adhesion molecules, and hemostasis, *i.e.*, antioxidants (53,54), may reduce the risk of arteriovenous fistula dysfunction.

In conclusion, this 2-yr prospective study demonstrates the influence of cytokines, hemostasis-derived vascular risk factors, hyperinsulinemia, and abnormalities of lipids and apolipoproteins on primary fistula survival. The assessment of these factors might be useful for the identification of the patients at risk of fistula stenosis and thrombosis.

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