

Risk Factors Related to Inflammation and Endothelial Dysfunction in the DCCT/EDIC Cohort and Their Relationship With Nephropathy and Macrovascular Complications

MARIA F. LOPES-VIRELLA, MD, PHD¹
 RICKEY E. CARTER, PHD²
 GREGORY E. GILBERT, MS²
 RICHARD L. KLEIN, PHD¹
 MIRAN JAFFA, PHD²
 ALICIA J. JENKINS, MD^{1,3}

TIMOTHY J. LYONS, MD^{1,3}
 W. TIMOTHY GARVEY, MD^{1,4}
 GABRIEL VIRELLA, MD, PHD⁵
 AND THE DCCT/EDIC COHORT STUDY
 GROUP

OBJECTIVE — Because endothelial cell dysfunction and inflammation are key contributors to the development of complications in type 1 diabetes, we studied risk factors related to endothelial dysfunction and inflammation (C-reactive protein and fibrinogen, soluble vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and E-selectin, and fibrinolytic markers) in a subgroup of patients from the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Intervention and Complications (EDIC) study cohort.

RESEARCH DESIGN AND METHODS — We determined which of these risk factors or clusters thereof are associated with the presence of and subsequent development of nephropathy and macrovascular complications (reflected by carotid intima-media thickness [IMT]).

RESULTS — After adjustment for conventional risk factors (age, sex, DCCT treatment group, diabetes duration, A1C, systolic blood pressure, waist-to-hip ratio, total and HDL cholesterol, and smoking status), fibrinogen remained strongly associated with progression of internal and common carotid IMT ($P < 0.01$) and soluble E-selectin had a strong association with nephropathy ($P < 0.01$).

CONCLUSIONS — The best predictor for IMT progression in the DCCT/EDIC cohort was plasma fibrinogen, and the levels of soluble E-selectin discriminate patients with albuminuria better than conventional risk factors.

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Biomarkers are circulating molecules, proteins, or enzymes whose levels provide independent diagnostic or prognostic value for an underlying disease state or for complications thereof (1). Studies over the last decade strongly support the idea that atherosclerosis and di-

abetic retinopathy and nephropathy result from endothelial cell dysfunction followed by lipid accumulation and an inflammatory process affecting both macro- and microvasculature. Early studies focused on quantitative and qualitative lipoprotein changes as biomarkers for the

development of diabetes complications (2). However, although lipoproteins are important biomarkers for vascular risk, they do not completely account for the excess of vascular complications in diabetes. Therefore, more recently, plasma biomarkers of inflammation and endothelial dysfunction have been investigated as possible risk factors for diabetes complications.

The majority of the studies performed to examine these parameters have included relatively small numbers of subjects and had discrepant results. Some studies showed elevation of inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) in patients with nephropathy and other microvascular but not macrovascular complications (3). However, increased levels of these inflammatory markers have also been reported in type 1 diabetic patients without clinical evidence of complications (4).

The most extensive study of risk factors to date was conducted by Schram et al. (5) in a subgroup of patients from the European Diabetes (EURODIAB) Prospective Complications Study. These authors compared diabetic patients with micro- and/or macrovascular complications with diabetic patients who were complication free. They found increased levels of CRP, IL-6, and tumor necrosis factor in the patients with complications. These authors also described a correlation between conventional vascular risk factors, including age, sex, diabetes duration, hypertension, lipid levels, and smoking, with markers of inflammation and endothelial dysfunction. Plasma levels of soluble vascular cell adhesion molecule (sVCAM-1) and soluble E-selectin (sE-selectin) were also strongly and independently associated with inflammatory markers, suggesting that endothelial dysfunction and inflammatory activity (reflected by predominantly nonendothelium-derived markers) are related in type 1 diabetes (5). The above study, however, did not properly examine the association

From the ¹Department of Medicine and Laboratory Services, Medical University of South Carolina and Ralph H. Johnson VA Medical Center, Charleston, South Carolina; the ²Department of Biostatistics, Bioinformatics, and Epidemiology, Medical University of South Carolina, Charleston, South Carolina; the ³Section of Endocrinology, Oklahoma University of Health Sciences Center, Oklahoma City, Oklahoma; the ⁴Department of Nutrition, University of Alabama, Birmingham, Alabama; and the ⁵Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina.

Corresponding author: Maria F. Lopes-Virella, virellam@muscc.edu.

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of complications with inflammation/endothelial dysfunction markers as no adjustment for conventional risk factors was performed, and micro- and macrovascular complications were grouped.

The current literature does not provide a clear picture of the value of biomarkers as predictors of diabetes complications, although some articles provide a basis for selecting biomarkers more likely to provide useful information, such as CRP (1,6–10) and fibrinogen levels (8,11–13), endothelial dysfunction markers (sVCAM-1, soluble intracellular adhesion molecule [sICAM-1], and sE-selectin) (7,12,14,15), and the modulators of fibrinolysis, plasminogen activator inhibitor 1 (PAI-1) (16), and tissue type-specific plasminogen activator (tPA) (17).

Thus, we studied parameters of inflammation and endothelial dysfunction in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Intervention and Complications (EDIC) cohort, a large well-characterized cohort of patients with type 1 diabetes, to determine which parameter or cluster of parameters are associated with the presence of nephropathy and macrovascular complications. Because the number of events related to macrovascular disease in the cohort was rather small, carotid intima-media thickness (IMT) was used as a surrogate. Data obtained in the DCCT/EDIC study have shown that carotid IMT is a sensitive marker for coronary and cerebrovascular disease in patients with type 1 diabetes (18). We chose CRP and fibrinogen as inflammatory markers and sICAM-1, sVCAM-1, sE-selectin, tPA, and PAI-1 as markers of endothelial dysfunction.

RESEARCH DESIGN AND METHODS

The DCCT enrolled and followed 1,441 patients with type 1 diabetes between 1983 and 1993 to determine whether intensive diabetes management would prevent complications (19). The study was stopped in June 1993 because it was conclusively shown that intensive management of type 1 diabetes delayed development and progression of retinopathy, neuropathy, and nephropathy. The DCCT was followed by the EDIC study, which is a noninterventional epidemiologic investigation designed to study the development of vascular diseases in patients who were part of the DCCT. EDIC has documented the development and progression of macrovascular disease and nephropathy through

repeated measurements of carotid IMT (macrovascular disease) and serum creatinine, estimated glomerular filtration rate, and albumin excretion rate (AER) (nephropathy) since its inception in 1994. In 1996 a Program Project Grant (PPG) was obtained by the Medical University of South Carolina to perform ancillary studies on the DCCT/EDIC cohort. A total of 1,063 EDIC participants agreed to participate in the PPG study. The first blood and urine collection for the PPG study, obtained in 1997–1999, was used to measure the inflammation/endothelial cell dysfunction parameters reported in this article, and these measurements were linked with clinical parameters from the DCCT/EDIC cohort, including history of clinical events and risk behaviors (such as smoking) and physical examination, as well as blood pressure, routine electrocardiograms, carotid IMT, lipid levels, A1C, and AER. The study was approved by the institutional review boards of all participating institutions, and each participant gave written informed consent.

Quantitation of biomarkers

Serum levels of sICAM-1, sVCAM-1, and sE-selectin were determined using SearchLight Proteome Arrays (Pierce Biotechnology, Rockford, IL) using Array-Vision software for data analysis. Interassay coefficients of variation (CVs) for sICAM-1, sVCAM-1, and sE-selectin were, respectively, 10.7, 10.6, and 5%, and intra-assay CVs were, respectively, 4.5, 4.3, and <2%. Plasma concentrations of fibrinogen were determined using a commercially available assay using an ST4 agglutinator (Diagnostica Stago) as described previously (11). Inter- and intra-assay CVs were 6.4 and 3.9%, respectively. Serum CRP was measured by high-sensitivity immunonephelometry with intra- and interassay CVs of <11 and <2% as described (20). Quantitation of tPA antigen and activity were determined using commercially available enzyme immunoassays (TintElize tPA and Chromolize tPA, respectively; Trinity Biotech, St. Louis, MO). Interassay CVs for the two assays averaged 6.6 and 5.3%, respectively, whereas the intra-assay CVs averaged 4.1 and 4.0%, respectively. PAI-1 antigen and activity levels were determined using commercially available enzyme immunoassays (TintElize PAI-1 and Chromolize PAI-1, respectively; Trinity Biotech). The interassay CVs for these two assays averaged 8.8 and 10.8%, whereas the intra-assay CVs averaged 4.7 and

3.5%. We performed masked determinations in 69 to 86 duplicate samples from EDIC patients and the coefficients of reliability (calculated by EDIC) were 0.938 for sVCAM-1, 0.918 for sVCAM-1, 0.946 for PAI-1, 0.926 for fibrinogen, and 0.999 for CRP.

Statistical analysis

Outcome variables. According to the EDIC protocol, renal assessment and blood collection were performed in alternate years. Thus, AER was calculated as the average of measurements performed on urine specimens collected within the 2-year period around the time of collection of blood samples used for biomarker assays. Patients with AER <40 mg/24 h were considered to have normal urine albumin levels; those with AER \geq 40 mg/24 h were classified as having abnormal urine albumin levels.

Macrovascular disease was assessed by internal and common carotid IMT assessed by ultrasonography between 1994 and 1996, at approximately year 6 of EDIC and 3 years before the collection of the samples used for this report (21). Corresponding year 1 IMT measurements for internal carotid and common carotid were used as covariables to assess progression of IMT. A description of IMT measurements was detailed earlier (21,22)

Modeling process. A multistage modeling process was used to test the primary hypothesis that inflammation/endothelial dysfunction markers predict diabetes complications after adjustment for known risk factors. The first stage of analysis was an univariate analysis in which each outcome variable was associated with each candidate marker. The candidate biomarkers were as follows: sVCAM-1, sICAM-1, sE-selectin, PAI-1 activity and mass, tPA activity and mass, CRP, and fibrinogen. The natural logarithms tPA mass, PAI-1 activity and mass, sE-selectin, and sVCAM-1 were taken because of highly skewed levels. All regression models controlled for age, sex, DCCT treatment group (intensive and conventional randomization groups), diabetes duration, concurrent A1C, systolic blood pressure, waist-to-hip ratio, total and HDL cholesterol levels, and smoking status after initial univariate associations were examined in stage 1.

During the latter stages of the analysis, biomarkers were grouped for analysis according to their perceived biological function. Three subgroups were identi-

fied: 1) adhesion molecules, 2) biomarkers of fibrinolysis, and 3) inflammatory mediators (fibrinogen and CRP). For fibrinolysis, two different models were created (one with mass measurements and another with activity measurements). Mass and activity measurements of PAI-1 were not included together in the same model as they are highly correlated ($r = 0.49$) and thus would contribute to multicollinearity in the model. Similarly, the mass and activity measures of tPA were not included together in the models. Each subgroup of variables was entered into regression models separately. Collinearity within a biological domain was assessed by the variance inflation factors; no significant collinearity was detected. Partial F testing (or likelihood ratio testing) was used to determine whether addition of a biomarker(s) significantly enhanced outcome prediction over conventional variables.

All sets of multivariable regression models that examined associations between investigational variables and nephropathy, internal IMT, and common carotid IMT after adjustment for conventional risk factors were constructed, and optimal models were chosen on the basis of partial F testing and likelihood ratio testing. Investigational variables were added by category (e.g., inflammation, fibrinolysis variables [activity or mass], and adhesion molecules).

To measure the strength of the associations in the logistic regression models, the Nagelkerke's pseudo- R^2 (23) and Harrell's C statistic (24) were used. A value of 0.5 represents a chance prediction, values >0.8 are considered discriminative, and the discrimination of the model is improved as the C value approaches 1.0.

All data management and analyses were done using the SAS system (version 9.1.3; SAS Institute, Cary, NC). The type I error rate was determined to be 0.05 a priori, and no correction for multiple comparisons was applied to the reported P values.

RESULTS— At the time of blood sample collection, the mean \pm SD age of the patients was 39 ± 7 years and 55% were male. Duration of diabetes was 17.5 ± 4.8 months and A1C was $8.2 \pm 1.3\%$. BMI was 27.2 ± 4.2 kg/m² and natural waist-to-hip ratio was 0.8 ± 0.1 . Systolic and diastolic blood pressures were 120 ± 14 and 75 ± 9 mmHg. Total, HDL, and LDL cholesterol levels were, respectively, 189.0 ± 35.3 , 56.0 ± 14.7 , and 115.0 ± 30.5 mg/dl. Tri-

Table 1—Descriptive statistics of demographic variables, covariates, and outcome variables

	Sample size	Mean \pm SD	Interquartile range
Conventional risk factors			
Age (years)	1,063	39.2 \pm 7.0	10.0
DCCT experimental group (%)	1,063	51	—
Type 1 diabetes duration (years)	1,063	17.5 \pm 4.8	7.7
A1C (% of hemoglobin)	1,045	8.2 \pm 1.3	1.7
Male sex (%)	1,063	55	—
Smoker (%)	1,042	19	—
Systolic blood pressure (mmHg)	1,045	120.0 \pm 13.9	18.0
Diastolic blood pressure (mmHg)	1,045	75.0 \pm 9.3	12.0
Height (cm)	1,046	172.5 \pm 9.4	13.7
Weight (kg)	1,046	81.3 \pm 15.5	21.7
BMI (kg/m ²)	1,044	27.2 \pm 4.2	5.1
Natural waist-to-hip ratio	1,045	0.8 \pm 0.1	0.1
Total cholesterol (mg/dl)	1,027	189 \pm 35.3	44.0
HDL cholesterol (mg/dl)	1,027	56 \pm 14.7	19.0
LDL cholesterol (mg/dl)	1,021	115 \pm 30.5	39
Triglycerides (mg/dl)	1,027	89 \pm 63.3	51.0
Biomarkers			
PAI-1 activity (IU/ml)	928	4.5 \pm 6.9	4.5
PAI-1 mass (ng/ml)	990	32.2 \pm 24.8	30.6
sE-Selectin (ng/ml)	752	58.7 \pm 35.7	38.4
sICAM-1 (ng/ml)	812	332.4 \pm 123.7	158.6
sVCAM-1 (ng/ml)	782	685.8 \pm 442.4	418.1
tPA activity (IU/ml)	988	1.0 \pm 0.5	0.5
tPA mass (ng/ml)	964	6.3 \pm 5.0	4.3
Fibrinogen (mg/dl)	911	309.5 \pm 106.9	128.0
CRP (mg/l)	951	3.1 \pm 4.6	3.0
Outcomes			
AER (mg/24 h)	1,020	87 \pm 441.7	14.4
Micro-/macroalbuminuria (AER >40 mg/24 h) (%)	1,020	14	—
Internal IMT year 6 (units)	958	0.7 \pm 0.3	0.1
Common IMT at year 6 (units)	970	0.6 \pm 0.1	0.2

glyceride levels were 89.0 ± 63.3 mg/dl. In some patients collection of blood for biomarkers was not performed or was incomplete in 1997–1999, and, therefore, some biomarkers were assayed in a reduced number of samples. Not all outcome variables were obtained for a few patients. Conventional risk factors for 1997–1999 in all patients enrolled in the PPG as well as descriptive statistics of the outcomes and markers examined in this study are summarized in Table 1.

Logistic and linear regression analyses between the investigational variables, AER, and internal and common carotid IMT without adjustment for any other variables were performed (see supplemental Table A1 of the online appendix available at <http://dx.doi.org/10.2337/dc08-0659>). A significant positive association of PAI-1 activity with both internal

($P < 0.01$) and common carotid IMT ($P < 0.01$) and a negative association of tPA activity and internal carotid IMT ($P < 0.01$) were observed. tPA mass was associated with nephropathy ($P = 0.03$) as well as with internal ($P < 0.01$) and common ($P < 0.01$) carotid IMT. Of the adhesion molecules, sE-selectin was significantly associated with nephropathy ($P < 0.01$), and sICAM-1 was associated with internal carotid IMT ($P = 0.03$). No significant associations of fibrinogen and CRP levels with nephropathy were observed ($P = 0.13$ and $P = 0.77$, respectively). A strong association with internal and common carotid IMT was observed for fibrinogen ($P < 0.01$ for both IMT measurements) but not for CRP ($P = 0.54$ and $P = 0.84$; respectively). Because CRP was not associated by simple logistic and linear regression analysis with either

Table 2—Significance of the association of endothelial cell dysfunction and inflammation markers with nephropathy and cardiovascular disease in the presence of conventional risk factors

Biological domain	Nephropathy			Internal carotid IMT (year 6)			Common carotid IMT (year 6)		
	LRT	d.f.	P	Partial F test	d.f.*	P	Partial F test	d.f.*	P
Fibrinolysis (activity)	2.62	2	0.27	1.76	2, 756	0.17	4.43	2, 777	0.01
Fibrinolysis (mass)	0.17	2	0.92	0.47	2, 790	0.62	0.09	2, 817	0.91
Adhesion molecules	10.25	3	0.02	1.03	3, 605	0.38	0.07	3, 628	0.98
Inflammation	0.44	1	0.51	12.72	1, 762	<0.01	7.90	1, 787	<0.01

d.f., degree of freedom. *Numerator, denominator.

macrovascular complications or with nephropathy, CRP was not entered in the multivariate linear or logistic models that examined associations between the investigational variables and micro- and macrovascular complications.

Table 2 summarizes the significance of the association of each group of variables studied with nephropathy and internal and common carotid IMT (year 6) after adjustment for conventional cardiovascular disease (CVD) risk factors. Table 2 shows a statistically significant association ($P = 0.02$) of nephropathy with the set of adhesion molecules, after adjust-

ment for conventional CVD risk factors, as well as a significant association ($P = 0.01$) of fibrinogen with progression of internal and common carotid IMT (year 6), after adjustment for conventional CVD risk factors. The association between outcomes and biomarkers was not improved by combining biomarkers from different domains.

Table 3 summarizes the multivariate logistic and linear regression analyses examining the relationship of all biomarkers studied and the vascular outcomes (nephropathy assessed by AER and internal and common carotid IMT progression).

As seen in Table 3, sE-selectin shows strong evidence for an association with nephropathy, even after correction for conventional risk factors (total and HDL cholesterol, smoking, age, sex, A1C, blood pressure, and waist-to-hip ratio), duration of diabetes, and DCCT randomization group ($P < 0.01$). sE-selectin enhanced discrimination over that provided by conventional risk factors of patients with abnormal AER from patients with normoalbuminuria (C statistic increased from 0.80 to 0.84). Adding either fibrinolytic factors or acute inflammatory markers such as fibrinogen to sE-selectin

Table 3—Final models of multivariate logistic and linear regression analysis

Outcome, method, and model*	β †	SE (β)	β_{STD} †	P	Sample size			Coefficient of determination			Concordance index		
					M1	M2	M3	M1	M2	M3	M1	M2	M3
Nephropathy													
Logistic	0.72	0.25	0.21	<0.01	987	711	690	0.24	0.08	0.32	0.80	0.68	0.84
sE-Selectin (ng/ml)**													
sVCAM-1 (ng/ml)**	-0.26	0.26	-0.07	0.31									
sICAM-1 (ng/ml)**	-0.40	0.36	-0.08	0.26									
Internal IMT (year 6)													
Linear													
Fibrinogen (mg/dl)	0.09	0.03	0.10	<0.01	897	825	775	0.43	0.02	0.41	—	—	—
Common IMT (year 6)													
Linear													
PAI-1 Activity (IU/ml)**	0.01	0.00	0.06	0.05	927	823	791	0.37	0.02	0.37	—	—	—
tPA Activity (IU/ml)**	0.02	0.01	0.09	<0.01									
Linear													
Fibrinogen	0.02	0.01	0.08	<0.01	927	834	800	0.37	0.02	0.38	—	—	—

*Estimates are adjusted for age (years), treatment group (reference: standard treatment), duration of type 1 diabetes (years), A1C (percentage), sex (reference: male), smoking status (reference: smoker), systolic blood pressure (reference: millimeters of mercury), natural waist-to-hip ratio, HDL cholesterol (milligrams per deciliter), and total cholesterol (milligrams per deciliter). IMT outcomes at year 6 are also adjusted for corresponding IMT readings at year 1. †The reported values are the change in the log odds for a 1-unit change (unstandardized β values) or a 1 SD change (standardized β values) in the biological parameter (for logistic regression models) or the change in IMT for either a 1-unit change (unstandardized) or 1 SD change (standardized) for linear regression models. Odds ratios for the same change can be obtained by exponentiating the estimated β values. For example, a 1 SD change in the natural logarithm of PAI-1 activity (International Units per milliliter) increases the odds of nephropathy by 1.0602 ($P = 0.4013$). ‡Only applicable to the logistic model. §Nagelkerke's pseudo- R^2 is reported for the logistic model and the adjusted R^2 for the linear regression models. Regression model 1: composed of conventional risk factors: age (years), treatment group (reference: standard treatment), duration of diabetes (years), A1C (percentage), sex (reference: male), smoking status (reference: smoker), systolic blood pressure (reference: millimeters of mercury), natural waist-to-hip ratio, HDL cholesterol (milligrams per deciliter), and total cholesterol (milligrams per deciliter). IMT outcomes at year 6 are also adjusted for corresponding IMT readings at year 1. This model is denoted as M1 for sample size, coefficient of determination, and concordance index. Regression model 2: composed of only the specified biomarkers. This model is denoted as M2 for sample size, coefficient of determination, and concordance index. Regression model 3 (M3): models 1 and 2 combined. **Natural log transformed.

did not enhance the potential for discrimination of sE-selectin. Additive or synergistic interactions with other adhesion molecules were not observed (data not shown). Thus, the final predictive model for nephropathy consisted of the main effects of the three adhesion molecules.

Multivariable linear regression investigating the association of biomarkers and internal and carotid IMT incorporated, as correction factors, the conventional risk factors mentioned for nephropathy and the measurements performed for IMT at year 1 of EDIC. This way progression between the measurements of years 1 and 6 could be assessed. Interestingly some of the associations were strongly affected by the correction by conventional risk factors. Only fibrinogen remained associated with year 6 internal carotid IMT ($P < 0.01$), but fibrinogen levels ($P < 0.01$), PAI-1 activities ($P = 0.05$), and tPA activity ($P < 0.01$) remained associated with year 6 common carotid IMT. Adding different biomarkers did not strengthen the association of the levels of single biomarkers with internal carotid or common carotid IMT progression.

CONCLUSIONS— The identification of inflammation and endothelial dysfunction markers associated with the development of nephropathy and CVD in patients with type 1 diabetes has considerable clinical implications. This study is the first conducted on a large cohort of type 1 diabetic patients (1,063 patients from the well-characterized DCCT/EDIC cohort) that addresses the association of these markers to the development of complications.

Simple logistic and linear regression analyses of our data suggested that the levels of fibrinogen, soluble cell adhesion molecules, and PAI-1 and both tPA activity and tPA mass are the most promising biomarkers to assess the risk for development of complications in type 1 diabetic patients. On the other hand, CRP levels were not associated with either macro- or microvascular complications in this cohort of type 1 diabetic patients. Based on these results, we performed additional statistical studies exploring the correlation of fibrinolytic factors (activity and mass), fibrinogen, and cell adhesion molecules with the development of complications after adjustment for conventional risk factors.

After the adjustment for conventional risk factors, the levels of sVCAM-1, sICAM-1, and sE-selectin, as a group,

showed a statistically significant association with nephropathy, in agreement with several published reports. Such associations had been reported previously in other smaller cross-sectional studies, in which sICAM-1 levels were reported to be significantly increased in patients with type 1 diabetes compared with nondiabetic subjects and in diabetic patients with complications compared with those without (7,12) and in a larger study ($n = 540$), in which sVCAM-1 and sE-selectin levels had positive associations with albuminuria and CVD (14). Costacou et al. (15) also found sE-selectin to be an independent predictor of coronary heart disease in a case-control study of patients with type 1 diabetes from the Pittsburgh Epidemiology of Diabetes Complications study, but we did not observe such an association. In agreement with our negative results, Leinonen et al. (25) reported in patients with type 2 diabetes that levels of soluble cell adhesion molecules, although elevated, do not correlate with internal carotid IMT or with clinical CVD (25).

The EURODIAB Prospective Complications Study recently investigated two determinants of inflammatory activity, conventional risk factors for atherothrombosis and plasma concentrations of sVCAM-1 and sE-selectin, as indexes of endothelial dysfunction. The markers of inflammatory activity (CRP, IL-6, and tumor necrosis factor) were associated with conventional risk factors including sex, diabetes duration, level of glycemic control, BMI, HDL cholesterol (inversely), triglycerides, and systolic blood pressure. Plasma levels of sVCAM-1 and sE-selectin were also strongly and independently associated with these inflammatory markers, suggesting that endothelial dysfunction and inflammatory activity are related events in type 1 diabetes (5). However, because inflammation is strongly linked with conventional risk factors as well as with biomarkers of endothelial cell dysfunction in type 1 diabetes when all factors are taken into consideration, the value of the biomarkers of endothelial cell dysfunction to predict cardiovascular complications is not superior to that of conventional risk factors.

The value of inflammation markers as predictors of macro- or microvascular complications in diabetic patients is not clear, particularly in the case of CRP, which did not show any correlation with micro- or macrovascular disease in our study. These results are in keeping with

our cross-sectional study of DCCT-EDIC participants, in which serum CRP levels were correlated with BMI and glycemia but were not associated with micro- or macrovascular complications (20). Although CRP has been heralded as the optimal biomarker for improving vascular risk prediction (1), the data for type 1 diabetes are contradictory. Several groups have reported elevated CRP levels in diabetic patients (6,7), whereas others failed to reproduce this finding (8). Some studies have shown a correlation between high CRP levels with increased IMT of the carotid artery (9). Other studies reported that CRP is independently associated with development of diabetic nephropathy (6,10). However, elevated CRP levels have also been detected in patients with type 1 diabetes without evidence of macrovascular disease (7), and other studies have failed to show a significant correlation between CRP levels and small vessel vasculitis (13). Further, these studies failed to demonstrate that CRP levels are an independent marker for the development of vascular complications or nephropathy in patients with type 1 diabetes (8,12) in agreement with our results.

Fibrinogen levels have been shown to be associated with glomerular basal membrane thickening and to be increased in patients with diabetic nephropathy after correction of other markers of inflammation (8). However, it has also been reported that the increase in fibrinogen levels observed in type 1 diabetic patients with microvascular complications loses significance when the analysis is adjusted for diabetes duration and glycemic control (12). A previous study performed in a group of 909 patients from the DCCT/EDIC cohort showed significant correlations between fibrinogen levels and urinary AER in men ($r = 0.13$, $P = 0.003$) and a low average brachial index in women ($r = -0.13$, $P = 0.00$) (11). In the present study, fibrinogen levels had a strong predictive value for the development of macrovascular disease, showing the strongest association with progression of internal carotid IMT, whereas a weaker association was seen with PAI-1 and tPA activities. Other groups had reported that patients with type 1 diabetes of <1 year duration had higher levels of tPA than those with duration of diabetes of >1 year, suggesting that endothelial dysfunction is a very early event in type 1 diabetes (17). PAI-1 activity has also been reported to be decreased in patients with type 1 diabetes (16). It is possible that these cor-

relations were lost in our population, given that the mean duration of diabetes at admission to the original DCCT trial was 5.6 years.

In summary, the measurement of plasma fibrinogen levels and of the levels of soluble cell adhesion molecules are strongly correlated with the presence or development of vascular disease and diabetic nephropathy in the DCCT/EDIC cohort of type 1 diabetic patients. However, our study, as with previously published studies, has limitations because it is mostly cross-sectional with a very narrow period of follow-up for IMT, and, therefore, the predictive value of our findings needs to be confirmed and validated. Studies that we have now initiated will investigate the predictive power of these markers by measuring them in samples collected at enrollment into the DCCT/EDIC cohort and in this way clearly defining whether the measurements can predict the development of complications 10–20 years later.

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