

Baseline Markers of Inflammation Are Associated With Progression to Macroalbuminuria in Type 1 Diabetic Subjects

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OBJECTIVE—The current study aimed to determine in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications cohort whether or not abnormal levels of markers of inflammation and endothelial dysfunction measured in samples collected at DCCT baseline were able to predict the development of macroalbuminuria.

RESEARCH DESIGN AND METHODS—Levels of inflammation and endothelial cell dysfunction biomarkers were measured in 1,237 of 1,441 patients enrolled in the DCCT study who were both free of albuminuria and cardiovascular disease at baseline. To test the association of log-transformed biomarkers with albuminuria, generalized logistic regression models were used to quantify the association of increased levels of biomarkers and development of abnormal albuminuria. Normal, micro-, and macroalbuminuria were the outcomes of interest.

RESULTS—In the logistic regression models adjusted by DCCT treatment assignment, baseline albumin excretion rate, and use of ACE/angiotensin receptor blocker drugs, one unit increase in the standardized levels of soluble E-selectin (sE-selectin) was associated with an 87% increase in the odds to develop macroalbuminuria and one unit increase in the levels of interleukin-6 (IL-6), plasminogen activator inhibitor 1 (PAI-1; total and active), and soluble tumor necrosis factor receptors (TNFR)-1 and -2 lead to a 30–50% increase in the odds to develop macroalbuminuria. Following adjustment for DCCT baseline retinopathy status, age, sex, HbA_{1c}, and duration of diabetes, significant associations remained for sE-selectin and TNFR-1 and -2 but not for IL-6 or PAI-1.

CONCLUSIONS—Our study indicates that high levels of inflammatory markers, mainly E-selectin and sTNRF-1 and -2, are important predictors of macroalbuminuria in patients with type 1 diabetes.

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Nephropathy has been recognized as a major cause of morbidity and mortality in diabetes (1). Overt nephropathy is usually preceded by increased levels of albumin in the urine (2,3). Microalbuminuria is associated not only with risk of developing renal insufficiency (4,5) but also cardiovascular

disease (6) in patients with diabetes. Therefore, there is considerable interest in determining the mechanisms responsible for albuminuria and in identifying early biomarkers that may be predictive of this complication of diabetes.

The pathological mechanism(s) responsible for the development and progression of albuminuria in diabetic patients are poorly understood. Several metabolic and hormonal intermediates have been proposed as important mechanisms responsible for initiating glomerular disease in diabetes (7). In this context, there is strong evidence implicating abnormalities in endothelial function and inflammation as early events leading to diabetes-related renal disease (8–12).

Schram et al. (10) compared diabetic patients with micro- and/or macrovascular complications with diabetic patients who were complication-free in a subgroup of participants from the EURODIAB Prospective Complications Study. They found that the combination of increased levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor (TNF) was associated with albuminuria, retinopathy, and cardiovascular disease (10,11). These authors also reported that plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin (sE-selectin), markers of endothelial dysfunction, were strongly and independently associated with inflammatory markers, suggesting that endothelial dysfunction and inflammatory activity are closely related in type 1 diabetes mellitus. However, in the EURODIAB Study, the authors did not find a significant association between albuminuria and endothelial dysfunction markers.

In a previous cross-sectional study of a subgroup of patients 8–16 years after enrollment in the Diabetes Control and Complications Trial (DCCT), we examined the association of risk factors related to endothelial dysfunction and inflammation, including CRP and fibrinogen, sVCAM-1, intracellular adhesion

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molecule-1 (ICAM-1), E-selectin, and fibrinolysis markers, with diabetic nephropathy. We found that after adjusting for conventional risk factors (age, sex, DCCT treatment group, diabetes duration, HbA_{1c}, systolic blood pressure, waist-to-hip ratio, total and HDL cholesterol [HDL-C], and smoking status), sE-selectin was strongly associated with abnormal albuminuria (8).

The objective of the present prospective evaluation was to confirm and expand our previous observations and determine whether or not DCCT baseline values of markers of inflammation and endothelial dysfunction would be associated with the subsequent development of albuminuria. In addition to the traditional markers of inflammation (CRP, IL-6, and fibrinogen) we measured soluble TNF receptors (sTNFR)-1 and -2 as well as soluble ICAM-1 (sICAM-1), sVCAM-1, and sE-selectin, which are markers of endothelial dysfunction. We also assessed the possible predictive value of both total and active plasminogen activator inhibitor 1 (PAI-1), an important component in fibrinolysis (13).

RESEARCH DESIGN AND METHODS

The DCCT was a randomized controlled trial of 1,441 patients who were 13–39 years of age and had type 1 diabetes for 1–15 years at study entry (2). Subjects in the primary prevention cohort were retinopathy-free (Early Treatment Diabetic Retinopathy Study score of 1), had diabetes for 1–5 years duration, and did not have microalbuminuria (<40 mg/24 h). The subjects in the secondary intervention cohort had mild to moderate nonproliferative diabetic retinopathy (Early Treatment Diabetic Retinopathy Study score of 2–9), had diabetes for 1–15 years, and albumin excretion rates (AERs) ≤200 mg/24 h. The participants were randomized to intensive or conventional insulin therapy and followed for an average of 6.5 years before the study stopped in 1993 because of the beneficial impact of intensive therapy on microvascular complications. In 1994, ~95% of the DCCT participants were enrolled into an observational study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study. The goal of EDIC was to assess the development of more advanced microvascular and macrovascular complications in type 1 diabetes (14). During EDIC, diabetes care was transferred from DCCT to the patients' health care provider, and implementation

of intensive insulin therapy was recommended. At DCCT baseline, each participant completed a physical examination, medical history, electrocardiogram, and routine laboratory analysis that included serum creatinine, lipid profile, and HbA_{1c} (14). Four-hour urine collections for measurement of AER and creatinine clearance were also obtained during EDIC on alternate years (14,15).

The current study was performed to determine whether DCCT baseline markers of inflammation and endothelial dysfunction would be associated with the development of albuminuria. Participants included in the study did not have microalbuminuria (<40 mg/24 h) (AER) at DCCT baseline and had sufficient volume in stored specimens to perform the measurements. Outcomes of interest were defined as persistent normal AER (all AER <40 mg/24 h); incident microalbuminuria (40 mg/24 h ≤ AER <300 mg/24 h) and macroalbuminuria (AER ≥300 mg/24 h). Of a total of 1,237 patients, 404 (32.7%) of them were considered to have developed abnormal albuminuria (micro-/macroalbuminuria) during the study period, met sampling criteria, and were included in this analysis.

Samples

Fasting serum samples obtained during DCCT/EDIC were sent to the DCCT/EDIC central laboratory for standard lipid analysis. Aliquots of these samples were archived for future research purposes. In 1999–2000, as part of a Medical University of South Carolina Program Project Grant funded by the National Institutes of Health/Juvenile Diabetes Research Foundation, serum samples collected during DCCT were provided by the DCCT/EDIC Coordinating Center and NIDDK to complement the serum samples collected during EDIC. The serum samples had been stored at –70°C, and refreezing effects were minimized by preparing aliquots of the serum when thawed for the first time and using a new frozen aliquot for each new test performed. The Institutional Review Board at the Medical University of South Carolina and all participating DCCT/EDIC centers approved the sample collection procedures. Written informed consent was obtained from all participants.

Biomarker assays

Serum levels of CRP, PAI-1 total and active, sICAM-1, sVCAM-1, sE-selectin, IL-6, sTNFR-1, and sTNFR-2 were assayed

using the Signature Plus Protein Array imaging and Analysis System (Aushon Biosystems) using ArrayVision software for data analysis. Coefficients of variation were, respectively, 2.6% for CRP, 3.4% for PAI-1 total, 5.9% for PAI-1 active, 3% for sICAM-1, 4% for sVCAM-1, 4% for sE-selectin, 7.5% for IL-6, 5.9% for sTNFR-1, and 2.7% for sTNFR-2. Plasma concentrations of fibrinogen were determined in a Beckman IMMAGE 800 Immunochemistry Analyzer (Beckman Coulter) using the Fibrinogen SPQ II Test System (Diagnostica Stago S.A.S, Asnieres sur Seine, France). The coefficient of variation of this assay was 5.6%.

Statistical analysis

The concentrations of markers of inflammation and endothelial dysfunction were measured at DCCT baseline and used to determine whether increases in these measures could predict elevated risk to develop albuminuria. All biomarker levels were assessed for normality and transformed when necessary.

Following data normalization, all biomarkers were standardized, and the analysis results represent the association between a change of 1 SD in each biomarker and the odds to develop macroalbuminuria.

The analysis results were determined for one unit change of each measure. Baseline covariates for the current analyses were obtained from DCCT baseline history, physical examination, and laboratory data (fasting lipids and renal function). Model end points were persistent normal AER (all AER <40 mg/24 h); incident microalbuminuria without further progression to macroalbuminuria (40 mg/24 h ≤ AER <300 mg/24 h); and incident macroalbuminuria (AER ≥300 mg/24 h). Standard descriptive statistics were used to summarize the general demographic and clinical data. A linear trend test was used to evaluate continuous baseline demographic and clinical measures across albuminuria outcomes; the Cochran-Armitage trend test was used to assess the relationship for categorical variables. Correlations between the biomarkers and known microvascular predictors (AER, triglycerides, HbA_{1c} percentage, serum creatinine, HDL-C, LDL-cholesterol [LDL-C], and age) at DCCT baseline were done using Spearman correlation coefficients. An unadjusted linear trend test was used to examine a possible dose-response relationship between baseline biomarker levels and the albuminuria

severity classification. Parallel pairwise comparisons of biomarker levels were also assessed between albuminuria severity groups.

Generalized logistic regression models were used to quantify the association of increased baseline biomarker levels on the subsequent development of micro- and macroalbuminuria. The primary parameter of interest in the logistic regression models was the change in the log-odds (with 95% Wald CI) for the progression to micro- or macroalbuminuria as compared with those that remained normal throughout follow-up for the main effect of baseline biomarker levels; initial design models were adjusted for DCCT randomized treatment, baseline levels of AER, and the use of any ACE/angiotensin receptor blocker (ARB) drugs during the study. Covariate adjusted models additionally contain baseline retinopathy cohort, sex, and baseline measures of age, HbA_{1c} percentage, AER, and diabetes duration. Covariates were

selected based on study design, significance within the model, or modification of one or more of the biomarkers effect on the development of albuminuria. Levels of baseline lipids (LDL-C, HDL-C, and triglycerides) and baseline blood pressures (systolic blood pressure and diastolic blood pressure) were individually and jointly entered into the covariate adjusted models; all were found to be highly insignificant, did not significantly modify the relationship between the biomarkers and development of abnormal albuminuria, and thus were omitted from the final parsimonious model. To further assess the relationship between baseline biomarker levels and the development of macroalbuminuria, Cox proportional hazard regression models were developed for both design and covariate adjusted models. For the models evaluating the time to development of macroalbuminuria, the effects of ACE/ARB therapy at any time leading up to progression or censor time is entered into the model

as a time-varying covariate. Modifying effects of DCCT treatment group, baseline retinopathy cohort, and sex on the effects of the biomarker levels were examined in all models.

All statistical analyses were performed using the SAS System version 9.3 (SAS Institute). The type I error rate was controlled at 0.05 for all analysis.

RESULTS—The concentrations of markers of inflammation and endothelial dysfunction were collected at DCCT baseline for all 1,441 subjects randomized in the study. Of these subjects, 1,237 (85.8%) had normal AER values at DCCT baseline (<40 mg/24 h) and were included in the analysis. Demographic and clinical differences between persistently normal subjects and those that developed micro- or macroalbuminuria are summarized in Table 1. At DCCT baseline, the mean age of the study cohort was 27.0 ± 7.1 years with an average duration of diabetes of 5.4 ± 4.1 years, 649

Table 1—Clinical and demographic characteristics by albuminuria status during the follow-up period

Demographics	Overall (n = 1,237)	Albuminuria			P value
		Normal (n = 833)	Micro- (n = 304)	Macro- (n = 100)	
Baseline characteristics					
Age (years)	27.0 (7.1)	27.6 (6.7)	25.6 (7.6)	26.0 (7.8)	<0.001
Male [% (n)]	52.5 (649)	53.5 (446)	45.7 (139)	64.0 (64)	0.818
Intensive treatment (%)	49.9 (617)	54.9 (457)	46.7 (142)	18.0 (18)	<0.001
Primary prevention cohort (%)	53.4 (660)	56.8 (473)	47.7 (145)	42.0 (42)	<0.001
Duration of T1DM (years)	5.4 (4.1)	5.1 (4.0)	6.0 (4.3)	6.0 (3.7)	<0.001
BMI (kg/m ²)	23.3 (2.8)	23.3 (2.7)	23.1 (2.9)	24.2 (2.9)	0.106
Weight (kg)	69.0 (11.9)	69.4 (11.5)	67.4 (12.4)	71.3 (13.1)	0.857
Diastolic blood pressure (mmHg)	72 (8.8)	72 (9.0)	72 (8.6)	74 (8.3)	0.734
Systolic blood pressure (mmHg)	114 (11.5)	114 (11.6)	113 (11.3)	116 (10.9)	0.437
Total cholesterol (mg/dL)	176 (33)	176 (33)	174 (33)	182 (33)	0.271
HDL-C (mg/dL)	51 (12)	51 (13)	50 (12)	48 (13)	0.026
LDL-C (mg/dL)	109 (29)	109 (29)	108 (29)	115 (29)	0.374
Triglycerides (mg/dL)	79 (46)	76 (43)	82 (42)	100 (74)	<0.001
HbA _{1c} baseline (%)	8.8 (1.6)	8.6 (1.4)	9.1 (1.6)	10.2 (1.7)	<0.001
HbA _{1c} baseline (mmol/mol)	73 (17.5)	70 (15.3)	76 (17.5)	88 (18.6)	<0.001
Serum creatinine (mg/dL)	0.81 (0.15)	0.82 (0.15)	0.77 (0.15)	0.79 (0.16)	<0.001
AER (baseline)	12.6 (8.3)	11.0 (7.2)	16.2 (9.5)	15.2 (9.2)	<0.001
Study characteristics					
HbA _{1c} percentage (study mean)	8.2 (1.1)	7.9 (0.9)	8.5 (1.2)	9.5 (1.1)	<0.001
HbA _{1c} study mean (mmol/mol)	66 (12.0)	63 (9.8)	69 (13.1)	80 (12.0)	<0.001
AER (study mean)	40.1 (141.3)	21.0 (3.7)	25.8 (14.4)	332 (393)	<0.001
Use of ACE/ARB during study	42.0 (519)	33.6 (280)	51.0 (155)	84.0 (84)	<0.001
Use of statins during study	33.0 (408)	32.4 (270)	29.9 (91)	47.0 (47)	0.074
Incident low GFR (<60 mL/min/1.73 m ²) [% (n)]	6.8 (84)	4.1 (34)	6.3 (19)	31.0 (31)	<0.001
Persistent low GFR† (<60 mL/min/1.73 m ²) [% (n)]	3.6 (45)	1.3 (11)	2.6 (8)	26.0 (26)	<0.001
Serum creatinine doubling since baseline [% (n)]	1.1 (14)	0.6 (5)	1.0 (3)	6.0 (6)	0.001

GFR, glomerular filtration rate; T1DM, type 1 diabetes mellitus. Italicized boldface numbers indicate $P < 0.05$. †Persistent low GFR defined as having estimated GFR <60 mL/min/1.73 m² for two consecutive visits during follow-up.

(52.5%) of the 1,237 subjects were male, and 617 (49.9%) were assigned to the DCCT intensive-treatment group. There were statistically and clinically significant trends across disease progression groups in several baseline factors: participants who progressed to more severe levels of albuminuria during the study were more likely to have received conventional treatment during DCCT, to have mild retinopathy at the DCCT baseline evaluation, and longer duration of diabetes, as well as elevated levels of AER, HbA_{1c} percentage, and triglycerides at baseline (all *P* < 0.002).

Prior to biomarker standardization, values were assessed for normality through the use of histograms and quantile–quantile plots. Due to the skewed nature of some of the biomarkers, natural log transformations were applied and the data rechecked for normality. CRP, sE-selectin, IL-6, PAI-1 (active), and PAI-1 (total) were transformed using the natural logarithm, whereas sICAM-1, sVCAM-1, sTNFR-1 and -2, and fibrinogen met criteria for normality without the need for transformation.

Baseline biomarker levels were assessed for correlation with other baseline factors associated with microvascular disease (AER, triglycerides, HbA_{1c} percentage, serum creatinine, HDL-C, LDL-C, and age; Supplementary Table 1). None of the measured biomarkers were associated with baseline levels of AER. Although not of great magnitude, there were statistically significant positive associations between many of the biomarkers and the baseline levels of triglycerides and HbA_{1c} percentage and negative

associations with baseline serum creatinine levels (all *P* < 0.001). CRP levels were additionally correlated with baseline age and LDL levels. Interestingly, sVCAM-1 levels were negatively correlated with triglycerides, HbA_{1c} percentage, and LDL-C (all *P* < 0.001). Table 2 shows the means and SDs of the unadjusted mean biomarker levels by albuminuria severity group. Results of the univariable analysis show increased levels of sE-selectin, IL-6, PAI-1 (active and total), and sTNFR-1 and -2 in those who develop macroalbuminuria as compared with those who remain normal during follow-up. There is also a pronounced decrease in baseline sVCAM-1 levels in those who progress to macroalbuminuria as compared with those who remain normal during follow-up.

In the design-adjusted logistic regression models (adjusted for DCCT treatment assignment, baseline levels of AER, and the use of ACE/ARB drugs during the study), a one unit increase in the standardized levels of soluble sE-selectin increased the odds of subsequent development of macroalbuminuria by a factor of 1.9 (odds ratio [OR] 1.87 [CI 1.42–2.47]). Similarly, one unit increase in the standardized levels of IL-6, PAI-1 (total and active), and sTNFR-1 and -2 were all associated with a 30–50% increase in the odds for the development of macroalbuminuria during the study period (OR 1.28 [1.04–1.56], 1.27 [1.04–1.56], 1.31 [1.00–1.71], 1.32 [1.02–1.71], and 1.52 [1.16–2.00], respectively) [Table 3]. Following adjustment for covariates of interest (additionally adjusted for DCCT baseline

retinopathy cohort, sex, baseline measures of age, HbA_{1c} percentage, and duration of diabetes), significant associations with progression to macroalbuminuria remained for sE-selectin (OR 1.42 [1.06–1.91]) as well as sTNFR-1 and -2 (OR 1.33 [1.01–1.76] and 1.43 [1.07–1.91], respectively). Interestingly, for each one-unit increase in the standardized levels of sVCAM-1, there was a decrease in the odds of progression to macroalbuminuria in both the design- and covariate-adjusted models (OR 0.54 [0.37–0.78] and 0.62 [0.42–0.93], respectively).

The modifying effects of DCCT treatment group assignment, sex, and baseline retinopathy cohort were assessed through the use of model–interaction terms. No modifying effects of DCCT treatment group or sex were found in the examined models. However, a significant interaction was noted between PAI-1 total levels and baseline retinopathy cohort (*P* = 0.009). Results indicate that there exists a relationship between total levels of PAI-1 and progression to micro-/macroalbuminuria in subjects without any retinal complications at baseline, but no relationship in those with early retinopathy and longer duration of type 1 diabetes mellitus at baseline (primary cohort: OR 1.48 [1.09–2.00] vs. secondary cohort: OR 0.82 [0.60–1.13]). The levels of PAI-1 active showed similar patterns of significance with respect to baseline retinopathy cohort as PAI-1 total, but the interaction between the marker levels and retinopathy cohort failed to meet significance (*P* = 0.12).

Other risk factors associated with progression to macroalbuminuria included intensive DCCT treatment group (vs. conventional treatment OR 0.17 [95% CI 0.09–0.30]). Additionally, there was an increased odds of progression to macroalbuminuria with increases in baseline HbA_{1c} (1% OR 1.84 [1.57–2.16]) and baseline levels of AER (1 mg/24 h [Ln transformed] OR 2.25 [1.77–2.85]).

Analysis of the time to the development of the more severe outcome, macroalbuminuria, was assessed through Cox proportional hazard regression models. In the design-adjusted models (adjusted for DCCT treatment assignment, baseline AER, and the time-dependent effect of ACE/ARB use), a one unit increase in the standardized levels of sE-selectin, IL-6, PAI-1 (active), and sTNFR-1 and -2 significantly increased the hazard of subsequent progression to macroalbuminuria (hazard ratio [HR] 1.64 [95% CI 1.30–2.05], 1.24

Table 2—Baseline inflammatory biomarker levels with categorization by albuminuria status during the follow-up period

Biomarkers	Overall	Albuminuria			<i>P</i> value*
		Normal	Micro-	Macro-	
CRP (mg/L)	0.39 (0.75)	0.40 (0.78)	0.33 (0.67)	0.44 (0.72)	0.202
sE-selectin (ng/mL)	63 (58)	58 (52)	69 (67)†	83 (67)†‡	<0.001
sICAM-1 (ng/mL)	357 (127)	355 (134)	360 (111)	358 (118)	0.834
sVCAM-1 (ng/mL)	1,022 (396)	1,048 (395)	1,001 (384)	844 (390)†‡	<0.001
IL-6 (ng/mL)	9.7 (18.6)	8.9 (15.9)	11.8 (26.1)	10.7 (12.1)†	0.054
PAI-1 total (ng/mL)	181 (104)	178 (95)	183 (115)	205 (133)†	0.056
PAI-1 active (ng/mL)	8.7 (5.8)	8.5 (5.7)	8.6 (5.9)	10.0 (6.6)†‡	0.041
sTNFR-1 (ng/mL)	1.44 (0.60)	1.42 (0.55)	1.49 (0.73)	1.54 (0.62)†	0.053
sTNFR-2 (ng/mL)	1.43 (0.54)	1.41 (0.53)	1.46 (0.53)	1.55 (0.56)†	0.021
Fibrinogen (ng/mL)	196 (59)	194 (58)	198 (63)	204 (55)	0.495

**P* value from unadjusted linear trend test. Italicized boldface numbers indicate *P* < 0.05. Pairwise comparisons: †*P* < 0.05 vs. normal and ‡*P* < 0.05 vs. microalbuminuria.

Table 3—Odds of abnormal albuminuria for patients with increased levels of inflammatory biomarkers

Biomarker	Design adjusted*		Covariate adjusted†	
	Microalbuminuria	Macroalbuminuria	Microalbuminuria	Macroalbuminuria
CRP (mg/L)	0.89 (0.77–1.03)	1.10 (0.86–1.41)	0.86 (0.74–1.01)	1.07 (0.82–1.39)
sE-selectin (ng/mL)	1.29 (1.09–1.53)	1.87 (1.42–2.47)	1.22 (1.02–1.45)	1.42 (1.06–1.91)
sICAM-1 (ng/mL)	1.04 (0.90–1.20)	1.10 (0.85–1.42)	0.99 (0.85–1.15)	0.98 (0.75–1.29)
sVCAM-1 (ng/mL)	0.90 (0.73–1.11)	0.54 (0.37–0.78)	0.97 (0.78–1.20)	0.62 (0.42–0.93)
IL-6 (ng/mL)	1.05 (0.92–1.21)	1.28 (1.04–1.56)	1.01 (0.88–1.16)	1.21 (0.97–1.50)
PAI-1 total (ng/mL)	1.06 (0.92–1.22)	1.27 (1.04–1.56)	1.03 (0.89–1.19)	1.10 (0.89–1.37)
PAI-1 active (ng/mL)	1.03 (0.85–1.24)	1.31 (1.00–1.71)	0.99 (0.82–1.21)	1.20 (0.89–1.60)
sTNFR-1 (ng/mL)	1.09 (0.94–1.27)	1.32 (1.02–1.71)	1.09 (0.94–1.27)	1.33 (1.01–1.76)
sTNFR-2 (ng/mL)	1.16 (0.99–1.37)	1.52 (1.16–2.00)	1.15 (0.97–1.36)	1.43 (1.07–1.91)
Fibrinogen (ng/mL)	1.04 (0.83–1.31)	1.23 (0.81–1.86)	0.98 (0.67–1.66)	1.06 (0.67–1.66)

Data are OR (95% CI). Italicized boldface numbers indicate $P < 0.05$. *Design-adjusted models contain standardized biomarker level, DCCT treatment-group assignment, baseline AER measure, and treatment with ACE/ARB drugs during study period. †Covariate-adjusted models contain standardized biomarker level, DCCT treatment group, baseline retinopathy cohort, use of ACE/ARB drugs during study period, sex, and baseline measures of duration of type 1 diabetes mellitus, age, HbA_{1c} percentage, and AER.

[1.03–1.49], 1.31 [1.02–1.67], and 1.38 [1.10–1.75], respectively). Following further adjustment for covariates (additionally adjusted for DCCT baseline retinopathy cohort, sex, baseline measures of age, HbA_{1c} percentage, and duration of diabetes), significant associations remained for sE-selectin as well as sTNFR-1 and -2 (HR 1.34 [1.04–1.72], 1.38 [1.08–1.76], and 1.34 [1.06–1.71], respectively). The data also show a decreased HR for an incremental increase in sVCAM-1 levels in the design-adjusted hazard models (0.59 [0.42–0.82]); however, this relationship does not persist following adjustment for other covariates (0.71 [0.50–1.02]). The cumulative incidence of progression to macroalbuminuria during the study period is presented for the three biomarkers that retained significance in the hazard models following adjustment for design and prognostic covariates (Fig. 1). The figure shows the increased incidence rate in those in the upper quartile of sE-selectin and sTNFR-1 and -2 as compared with the lower three quartiles (Fig. 1).

CONCLUSIONS—The association of inflammatory and endothelial dysfunction markers with abnormal albuminuria in patients with type 1 diabetes had been previously reported by our group (8) as well as by other investigators (16–22). The current report is noteworthy in that we have shown in a large cohort of type 1 diabetes that high levels of sE-selectin, sTNFR-1, and sTNFR-2 can predict progression to macroalbuminuria in patients completely free of disease at baseline. Gohda et al. (23) have previously reported in a study of 628 patients

with type 1 diabetes, from two cohorts recruited at the Joslin clinic, that high levels of sTNFR-1 and -2 at baseline were strongly associated with early loss of renal function and progression of chronic kidney disease to stage 3 or higher. The same group also performed a study in 410 patients with type 2 diabetes, showing that elevated levels of sTNFRs, particularly TNFR-2, were strong predictors of progression to end-stage renal disease (24). Interestingly, the fact that the association of high levels of sTNFR1 and 2 with early renal function loss in type 1 diabetes is independent of the levels of either free or total circulating TNF levels (25) argues for the direct involvement of sTNFRs through a specific but not-yet-defined pathway leading to deterioration of kidney function and progression to end-stage kidney disease. Fernández-Real et al. (25), in studies aimed at evaluating the role of the TNF system activity on structural kidney damage in type 2 diabetes, reported a direct correlation between the levels of sTNFR-1 and mesangial expansion. The above studies together with our own data strongly support the lack of association of general markers of inflammation like CRP and IL-6 and initiation and progression of kidney disease in diabetes.

Other markers of inflammation and endothelial dysfunction that have been found to be elevated in patients with type 1 diabetes and nephropathy or found to be present in patients with macrovascular disease, such as IL-6 (11,26), CRP (11,20,22), and fibrinogen (8,19,21), did not show the same robust predictive power for progression to macroalbuminuria. This was also the case for other markers of

endothelial dysfunction included in our study, sICAM-1 (18) and PAI-1 (18).

An interesting paradox was observed with sVCAM-1 measurements. While several groups have reported that high levels of sVCAM-1 are positively associated with albuminuria (16,17) or inflammation (10), our data showed that low levels, not high levels, correlated with progression to macroalbuminuria, although the relationship did not persist after adjustment for other covariates in the time to event model. However, since the low levels of sVCAM-1 at baseline were correlated with high levels of LDL-C, HbA_{1c}, and triglycerides (Table 2), it is possible that the association is secondary to the cluster of these three markers in the patients than with sVCAM-1. This is supported by the fact that the association is no longer significant when these covariates are included in the model.

In conclusion, the measurement of sE-selectin, sTNFR-1, and sTNFR-2 levels provide valuable information concerning the risk for development of macroalbuminuria in patients with type 1 diabetes. This finding is important from two perspectives: it could be the basis for therapeutic interventions aimed at slowing the rate of progression of kidney disease, and it suggests that vascular dysfunction and the as-yet-undefined effects of sTNFRs are either directly responsible or more closely related to the development of diabetic nephropathy than other markers of inflammation, with the exception of antigen-antibody complexes containing modified LDL, which are also predictive of the evolution to macroalbuminuria (27).

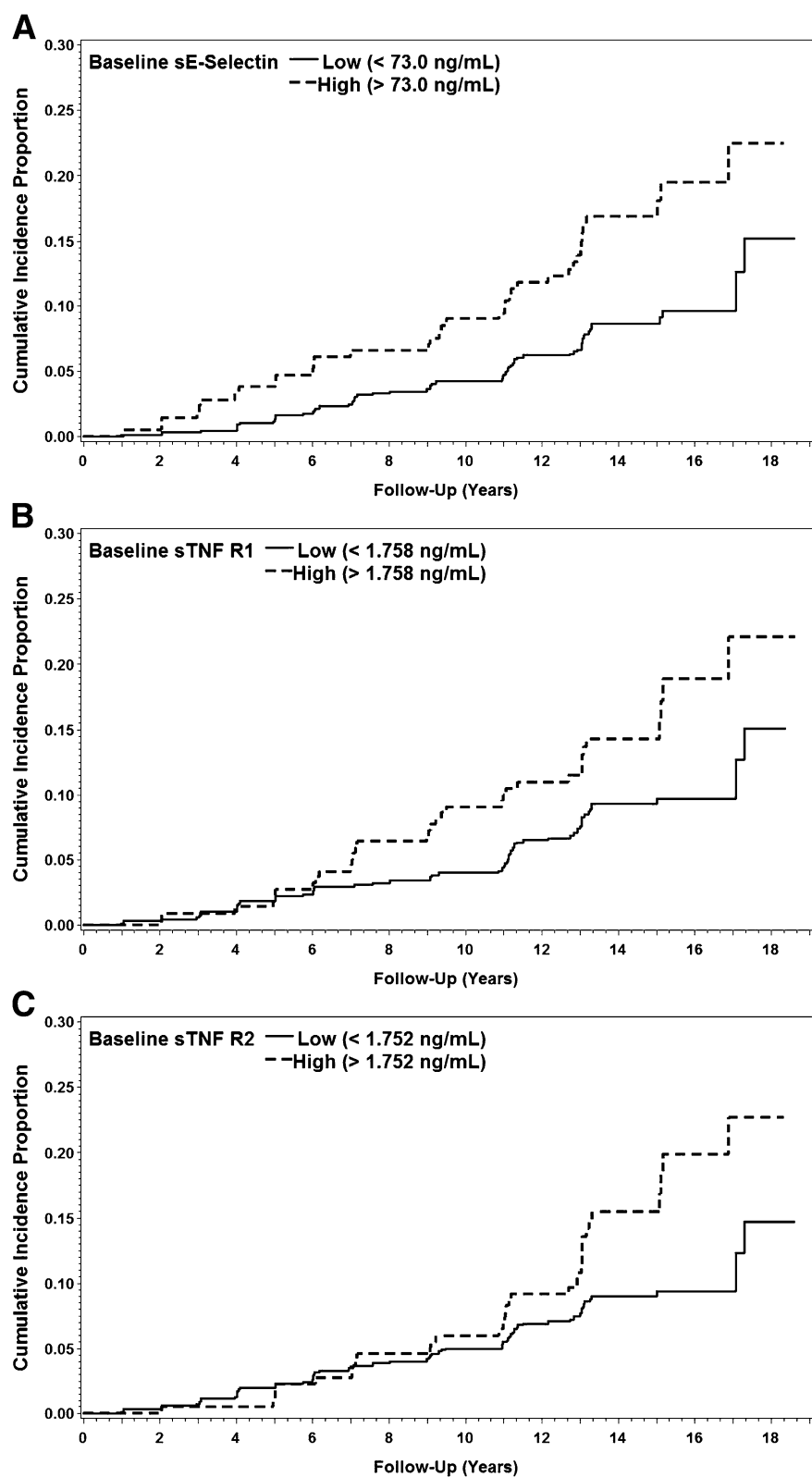


Figure 1—Cumulative incidence of progression to macroalbuminuria for high (upper quartile) versus low (lower quartiles) for sE-selectin (A), sTNFR-1 (B), and sTNFR-2 (C).

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M.F.L.-V. supervised the performance of all the biomarker assays performed in this study, assembled the resulting data, and wrote, reviewed, and edited the manuscript. N.L.B. and K.J.H. analyzed data and wrote, reviewed, and edited the manuscript. P.A.C. provided the clinical data from the EDIC cohort and assisted in the writing and revision of the manuscript. R.K. supervised the performance of all of the biomarker assays performed in this study and assembled the resulting data. G.V. wrote, reviewed, and edited the manuscript. The DCCT/EDIC group provided samples and reviewed and edited the manuscript. M.F.L.-V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. The importance of diabetic nephropathy in current nephrological practice. *Nephrol Dial Transplant* 2003;18:1716–1725
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977–986
3. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39(Suppl. 1):S1–S266
4. Mogensen CE. Microalbuminuria as a predictor of clinical diabetic nephropathy. *Kidney Int* 1987;31:673–689

5. Viberti GC, Jarrett RJ, Keen H. Microalbuminuria as prediction of nephropathy in diabetics. *Lancet* 1982;2:611
6. Gerstein HC, Mann JF, Yi Q, et al.; HOPE Study Investigators. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001;286:421–426
7. Jawa A, Kcomt J, Fonseca VA. Diabetic nephropathy and retinopathy. *Med Clin North Am* 2004;88:1001–1036, xi
8. Lopes-Virella MF, Carter RE, Gilbert GE, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Cohort Study Group. Risk factors related to inflammation and endothelial dysfunction in the DCCT/EDIC cohort and their relationship with nephropathy and macrovascular complications. *Diabetes Care* 2008;31:2006–2012
9. Araki S, Haneda M, Koya D, et al. Predictive impact of elevated serum level of IL-18 for early renal dysfunction in type 2 diabetes: an observational follow-up study. *Diabetologia* 2007;50:867–873
10. Schram MT, Chaturvedi N, Schalkwijk C, et al.; EURODIAB Prospective Complications Study. Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetes Care* 2003;26:2165–2173
11. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD; EURODIAB Prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes—the EURODIAB Prospective Complications Study. *Diabetologia* 2005;48:370–378
12. Dalla Vestra M, Mussap M, Gallina P, et al. Acute-phase markers of inflammation and glomerular structure in patients with type 2 diabetes. *J Am Soc Nephrol* 2005;16 (Suppl. 1):S78–S82
13. Gils A, Declercq PJ. Plasminogen activator inhibitor-1. *Curr Med Chem* 2004;11: 2323–2334
14. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. *Diabetes Care* 1999;22:99–111
15. The DCCT Research Group. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. *Clin Chem* 1987;33:2267–2271
16. Nelson CL, Karschimkus CS, Dragicevic G, et al. Systemic and vascular inflammation is elevated in early iga and type 1 diabetic nephropathies and relates to vascular disease risk factors and renal function. *Nephrol Dial Transplant* 2005; 20:2420–2426
17. Soedamah-Muthu SS, Chaturvedi N, Schalkwijk CG, Stehouwer CD, Ebeling P, Fuller JH; EURODIAB Prospective Complications Study group. Soluble vascular cell adhesion molecule-1 and soluble E-selectin are associated with micro- and macrovascular complications in Type 1 diabetic patients. *J Diabetes Complications* 2006;20:188–195
18. Targher G, Bertolini L, Zoppini G, Zenari L, Falezza G. Increased plasma markers of inflammation and endothelial dysfunction and their association with microvascular complications in Type 1 diabetic patients without clinically manifest macroangiopathy. *Diabet Med* 2005;22:999–1004
19. Klein RL, Hunter SJ, Jenkins AJ, et al.; DCCT/ECIC STUDY GROUP. Fibrinogen is a marker for nephropathy and peripheral vascular disease in type 1 diabetes: studies of plasma fibrinogen and fibrinogen gene polymorphism in the DCCT/EDIC cohort. *Diabetes Care* 2003;26: 1439–1448
20. Skriverhaug T, Bangstad HJ, Stene LC, Sandvik L, Hanssen KF, Joner G. Low risk of overt nephropathy after 24 yr of childhood-onset type 1 diabetes mellitus (T1DM) in Norway. *Pediatr Diabetes* 2006;7:239–246
21. Myrup B, de Maat M, Rossing P, Gram J, Kluft C, Jespersen J. Elevated fibrinogen and the relation to acute phase response in diabetic nephropathy. *Thromb Res* 1996; 81:485–490
22. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. *Diabetologia* 2003;46:1402–1407
23. Gohda T, Niewczas MA, Ficociello LH, et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. *J Am Soc Nephrol* 2012;23:516–524
24. Niewczas MA, Gohda T, Skupien J, et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. *J Am Soc Nephrol* 2012;23:507–515
25. Fernández-Real JM, Vendrell J, García I, Ricart W, Vallès M. Structural damage in diabetic nephropathy is associated with TNF- α system activity. *Acta Diabetol* 2012;49:301–305
26. Shikano M, Sobajima H, Yoshikawa H, et al. Usefulness of a highly sensitive urinary and serum IL-6 assay in patients with diabetic nephropathy. *Nephron* 2000;85:81–85
27. Lopes-Virella MF, Carter RE, Baker NL, Lachin J, Virella G; DCCT/EDIC Research Group. High levels of oxidized LDL in circulating immune complexes are associated with increased odds of developing abnormal albuminuria in Type 1 diabetes. *Nephrol Dial Transplant* 2012;27:1416–1423