Association Between Inflammatory Markers and Progression to Kidney Dysfunction: Examining Different Assessment Windows in Patients With Type 1 Diabetes

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OBJECTIVE

To determine whether biomarkers of inflammation and endothelial dysfunction are associated with the development of kidney dysfunction and the time frame of their association.

RESEARCH DESIGN AND METHODS

Biomarkers were measured at four time points during 28 years of treatment and follow-up in patients with type 1 diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort. In addition to traditional biomarkers of inflammation (C-reactive protein and fibrinogen), we measured interleukin-6 (IL-6) and soluble tumor necrosis factor receptors 1 and 2 (sTNFR-1/2), markers of endothelial dysfunction (soluble intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin [sE-selectin]), and fibrinolysis (total and active plasminogen activator inhibitor-1 [PAI-1]). Renal outcomes were defined as progression to incident chronic kidney disease (stage 3 or more severe) or macroalbuminuria (albumin excretion rate ≥300 mg/24 h). Prospective multivariate event-time analyses were used to determine the association of each biomarker with each subsequent event within prespecified intervals (3-year and 10-year windows).

RESULTS

Multivariate event-time models indicated that several markers of inflammation (sTNFR-1/2), endothelial dysfunction (sE-selectin), and clotting/fibrinolysis (fibrinogen and PAI-1) are significantly associated with subsequent development of kidney dysfunction. Although some markers showed variations in the associations between the follow-up windows examined, the results indicate that biomarkers (sTNFR-1/2, sE-selectin, PAI-1, and fibrinogen) are associated with progression to chronic kidney disease in both the 3-year and the 10-year windows.

CONCLUSIONS

Plasma markers of inflammation, endothelial dysfunction, and clotting/fibrinolysis are associated with progression to kidney dysfunction in type 1 diabetes during both short-term and long-term follow-up.

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**A complete list of the DCCT/EDIC Research Group can be found in the Supplementary Data online.*

Although the pathological mechanisms associated with the development and progression of kidney disease in patients with diabetes are not well understood, both endothelial dysfunction and inflammation appear to play important pathogenic roles (1). The EURODIAB Prospective Complications Study has provided strong supporting evidence for the clinical significance of biomarkers of inflammation and endothelial dysfunction as predictors of a variety of diabetes complications, including albuminuria, retinopathy, and cardiovascular disease. Schram and colleagues (2,3) found that the combination of increased levels of C-reactive protein (CRP), interleukin-6 (IL-6), and soluble tumor necrosis factor (sTNF) is associated with albuminuria, retinopathy, and cardiovascular disease. The same group also reported that plasma levels of markers of endothelial dysfunction (soluble vascular cell adhesion molecule-1 [sVCAM-1] and soluble E-selectin [sE-selectin]) were strongly and independently associated with inflammation markers, suggesting that endothelial dysfunction and inflammatory activity are closely related in the pathogenesis of complications classically associated with type 1 diabetes.

In a previous cross-sectional study of a subgroup of patients with samples taken between 8 and 16 years after enrollment in the Diabetes Control and Complications Trial (DCCT), we assessed the cross-sectional association of risk factors of endothelial dysfunction and inflammation, including CRP, fibrinogen, soluble intracellular adhesion molecule-1 (sICAM-1), sVCAM-1, sE-selectin, and fibrinolytic markers with prevalent diabetic nephropathy. 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, and smoking status), sE-selectin remained strongly associated with concurrent abnormal albuminuria (1). Similarly, in a subsequent study, we examined the ability of these biomarkers measured at DCCT baseline to predict the development of nephropathy during an average of 14.5 years of follow-up (i.e., average of 6.5 years during DCCT and 8 years as part of the Epidemiology of Diabetes Interventions and Complications [EDIC] follow-up). Results of these analyses showed that higher levels of sE-selectin and sTNF receptors 1 and 2 (sTNFR-1/2) are strongly associated with long-term progression to macroalbuminuria (MA) (4).

The objective of the current prospective evaluation is to expand our previous observations and determine whether markers of inflammation and endothelial dysfunction are associated with the subsequent development of kidney dysfunction in two follow-up windows (up to 3 years and up to 10 years) and with varying levels of baseline kidney function. Characterization of the time frame in which these biomarkers are associated with progression to kidney dysfunction will aid in the development and design of future clinical studies. In addition to the traditional markers of inflammation (CRP, IL-6, and fibrinogen), we measured sTNFR-1/2 as well as sICAM-1, sVCAM-1, and sE-selectin, markers of endothelial dysfunction. Additional models assessed the possible association of both total and active plasminogen activator inhibitor-1 (PAI-1), an important risk factor in thrombosis and atherosclerosis (5).

RESEARCH DESIGN AND METHODS

The DCCT (1983–1993) was a randomized controlled trial of 1,441 patients age 13–39 years who had type 1 diabetes for 1–15 years at study entry (6). Participants were randomly assigned from two study cohorts. Participants in the primary prevention cohort had no retinopathy on the basis of fundus photography, diabetes for 1–5 years, and no microalbuminuria ($<$40 mg/24 h). Participants in the secondary intervention cohort had mild to moderate nonproliferative diabetic retinopathy (at least one microaneurysm in either eye), diabetes for 1–15 years, and an albumin excretion rate (AER) $<$200 mg/24 h. None of the participants had hypertension ($\geq$140/90 mmHg) or dyslipidemia (total cholesterol $>$200 mg/dL and/or LDL $>$160 mg/dL) at baseline. At the baseline visit of DCCT, each participant underwent a physical examination, medical history, and routine laboratory analysis that included serum creatinine, lipid profile, and HbA_{1c} (6).

The participants were randomly assigned to either intensive or conventional insulin therapy and followed for an average of 6.5 years before the study was halted in 1993, 1 year ahead of its scheduled end because of the consistent beneficial impact of intensive therapy on diabetes complications (6). After the completion of DCCT, intensive diabetes therapy was taught to the original conventional treatment group and recommended for all participants, and diabetes care was transferred from DCCT study staff to the participants' own health care providers. In 1994, ~95% of the DCCT participants were enrolled in the EDIC observational follow-up study. The goal of EDIC was to assess the development and progression of more-advanced complications in the type 1 diabetes population, including cardiovascular disease (7). AER and creatinine clearance were determined in 4-h urine collection specimens obtained annually during DCCT; estimated glomerular filtration rate (eGFR) was calculated on the basis of annual serum creatinine levels, and AER was measured every other year during EDIC (7,8).

The current study was performed to determine whether markers of inflammation, endothelial dysfunction, and fibrinolysis taken at four study time points (DCCT baseline, DCCT closeout, EDIC years 4–6, and EDIC years 8–11) would be associated with the development of kidney dysfunction within two follow-up windows (i.e., up to 3 years [short term], up to 10 years [long term]). The outcomes of interest were incident MA and chronic kidney disease (CKD) stage 3 or worse. During DCCT, 1,396 of the 1,441 randomized participants (96.9%) had biomarker data at either the baseline (n = 1,345) or the closeout (n = 1,353) visit. Of the 1,425 surviving DCCT participants, 1,375 (96%) joined EDIC. In addition, 886 of these individuals agreed to participate in a biomarker substudy and had blood collected during EDIC. Of the 1,396 participants with at least one available measure, 830 had data at all four biomarker measurements. Thus, the analysis cohort comprised 4,378 paired biomarker and disease outcome measurements taken in 1,396 participants. The 886 substudy participants with available biomarker data during EDIC did not differ significantly from the 510 with only DCCT data with respect to DCCT treatment arm, primary prevention cohort, and age; however, women were less likely to be included in the sample than men ($P <$ 0.05) (data not shown). Additional clinical characteristics of these participants have been reported previously (9–13).

Samples and Biomarker Assays

Fasting serum samples obtained during DCCT/EDIC were sent to a central laboratory for standard lipid analysis, with

excess samples sent to the Medical University of South Carolina (MUSC) where they were aliquoted and stored at −70°C to minimize refreezing effects. A new frozen aliquot was used for each new test performed. The institutional review boards at MUSC and all participating DCCT/EDIC centers approved the sample collection procedures. Written informed consent was obtained from all participants.

Serum levels of CRP, total and active PAI-1, sICAM-1, sVCAM-1, sE-selectin, IL-6, and sTNFR-1/2 were assayed by using the SignaturePLUS Protein Array Imaging and Analysis System (Aushon BioSystems), and ArrayVision software was used for data analysis. Coefficients of variation were 2.6% for CRP, 3.4% for total PAI, 5.9% for active PAI-1, 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 by immunonephlometry (catalog number 86086; DiaSorin, Stillwater, MN) with a Beckman IMMAGE 800 analyzer.

Outcomes

The two primary outcomes for each participant were characterized from the baseline DCCT visit (1983–1989) until year 18 of the EDIC study (2010–2012). Levels of eGFR defined progression to CKD, and AER values defined progression to MA. Although AER is associated with progression to CKD, with increases in AER generally preceding declines in measured eGFR; in some patients, renal failure occurs with a normal AER. Thus, each outcome was analyzed independently of the progression of the other. Each participant had eGFR values calculated annually from serum creatinine levels by using the Chronic Kidney Disease Epidemiology Collaboration equation (14). During both DCCT and EDIC, CKD stage 3 or worse was defined as any two consecutive eGFR measures $<$60 mL/min/1.73 m^2, and time to CKD was defined as the date of the first of two consecutive low eGFR values. Incident MA was defined as an AER $\geq$300 mg/24 h at any time during the study. Outcomes were independently determined after each of the four biomarker measurements and assessed from the day of the biomarker measurement up to the observation of an event or date of censoring. Censoring was defined as the day before the next available biomarker measurement or the end of available follow-up data. However, when a measurement was missing, the time to event from the previous measure was limited to 120 months of follow-up (maximum follow-up between two measures given complete follow-up data). Therefore, the 510 participants with only DCCT baseline and/or closeout biomarker data available who chose not to participate in the MUSC program project grant substudy had follow-up data censored 120 months after the last available DCCT biomarker measurement (hence the lower number of participants with later study time points). By using the 10-year follow-up window, 166 of the 1,396 participants (11.9%) progressed to either CKD (n = 74) or MA (n = 143), 51 of the 166 (30.7%) progressed to both CKD and MA, 23 (31.1%) progressed to CKD without MA, and 92 (64.3%) progressed to MA without CKD. When the follow-up window was limited to 3 years, 68 of the 1,396 (4.9%) participants progressed to either CKD (n = 25) or MA (n = 44), and 19 of the 68 (27.9%) progressed to both. In addition to the primary outcomes, 21 participants progressed to end-stage renal disease (eGFR $<$10 mL/min/1.73 m^2 or renal dialysis/transplantation) within a 10-year window of the prior biomarker measurement (data not shown).

Statistical Analysis

Trends in demographic, clinical, and biomarker measurements over time were assessed by using methods of maximum likelihood through mixed-effects regression models (Table 1). Concentrations of markers were measured longitudinally during DCCT and EDIC and used to determine whether increased levels in these measures were associated with risk of progression to kidney dysfunction. Before analysis, all biomarker levels were assessed for normality and transformed when necessary. After data normalization, all biomarkers were standardized (z scores), and the results represent the association between a change of 1 SD in each biomarker and the risk of progression to kidney dysfunction before the next measurement or the end of follow-up/censoring. Covariates for the analysis were obtained from clinical measures taken at baseline as well as concurrently with each biomarker measurement (i.e., at the beginning of each follow-up window) whenever possible, and when a clinical characteristic was unavailable at the time of biomarker measurement, the temporally nearest measure was included (usually within 12 months before or after the biomarker measurement).

Prospective multivariate event-time analysis was used to account for the within-participant correlation as a result of the repeated biomarker measurements. Each study participant had from one to four ordered biomarker measurements during DCCT and the first 18 years of the EDIC follow-up study (DCCT baseline, DCCT closeout [average 6.5 years, maximum 10 years], and two measurements during EDIC). The primary hypothesis that biomarkers measured during DCCT and EDIC are associated with progression to kidney disease was assessed by using marginal Cox proportional event-time models for clustered data (15). This modeling strategy allowed for multiple follow-up windows for each participant to be included in one model, whereas robust variance estimates (sandwich estimates) were used to account for the clustering of measurements by participant. Data were structured to allow each measured biomarker and associated follow-up event status to be modeled as a single observation while accounting for the clustering of multiple biomarker measures for each participant. Each biomarker follow-up window began on the day of the measure and ended the day before the next measure or at the time of an event or censoring. After the occurrence of an incident event, measures taken after progression to that event were removed from the analysis. We evaluated the association of biomarker levels on subsequent events taken during follow-up windows of up to 3 and 10 years to determine whether associations between biomarkers and outcomes changed depending on assessment window length. Next, composite biomarker scores were created to assess the combined impact of multiple biomarkers acting on the same pathway. Specifically, four scores were created by combining standardized individual biomarkers: acute phase reactants (fibrinogen and CRP), cytokines/adipokines (sTNFR-1/2, active PAI-1, total PAI-1, and IL-6), thrombosis (fibrinogen, active PAI-1, and total PAI-1), and endothelial dysfunction (sICAM-1, inverse of sVCAM-1, and sE-selectin). The inverse z score of sVCAM-1 was used because of its consistent inverse relationship with the outcomes of interest (4).

Table 1—Participant demographic and clinical levels taken at each measurement time point

Characteristic	DCCT baseline (n = 1,346)	DCCT closeout (n = 1,354)	EDIC years 4–6 (n = 816)	EDIC years 8–11 (n = 830)	P value
Age (years)	27.0 (7.1)	33.2 (7.1)	39.4 (6.9)	43.6 (6.9)	—
Male sex	52.9 (712)	52.7 (714)	56.1 (455)	55.4 (456)	—
Intensive treatment	49.9 (671)	49.5 (670)	51.7 (419)	51.0 (420)	—
Primary prevention cohort	50.9 (685)	50.5 (683)	51.8 (420)	51.4 (423)	—
Duration of type 1 diabetes (years)	5.6 (4.1)	12.0 (4.9)	17.5 (4.8)	21.8 (4.9)	—
Study duration (years)	—	6.4 (1.8)	12.1 (2.0)	16.3 (2.1)	—
Current HbA_{1c} (%)	9.0 (1.6)	8.3 (1.6)	8.1 (1.3)	7.9 (1.3)	<0.001
Current HbA_{1c} (mmol/mol)	75 (17.5)	67 (17.5)	65 (14.2)	63 (14.2)	<0.001
Study-weighted mean HbA_{1c} (%)	9.0 (1.6)	8.2 (1.5)	8.1 (1.1)	8.1 (1.1)	<0.001
AER (mg/24 h)*	11.3 (2.2)	11.6 (3.1)	13.6 (3.8)	15.1 (3.6)	<0.001
eGFR*	125 (1.1)	116 (1.1)	107 (1.2)	101 (1.2)	<0.001
Serum creatinine	0.7 (0.1)	0.7 (0.2)	0.8 (0.2)	0.8 (0.2)	<0.001
LDL cholesterol (mg/dL)	110 (29)	113 (29)	114 (31)	109 (28)	0.047
HDL cholesterol (mg/dL)	51 (12)	51 (13)	56 (15)	55 (15)	<0.001
Triglycerides (mg/dL)*	73 (1.5)	74 (1.6)	75 (1.7)	77 (1.7)	0.002
SBP (mmHg)	115 (11)	116 (12)	120 (14)	121 (14)	<0.001
MBP (mmHg)	87 (8)	88 (9)	90 (10)	90 (10)	<0.001
ACE/ARB taken to date	0.0 (0)	0.1 (1)	19.2 (156)	39.6 (326)	<0.001
Smoker (current)	18.7 (251)	20.1 (272)	16.7 (135)	14.3 (118)	0.005

Data are mean (SD) or % (n). P values shown are for trends over time by using methods of maximum likelihood through mixed-effects regression models. MBP, mean blood pressure; SBP, systolic blood pressure. *Presented as geometric means.

Associations were reported as hazard ratio (HRs) and 95% CIs. Because of the efficacy of DCCT treatment on reduction and control of blood glucose (16) and the potent effect of HbA_{1c} levels on renal outcomes, initial models were adjusted for concurrent HbA_{1c}. Expanded models explored the influence of additional clinical characteristics on the basis of univariate associations and known clinical associations with the overarching goal of identifying a parsimonious and clinically interpretable model in the presence of a relatively small number of events. All statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC). No corrections for multiple testing/comparisons were applied.

RESULTS

Demographics and Clinical Characteristics

Demographic and clinical measures taken concurrently with biomarker measurements are shown in Table 1. At DCCT study enrollment, the mean age of participants with available data was 27.0 (SD 7.1) years, with a mean duration of diabetes of 5.6 (4.1) years. Seven hundred thirty-one (52.9%) participants with available samples were male, 696 (49.9%) were randomly assigned to the intensive-therapy groups, and 704 (50.5%) were assigned to the primary prevention cohort. At the time of the final biomarker sample collection (EDIC years 8–11), the mean age was 43.6 (6.9) years, and the mean duration of diabetes was 21.8 (4.9) years. During the study period, the 1,396 included participants contributed 4,378 biomarker measurements, with a median of 6.1 (interquartile range [IQR] 4.7–8.7) years between each measurement. Biomarker measurements taken across the four time points were assessed for changes over the duration of the DCCT and EDIC follow-up period (Supplementary Table 1). Although levels of sTNFr-1, fibrinogen, and CRP increased during the study ($P < 0.001$), sTNFr-2 and total PAI-1 levels remained stable over time ($P = 0.764$ and 0.760, respectively). Levels of sE-selectin showed a clear decreasing trend over time ($P < 0.001$), whereas the remaining biomarker levels had inconsistent patterns of change over time.

Clinical Correlates

Univariate associations between baseline demographic characteristics (participant-specific covariates: DCCT treatment assignment, baseline disease cohort, and sex) as well as clinical characteristics taken concurrently with biomarker and outcome measurements during the subsequent follow-up window (observation-specific covariates) are presented in Table 2. Increased current and study-weighted mean HbA_{1c} levels, total and LDL cholesterol, triglyceride levels, and blood pressure levels concurrent with biomarker measurements were significantly associated with an increased risk of progression to CKD stage 3 and MA during the follow-up windows. Males were more likely than females to progress to MA (HR 2.1 [95% CI 1.5–3.0]; $P < 0.001$) but not to CKD. Participants who reported taking ACE inhibitor or angiotensin II receptor blocker (ARB) medication at the start of each follow-up window were likely to have increased kidney dysfunction and, thus, were significantly more likely to experience a kidney event than those who did not report taking the medications ($P < 0.05$). This positive association is likely due to the use of ACE/ARB medications as a first-step response to not only hypertension but also low-level proteinuria and decreases in eGFR before progression CKD (17,18).

Kidney Dysfunction

Table 3 depicts mean biomarker levels stratified by whether a participant progressed

Table 2—Univariate associations of clinical and demographic characteristics as well as biomarkers with each study outcome between available biomarker measurements

	Events					
	CKD (n = 74)			MA (n = 143)		
Characteristic	HR (95% CI)	χ^2	P value	HR (95% CI)	χ^2	P value
Age (years)	1.07 (1.04–1.10)	16.8	<0.001	0.99 (0.97–1.01)	1.1	0.286
Male sex*	1.22 (0.77–1.93)	0.7	0.396	2.11 (1.48–3.01)	16.8	<0.001
Intensive treatment*	0.48 (0.30–0.77)	9.0	0.003	0.36 (0.25–0.52)	30.2	<0.001
Primary prevention cohort*	0.99 (0.63–1.56)	0.0	0.962	1.68 (1.20–2.36)	9.1	0.003
Duration of type 1 diabetes (years)	1.06 (1.03–1.09)	15.6	<0.001	1.04 (1.01–1.07)	8.8	0.003
Study duration (years)	1.16 (1.11–1.22)	39.7	<0.001	1.04 (0.99–1.08)	2.7	0.097
BMI	1.04 (0.99–1.08)	2.7	0.102	1.01 (0.97–1.05)	0.2	0.639
Current HbA_{1c} (%)	1.55 (1.38–1.75)	52.9	<0.001	1.73 (1.60–1.87)	184.0	<0.001
Study-weighted mean HbA_{1c} (%)	2.00 (1.74–2.29)	98.4	<0.001	1.84 (1.68–2.01)	177.2	<0.001
AER (50 mg/24 h)	1.04 (1.03–1.04)	82.9	<0.001	1.73 (1.44–2.08)	34.8	<0.001
eGFR (10-unit decrease)	2.20 (1.91–2.54)	116.5	<0.001	1.16 (1.01–1.34)	4.4	0.036
Cholesterol (10 mg/dL)	1.20 (1.14–1.26)	54.4	<0.001	1.08 (1.03–1.13)	10.1	0.002
LDL cholesterol (10 mg/dL)	1.18 (1.08–1.28)	15.2	<0.001	1.05 (1.00–1.11)	3.7	0.055
HDL cholesterol (10 mg/dL)	0.94 (0.80–1.11)	0.5	0.495	0.85 (0.74–0.98)	5.1	0.024
Triglycerides (10 mg/dL)	1.06 (1.05–1.07)	110.6	<0.001	1.05 (1.04–1.06)	146.7	<0.001
SBP (10 mmHg)	1.98 (1.68–2.33)	67.8	<0.001	1.25 (1.10–1.42)	11.3	<0.001
DBP (10 mmHg)	2.41 (1.73–3.36)	26.7	<0.001	1.57 (1.29–1.91)	19.8	<0.001
Any ACE/ARB taken to date	5.39 (3.23–9.00)	41.5	<0.001	2.65 (1.56–4.50)	13.0	<0.001
Current smoker	1.33 (0.77–2.32)	1.0	0.308	1.72 (1.19–2.49)	8.4	0.004

A total of 4,378 measurements were taken in 1,396 participants, with a median time between measurements of 6.1 years. DBP, diastolic blood pressure; SBP, systolic blood pressure. *Time invariant characteristics; all other characteristics are measured at time points corresponding to each biomarker measurement.

to kidney dysfunction (i.e., CKD stage 3 or worse or MA) before the next biomarker measurement or the end of study follow-up. Ninety-four of 1,396 participants (6.7%) progressed to CKD stage 3 or worse during DCCT or during the first 10–18 years of EDIC follow-up (21–28 years of follow-up time since enrollment in the DCCT study); the median progression time was 19.3 (IQR 14.3–22.2) years since DCCT baseline. Progression to CKD stage 3 or worse occurred on average 5.3 (IQR 2.9–8.5) years after the last available biomarker measurement, and three CKD events of stage 3 or worse per 1,000 person-years of follow-up was calculated. Seventy-four participants progressed to CKD within a 10-year window since the last available measurement, and 25 progressed within a 3-year window. By using a 10-year window, increased levels of some markers of inflammation (cytokines) and endothelial dysfunction (sTNFr-1/2 and sE-selectin) were associated with an increased risk of progression to CKD (Table 4). When selecting a short-term follow-up (3 years) to monitor outcomes, the risk of progression to CKD associated with increases in the same markers of inflammation and endothelial dysfunction remained strong. Increased levels of markers of thrombosis (fibrinogen and total PAI-1) also were associated with an increased risk of progression to CKD. sTNFr-1/2 levels as well as sE-selectin, fibrinogen, and PAI-1 were similarly

Table 3—Biomarker means taken before follow-up windows with and without clinical events

	CKD		MA	
	Event (n = 74)	No event (n = 4,313)	Event (n = 143)	No event (n = 4,244)
sTNFR-1	2.10 (1.94–2.28)	1.50 (1.47–1.54)	1.67 (1.57–1.79)	1.48 (1.45–1.51)
sTNFR-2	1.82 (1.70–1.94)	1.38 (1.35–1.40)	1.51 (1.43–1.59)	1.36 (1.34–1.39)
sE-selectin	45.3 (39.9–51.3)	44.6 (42.8–46.4)	51.8 (46.6–57.5)	44.0 (42.3–45.9)
PAI-1 active	8.64 (7.27–10.26)	7.88 (7.61–8.16)	9.44 (8.25–10.80)	7.75 (7.48–8.03)
PAI-1 total	158.2 (140.8–177.8)	153.4 (148.8–158.1)	166.3 (151.3–182.7)	151.9 (147.3–156.6)
sICAM-1	293.8 (273.9–315.0)	306.9 (300.8–313.0)	309.8 (292.7–327.9)	306.4 (300.4–312.5)
sVCAM-1	879.6 (797.8–969.9)	945.4 (917.1–974.5)	893.4 (820.3–973.0)	948.2 (920.1–977.2)
IL-6	7.03 (5.96–8.29)	6.60 (6.35–6.85)	7.36 (6.46–8.38)	6.56 (6.31–6.83)
Fibrinogen	253.4 (233.9–274.5)	220.5 (217.0–224.0)	230.0 (215.3–245.8)	220.6 (217.1–224.1)
CRP	0.21 (0.16–0.28)	0.21 (0.19–0.22)	0.19 (0.15–0.24)	0.21 (0.20–0.23)

Data are geometric mean (95% CI). Event count across up to four measurements in 1,396 participants.

Table 4—Associations between biomarker measures and time to progression to kidney dysfunction during two window lengths

	10-Year window			3-Year window		
	Events	HR (95% CI)*	HR (95% CI)†	Events	HR (95% CI)*	HR (95% CI)†
CKD						
sTNFR-1	64	**1.29 (1.16–1.45)**	**1.36 (1.23–1.49)**	23	**1.61 (1.43–1.82)**	**1.56 (1.40–1.74)**
sTNFR-2	74	**1.55 (1.38–1.74)**	**1.63 (1.47–1.82)**	25	**1.77 (1.52–2.06)**	**1.97 (1.70–2.29)**
sE-selectin	61	**1.31 (1.14–1.50)**	**1.23 (1.08–1.40)**	23	**1.42 (1.19–1.70)**	**1.38 (1.18–1.61)**
PAI-1 active	74	**1.23 (1.06–1.43)**	1.15 (0.95–1.39)	25	**1.30 (1.03–1.65)**	1.18 (0.87–1.61)
PAI-1 total	74	**1.23 (1.02–1.49)**	1.14 (0.94–1.39)	25	**1.55 (1.23–1.94)**	**1.52 (1.24–1.87)**
sICAM-1	74	1.09 (0.86–1.37)	1.02 (0.79–1.31)	25	0.84 (0.53–1.32)	0.88 (0.56–1.38)
sVCAM-1	63	0.82 (0.60–1.12)	0.88 (0.65–1.17)	23	0.74 (0.42–1.30)	0.76 (0.41–1.40)
IL-6	61	0.85 (0.66–1.09)	0.91 (0.75–1.10)	21	1.11 (0.90–1.37)	1.13 (0.96–1.33)
Fibrinogen	46	**1.96 (1.36–2.83)**	**1.74 (1.24–2.45)**	18	**2.59 (1.50–4.47)**	**2.13 (1.29–3.51)**
CRP	64	0.93 (0.76–1.15)	0.88 (0.67–1.15)	23	1.06 (0.77–1.47)	0.99 (0.63–1.56)
Composite scores‡						
Acute-phase reagents	46	**1.34 (1.05–1.72)**	1.25 (0.99–1.57)	18	**1.51 (1.09–2.10)**	**1.56 (1.19–2.04)**
Cytokines/adipokines	61	**1.53 (1.27–1.85)**	**1.70 (1.34–2.15)**	21	**3.58 (2.45–5.23)**	**3.25 (2.20–4.81)**
Thrombosis	46	**1.92 (1.37–2.70)**	**1.77 (1.20–2.61)**	18	**2.91 (1.86–4.54)**	**2.45 (1.50–4.01)**
Endothelial dysfunction	60	1.82 (0.78–4.24)	**1.59 (1.06–2.38)**	23	**2.28 (1.21–4.28)**	**2.16 (1.27–3.68)**
MA						
sTNFR-1	116	1.09 (0.97–1.23)	1.08 (0.97–1.20)	39	1.19 (1.02–1.38)	1.10 (0.83–1.44)
sTNFR-2	143	**1.31 (1.18–1.46)**	**1.25 (1.10–1.42)**	44	**1.32 (1.15–1.52)**	1.19 (0.97–1.46)
sE-selectin	117	**1.20 (1.07–1.35)**	**1.17 (1.04–1.33)**	40	1.10 (0.92–1.33)	0.96 (0.70–1.32)
PAI-1 active	141	**1.22 (1.07–1.38)**	1.10 (0.96–1.27)	43	1.13 (0.90–1.42)	0.97 (0.70–1.34)
PAI-1 total	143	**1.22 (1.06–1.41)**	**1.17 (1.00–1.36)**	44	1.27 (0.99–1.63)	1.22 (0.93–1.61)
sICAM-1	143	1.16 (0.97–1.40)	1.19 (0.99–1.42)	44	1.02 (0.71–1.46)	1.07 (0.78–1.47)
sVCAM-1	100	0.85 (0.67–1.09)	0.93 (0.76–1.15)	36	1.03 (0.70–1.53)	1.11 (0.85–1.45)
IL-6	113	0.96 (0.84–1.08)	1.02 (0.93–1.13)	38	0.97 (0.72–1.30)	1.05 (0.84–1.32)
Fibrinogen	70	1.01 (0.80–1.27)	1.01 (0.79–1.30)	26	0.72 (0.46–1.12)	0.62 (0.38–1.03)
CRP	115	0.95 (0.79–1.14)	0.95 (0.77–1.18)	38	0.88 (0.63–1.23)	0.83 (0.48–1.43)
Composite scores‡						
Acute-phase reagents	70	0.84 (0.63–1.11)	0.86 (0.60–1.24)	26	0.57 (0.31–1.05)	**0.41 (0.18–0.92)**
Cytokines/adipokines	113	**1.26 (1.06–1.50)**	**1.30 (1.04–1.63)**	38	1.47 (0.99–2.16)	1.28 (0.75–2.20)
Thrombosis	70	**1.53 (1.12–2.08)**	1.26 (0.89–1.78)	26	0.96 (0.57–1.62)	0.87 (0.41–1.86)
Endothelial dysfunction	99	**1.61 (1.19–2.19)**	**1.47 (1.06–2.04)**	36	1.08 (0.60–1.95)	0.83 (0.48–1.45)

Boldface indicates significance at $P < 0.05$. *Initial model adjusted for concurrent HbA_{1c} %. †Covariate-adjusted model also adjusted for sex, concurrent duration of type 1 diabetes, cholesterol (CKD), triglycerides (MA), and blood pressure. Time to MA was also adjusted for AER values measured concurrently with biomarkers. ‡Composite scores were created by combining the standardized individual biomarkers as follows: acute-phase reactants (fibrinogen and CRP), cytokines/adipokines (sTNFR-1/2, active PAI-1, total PAI-1, and IL-6), thrombosis (fibrinogen, active PAI-1, and total PAI-1), and endothelial dysfunction (sICAM-1, inverse of sVCAM-1, and sE-selectin). The inverse *z* score of sVCAM-1 was used because of its consistent inverse relationship with the outcomes of interest.

associated with CKD progression for both the 10-year and the 3-year windows, despite the reduced power for the 3-year window.

One hundred sixty-one of the 1,396 participants (11.5%) progressed to MA during DCCT or the first 18 years of EDIC follow-up; the median progression time was 11.7 (IQR 6.3–16.1) years since DCCT baseline. MA occurred on average 5.1 (IQR 2.7–6.3) years after the last available biomarker measurement, and 5.5 MA events per 1,000 person-years of follow-up occurred. One hundred forty-three participants progressed to MA within a 10-year window since the last available measurement, and 44 progressed within a 3-year window. Similar to CKD, sTNFR-2, sE-selectin, and PAI-1 were associated with progression to MA during the 10-year follow-up window, but the association was less marked within the 3-year window.

CONCLUSIONS

The primary objective of this study was to determine whether biomarkers of inflammation and endothelial dysfunction are associated with an increased risk to develop incident CKD or MA and the time frame in which the association was significant. The choice of biomarkers was based on previously reported results (1,4,19–27) on inflammatory, thrombosis, and endothelial dysfunction biomarkers and how they relate to progression of diabetes complications. We have examined the long-term association of inflammatory markers, adhesion molecules, and clotting/fibrinolysis biomarkers on the development of MA by using the levels of biomarkers obtained at DCCT baseline and closeout (4). The current results show that both sE-selectin and sTNFR-1/2 are associated with the long-term development of MA. Higher levels of these biomarkers indicated the development of MA in participants who were completely free of clinically detectible kidney disease (i.e., with normal albuminuria and normal eGFR) at baseline. Identification of biomarkers associated with long-term disease progression is important to understanding the underlying mechanisms of disease development and may allow the identification of patients more likely to benefit from targeted therapeutic measures.

Although albuminuria is one of the more commonly used parameters to detect early damage of kidney function in diabetes, a few patients with diabetes may develop nephropathy without MA

(16); furthermore, albuminuria may represent a mixture of glomerular and tubular damage and may not be completely representative of classical diabetic nephropathy. Therefore, determination of whether the same biomarkers associated with incident cases of MA also are associated with progressive reduction of eGFR and whether the time frame for this relationship is similar was important. Also important was the determination of whether biomarkers associated with long-term development of CKD in participants free of apparent clinical renal disease maintained their association when measured closer to the development of CKD and with varying stages of disease. Biomarkers associated diabetes complications in a close temporal relationship with the event could be useful in clinical trials for the recruitment of patients more likely to have the event during the trial, thus increasing the number of events and, therefore, enhancing the power of the study and validity of the conclusions.

The results in the whole subset of participants studied indicate, as seen in Supplementary Table 1, that the levels of CRP, fibrinogen, sTNFR-1, and active PAI-1 increased over time, whereas sE-selectin levels decreased over time. Some of the other biomarkers did not show a sustained trend with increasing age. These results also show that the overall levels of the biomarkers were increased in participants who developed impaired eGFR and MA compared with those who maintained normal kidney function. The only exceptions were IL-6, which did not show an appreciable difference between the two groups, and VCAM-1, which was lower in participants who developed nephropathy.

As shown in Table 4, some differences existed between the association of the biomarkers according to the outcome chosen to assess kidney dysfunction and the follow-up window examined. sE-selectin was associated with both long-term and short term development of decreased eGFR but was only associated with long-term development of MA. Other adhesions molecules, like ICAM-1 and VCAM-1, were not associated with the development of nephropathy in this cohort. sTNFR-1/2 and total PAI-1, like sE-selectin, were short- and long-term predictors of reduced eGFR. Total PAI-1, like sE-selectin, was not associated with the development of MA within the 3-year (short-term) window. sTNFR-1/2 were, in contrast, strongly associated with reduced eGFR within both the 3- and 10-year windows.

A high percentage of participants who developed MA had normal eGFR, and those with reduced eGFR did not necessarily have MA. The physiopathology of these renal outcomes may be different, although they likely have common pathways, such as inflammation. MA is related to increased glomerular permeability and decreased tubular reabsorption of proteins and is likely strongly influenced by treatment with ACE inhibitors and ARBs. Reduction of eGFR is related to kidney mesangial cell expansion and fibrosis and subsequent deterioration of kidney function. Therefore, although albuminuria is useful to the identification of early stages of diabetic kidney disease, it does not necessarily identify early stages of reduced eGFR, and both end points should be watched closely in diabetes to gain more clear and precise information about progression of kidney disease in diabetes.

Overall, the results suggest that high sTNFR-1/2 levels are associated with short-term progression of kidney dysfunction and may aid in the recruitment of participants for trials in which the main end point is the development of CKD. Both sTNFR-1/2 and sE-selectin are useful biomarkers for identifying patients at increased risk for MA earlier in life and can be used to institute therapeutic measures to prevent the development of overt nephropathy.

Although this study population is well defined and followed for a significant amount of time, some limitations temper the results. Because the number of events in this cohort was relatively small, primarily in the 3-year follow-up window, the lack of statistical significance may have resulted from insufficient power. In addition, participant AER values were measured annually during DCCT but only every other year during EDIC, creating a challenge to measuring persistent MA in the presence of treatment with ACE/ARB medications. Furthermore, one-third of the DCCT study participants did not participate in the MUSC program project grant biomarkers study, which examined levels during the EDIC, and only had levels available for follow-up windows early in the study. This analysis does not provide added clinical utility for disease prediction beyond the traditional risk factors, and further classification analysis, including prediction models, should be conducted. Future analytic directions include classification models with traditional risk factors and evaluation of improvement in classification accuracy with inclusion of these biomarkers. A better determination of whether the association of the biomarkers associated with MA reflect glomerular disease or tubular disease would be interesting. Unfortunately, the current data do not allow us to answer this question.

Most of the biomarkers shown by us and others to correlate with renal function deterioration in patients with diabetes seem to have long-term predictive value. However, only a limited number of these markers can predict the onset of chronic disease closer to the development of the outcomes considered in this study. Of these markers, sTNFR-1 and sTNFR2 seem to be the most useful for inclusion in clinical trials of relatively short duration.

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References

1. 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
2. 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
3. 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
4. Lopes-Virella MF, Baker NL, Hunt KJ, Cleary PA, Klein R, Virella G; DCCT/EDIC Research Group. Baseline markers of inflammation are associated with progression to macroalbuminuria in type 1 diabetic subjects. Diabetes Care 2013;36:2317–2323
5. Gils A, Declerck PJ. Plasminogen activator inhibitor-1. Curr Med Chem 2004;11:2323–2334
6. Nathan DM, Genuth S, Lachin J, et al.; 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
7. Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. 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
8. 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
9. Jenkins AJ, Lyons TJ, Zheng D, et al.; DCCT/EDIC Research Group. Serum lipoproteins in the diabetes control and complications trial/epidemiology of diabetes intervention and complications cohort: associations with gender and glycemia. Diabetes Care 2003;26:810–818
10. Jenkins AJ, Lyons TJ, Zheng D, et al.; DCCT/EDIC Research Group. Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. Kidney Int 2003;64:817–828
11. Klein RL, McHenry MB, Lok KH, et al.; DCCT/EDIC Research Group. Apolipoprotein C-III protein concentrations and gene polymorphisms in type 1 diabetes: associations with lipoprotein subclasses. Metabolism 2004;53:1296–1304
12. Lopes-Virella MF, McHenry MB, Lipsitz S, et al.; DCCT/EDIC Research Group. Immune complexes containing modified lipoproteins are related to the progression of internal carotid intima-media thickness in patients with type 1 diabetes. Atherosclerosis 2007;190:359–369
13. Lyons TJ, Jenkins AJ, Zheng D, et al.; DCCT/EDIC Research Group. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. Diabet Med 2006;23:955–966
14. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
15. Lee EW, Wei LJ, Amato D. *Cox-Type Regression Analysis for Large Numbers of Small Groups of Correlated Failure Time Observations*. Dordrecht, the Netherlands, Kluwer Academic, p. 237–247
16. de Boer IH, Sun W, Cleary PA, et al.; DCCT/EDIC Research Group. Intensive diabetes therapy and glomerular filtration rate in type 1 diabetes. N Engl J Med 2011;365:2366–2376
17. Viberti G, Mogensen CE, Groop LC, Pauls JF; European Microalbuminuria Captopril Study Group. Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. JAMA 1994;271:275–279
18. Laffel LMB, McGill JB, Gans DJ; North American Microalbuminuria Study Group. The beneficial effect of angiotensin-converting enzyme inhibition with captopril on diabetic nephropathy in normotensive IDDM patients with microalbuminuria. Am J Med 1995;99:497–504
19. 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
20. 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
21. 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
22. 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
23. Skrivarhaug 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
24. 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
25. 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
26. 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
27. 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