Markers of endothelial dysfunction and inflammation predict progression of diabetic nephropathy in African Americans with type 1 diabetes

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Abstract

African Americans with early onset type 1 diabetes mellitus are at a high risk for severe diabetic nephropathy and end-stage renal disease. In order to determine whether baseline plasma levels of inflammatory markers predict incidence of overt proteinuria or renal failure in African Americans with type 1 diabetes mellitus we reexamined data of 356 participants in our observational follow-up **study** of **725** New Jersey African-Americans with Type 1 diabetes. At baseline and 6-year follow-up, a detailed structured clinical interview was conducted to document medical history including kidney dialysis or transplant, other diabetic complications, and renal-specific mortality. Plasma levels of 28 inflammatory biomarkers were measured using a multiplex bead analysis system. After adjusting for baseline age, glycohemoglobin and other confounders, the baseline plasma levels of intercellular adhesion molecule-1(s-ICAM-1) in the upper two quartiles were respectively associated with a 3 to 5-fold increases in the risk of progression from none or microalbuminuria to overt proteinuria. Baseline plasma levels of the chemokine eotaxin in the upper quartile were significantly associated with a 7-fold increase in risk of incident renal failure. These associations were independent of traditional risk factors for progression of diabetic nephropathy. Thus, in type 1 diabetic African Americans, s-ICAM-1 predicted progression to overt proteinuria and eotaxin predicted progression to renal failure.

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DISCLOSURE

As principal investigator, I, Monique Roy, declare there are no conflicts of interest to disclose and no conflicting relationship exists for any author.

INTRODUCTION

African Americans with early onset type 1 diabetes mellitus are at a high risk for severe diabetic nephropathy and end-stage renal disease (1–3). For example, in the New Jersey 725 study, 49.8% of type 1 diabetic African Americans had some proteinuria at the baseline examination, 17% progressed over 6 years from no albuminuria to overt proteinuria, and 8.7% to end-stage renal disease (3). Clinical risk factors for progression of diabetic nephropathy include albumin excretion rate (AER), hypertension, and hyperglycemia (3). High AER is used clinically as an indicator of progression to overt proteinuria (4). However, in a significant proportion of type 1 patients, remission from micro-albuminuria to normal albumin excretion may occur (5). Furthermore, renal function may be lost years before proteinuria develops suggesting an alternative pathway to severe diabetic nephropathy (5). Thus, there is a need to identify novel predictors of progression of diabetic nephropathy, particularly of advanced chronic kidney disease, in order to better understand the development of diabetic nephropathy and reduce morbidity and mortality from the disease.

In diabetic nephropathy, progressive thickening of the glomerular basement membrane is followed by mesangial cell expansion and gradual progression to glomerulosclerosis and interstitial fibrosis, eventually resulting in renal failure (6). There is clinical and experimental evidence suggesting that inflammation may be involved in the development or progression of diabetic nephropathy (7–10). Infiltration of macrophages in the glomeruli and tubular interstitium with over expression of adhesion molecules and proinflammatory molecules has been documented within the diabetic human kidney and occurs early after induction of experimental diabetes in rat models (11,12). Compared with diabetic intercellular adhesion molecule (ICAM) +/+ mice, ICAM deficient diabetic mice have lower AER and show less macrophage infiltration of the kidneys, less glomerular hypertrophy, less mesangial matrix expansion, and less fibrosis (13).

Previously published clinical studies examining the association of inflammatory biomarkers and diabetic nephropathy have been limited by their cross sectional design and the few prospective studies have yielded conflicting results (14–27). Thus, the purpose of the present study was to use our longitudinal data for the New Jersey 725 cohort to examine whether baseline plasma levels of markers of inflammation or endothelial dysfunction predict progression of diabetic nephropathy and, if so, whether there are different markers of overt proteinuria or renal failure in our African Americans with type 1 diabetes.

RESULTS

Baseline characteristics of the African American patients by follow-up renal status, progression to overt proteinuria or renal failure, are shown in table 1. Baseline mean arterial blood pressure (MAP), glycohemoglobin, high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate (eGFR), presence of heart disease or stroke, lower extremity arterial disease (LEAD), and microalbuminuria were associated with increased risk of progression to overt proteinuria. Older age, longer duration of diabetes, MAP, glycohemoglobin, total cholesterol, low-density liproprotein cholesterol (LDL-C), eGFR, presence of heart disease or stroke, LEAD, micro and overt-proteinuria, and the use of

angiotensin converting enzyme inhibitor (ACE) were associated with increased risk of incident renal failure.

Baseline plasma levels of the inflammatory biomarkers in relation to known risk factors for incidence of proteinuria are presented in table 2. C-reactive protein (CRP) was the only biomarker significantly associated with MAP (r=0.24, p<0.001), total cholesterol (r=0.16, p<0.01) and LDL-C (r=0.19, p<0.001).

Incidence of overt proteinuria

Of the 264 patients who had no proteinuria or micro-albuminuria at baseline, 51 (19.3%) developed overt proteinuria over 6 years. Baseline plasma levels of the soluble adhesion molecule sICAM-1 were significantly associated with the incidence of overt proteinuria on univariate analysis (table 3). When sICAM-1 was entered into the multiple regression model as a second step after entering clinical variables, it made an independent contribution to the prediction of overt proteinuria in addition to baseline micro-albuminuria and MAP (p= 0.03). Relative to the first quartile, sICAM-1 values above the median (164 pg/ml) were associated with 3 to 5-fold increase in risk of incident proteinuria (p=0.02 and p=0.006, respectively, for 3rd and 4th quartiles) (table 4).

Incidence of renal failure

Of the 356 patients who at baseline had an eGFR 60 mL/min and did not have end-stage diabetic renal failure requiring dialysis or kidney transplant, 63 (17.7%) developed renal failure over the 6-year follow-up [56 with eGFR<60 mL/min (14 of whom were on dialysis), 1 had a kidney transplant, and 6 died of diabetic renal failure]. Baseline plasma levels of the cytokine tumor necrosis factor-α (TNF-α), chemokines [eotaxin, interleukin-8 (IL-8), and monocyte chemo-attractant protein-1 (MCP-1), and soluble adhesion molecules [sICAM-1 and soluble vascular cell adhesion molecule-1(sVCAM-1)] were significantly associated with the incidence of renal failure on univariate analysis (table 5). When all six markers were entered into the multiple regression model as second step after entering clinical variables, only eotaxin made an independent and strong contribution to the prediction of renal failure in addition to baseline glycohemoglobin, AER, eGFR, and use of ACE (p=0.007). Relative to the first quartile, only eotaxin values in the upper quartile (>67.5 pg/ml) were associated with >7-fold increase in risk of renal failure (p=0.001) (table 4).

In order to understand why inflammatory markers (other than eotaxin) significant on univariate analysis were not independent predictors of renal failure, an exploratory stepwise logistic regression analysis was computed including inflammatory markers which were significant on univariate analysis (table 5), but omitting the clinical factors. Results (not shown) identified two independent predictors of renal failure: first, after eotaxin entered the model, IL-8, TNF-α, and MCP-1 became non-significant, suggesting that these biomarkers are part of the same pathway in relation to incidence of renal failure; on the second step, sVCAM-1 entered the model (p= 0.02) and sICAM-1 was not significant, indicating that these markers may be part of a second pathway. Because in the first regression model eotaxin is a predictor independent of the clinical variables, we can infer that sVCAM-1 failed to enter that regression because of its relationship with clinical variables.

DISCUSSION

In the present study of African Americans with type 1 diabetes, baseline plasma sICAM-1, a soluble adhesion molecule, was significantly associated with progression from none or micro-albuminuria to overt proteinuria, while the chemokine eotaxin was significantly associated with the incidence of renal failure. These associations were independent of known risk factors for incident diabetic nephropathy, i.e., baseline AER, eGFR, glycemic control, hyperlipidemia, or cardiovascular disease (CVD). The fact that these inflammatory markers differ for the two outcomes suggests that the pathway to overt proteinuria may be distinct from that to renal failure.

Studies examining inflammatory markers in relation to incident diabetic nephropathy have yielded conflicting results (14–27). In support of our data, cross-sectional studies have shown that plasma levels of s-ICAM-1 are elevated in type 1 diabetic patients with microalbuminuria when compared with non-diabetic persons, and are higher in patients with greater severity of diabetic nephropathy (14,15,19,20). Cross-sectional studies, however, are not sufficient to infer causal relationship(s). In a longitudinal study of type 2 diabetic patients, Persson et al. reported that sICAM-1 predicted progression from micro-albuminuria to overt proteinuria independently of traditional risk factors for progression of diabetic nephropathy (28). In Astrup's 10-year follow-up study of type 1 diabetic patients, the mean inflammatory score, which included sICAM-1, was independently associated with the decline in GFR (25). In the Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications cohort, another adhesion molecule, E-selectin was also significantly associated with progression to overt proteinuria in type 1 white diabetic patients (27).

The possible role of sICAM-1 in relation to the progression of diabetic nephropathy is supported by experimental data (12,13). In streptozotocin-induced diabetic rats, increased sICAM-1 expression in the glomeruli is associated with an increased mononuclear cell infiltration that can be prevented by either anti ICAM monoclonal antibody or insulin treatment (12). Treatment of these animals with aldose reductase prevents glomerular hyperfiltration and decreases glomerular ICAM expression and mononuclear infiltration, suggesting that in experimental diabetic nephropathy sICAM-1 is upregulated and promotes the recruitment of mononuclear cells in the glomerulus (12).

The adhesion molecule sICAM-1 is an endothelial ligand for integrin expressed on leukocytes and platelets. Its activity results in the firm adhesion of circulating leukocytes to the vascular endothelium (29). Thus, the present study adds to the literature suggesting that progression to overt proteinuria is related to a pathway involving soluble adhesion molecules. As previously suggested, the ensuing leukostasis in the glomerular capillaries and interstitial inflammation seen in the diabetic kidney may represent an early phase in the progression of renal disease (11,30).

In the present study, a different inflammatory marker, the chemokine eotaxin, was an independent predictor of the incidence of renal failure. To the best of our knowledge, there is no previously published report of increased *plasma* eotaxin as a predictor of progression

to renal failure in diabetic persons. In micro-albuminuric type 2 diabetic patients, urinary eotaxin levels were increased compared with normo-albuminuric patients or controls (31). Clamped hyperglycemia increases excretion of urinary eotaxin as well as of other mediators of inflammation (32).

Eotaxin is a potent eosinophilic chemokine and also has angiogenic properties through activation of its CC chemokine receptor-3 (33–35). Our data also indicate that eotaxin is part of a pathway involving other inflammatory markers with which it co-varies. Some of these markers have angiogenic properties, i.e., IL-8, and TNF-α, while others also contribute to leukostasis and interstitial inflammation, i.e., TNF-α and the chemokine MCP-1 (9,10,26,36,37). Thus, eotaxin and co-varying inflammatory markers may be part of a complex pathway resulting in glomerulosclerosis and interstitial fibrosis as seen in advanced chronic kidney disease (11,30).

Whether systemic inflammatory biomarkers cause direct renal injury or whether they are the manifestation of a disease process resulting in renal injury is not known. In the present study, sICAM-1 and eotaxin predict progression of diabetic nephropathy independently of glycemic control, hyperlipidemia, hypertension, or CVD, although one cannot exclude that repeated episodes of hyperglycemia could contribute overtime to progressive renal injury (32,38–40).

Strengths of the study include its prospective design; inclusion of a large (356) number of type 1 diabetic African Americans, for whom detailed clinical evaluations were available from two visits; and measurement of a large number of markers of both inflammation and endothelial dysfunction which were examined for their individual and combined actions after adjusting for potential confounders.

Limitations of the study include the fact that there is the possibility of protein degradation of the samples, despite storage at −70°C (41). Multiple urine collections were not obtained. Repeated documentation of changes in proteinuria, blood creatinine, GFR status, and inflammatory marker levels over time was not available. The imprecision of the Modification of Diet in Renal Disease (MDRD) equation for eGFR values within the normal range may have led to misclassification of the patients (42). Finally, we cannot exclude the possibility of survival bias for this group of patients despite the fact that the follow-up rate was good. Thus, our present data should only be considered applicable to this population.

In the present study of African Americans with type 1 diabetes, the adhesion molecule sICAM-1 predicted progression to overt proteinuria while the chemokine eotaxin predicted progression to renal failure suggesting that these two inflammatory markers may be part of separate pathways of progression of renal disease.

METHODS

Study population

The original cohort consisted of 725 African Americans with type 1 diabetes who participated in the New Jersey 725 study between 1993 and 1998 (43). Patients were

identified from among 68455 African Americans listed in the New Jersey Department of Health computerized Hospital Discharge Data as having a diagnosis of diabetes mellitus. Of those, a review of randomly chosen 13615 patient charts was conducted in 31 participating hospitals. Patients with a discharge diagnosis of type 1 diabetes, acute onset of diabetes mellitus, treated with insulin before 30 years of age, and currently on insulin were included. The use of insulin was confirmed at the time of first contact with the patient. Excluded were patients diagnosed with acute onset of diabetes mellitus before age 30 but not currently on insulin, those diagnosed with diabetes after age 30, and patients with a discharge diagnosis of maturity-onset diabetes of youth (44). Of the 875 eligible patients, 725 participated.

Of the 725, 508 (70.1%) underwent a 6-year follow-up examination, 44 (6.1%) could not be located, 34 (4.7%) refused examination, and 139 (19.2%) had died (45). At the 6-year follow-up, 25 (4.9%) participants no longer on insulin were excluded, leaving 483 eligible for analysis. Also excluded were one patient with systemic lupus, one with Buerger's disease, and one with multiple sclerosis in whom adequate urine collection could not be obtained. This report concerns 356 patients who had baseline plasma samples available for measurement of the markers, had a 6-year follow-up examination (or died), and were at risk for incident renal failure, and a subset of 264 patients with no proteinuria or micro-albuminuria at baseline who progressed to overt proteinuria at 6-years. The mean (± standard deviation) follow-up was 6.0 (±0.3) years.

Procedures

Patients were examined in University Hospital's Eye Clinic, Newark, NJ. The same procedures were followed at baseline and follow-up visits. Upon arrival, informed written consent was obtained. A structured clinical interview was conducted to document medical [dialysis and kidney transplant for diabetic renal failure, infections, use of ACE inhibitor and statin medications, and past history of coronary disease, stroke, or LEAD], and socio-demographic and life-style variables (smoking). Height and weight were obtained. Blood pressure was measured twice in sitting and standing positions, and the average was used.

A 4-h timed urine collection was obtained for measurement of AER and creatinuria, using spectrophotometry (Quest Diagnostics, Madison, NJ). Venous blood was drawn for measurement of creatinine by spectrophotometry, HDL-C, LDL-C and total cholesterol using an enzymatic assay and separation spectrophotometry (Quest Diagnostics), and total glycosylated hemoglobin using high-pressure liquid chromatography (Labcorp, Burlington, NC). eGFR was calculated using serum creatinine and the Modification of Diet in Renal Disease equation (MDRD) (42). A 2 ml venous blood sample was collected by venipuncture in an EDTA-coated vacutainer tube, the content was thoroughly mixed and plasma separated by centrifugation and stored frozen at −70°C for future assay.

The research followed the tenets of the Declaration of Helsinki. The Institutional Review Board of the New Jersey Medical School, Newark, NJ approved the study.

Measurements of the inflammatory biomarkers

Juan Crosby, who measured the markers, was masked to the clinical data. Baseline plasma samples stored at −70°C were analyzed for 28 inflammatory biomarkers: 9 cytokines and

soluble receptors [interleukin-1α (IL-1α), IL-2 receptor (IL-2R), IL-6, IL-10, IL-12p40, IL-12p70, soluble CD40 ligand (sCD40), TNF-α, interferon-γ (IFN-γ)]; three growth factors, granulocyte macrophage colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF); 12 chemokines [eotaxin, fractalkine, growth-related oncogene-α (GRO-α), IL-8, MCP-1, MCP-3, regulated on activation normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1ß (MIP-1β), interferon-inducible protein-10 (IP-10), stromal-derived factor-1 (SDF-1), neutrophil-activating peptide (ENA-78)]; 4 soluble adhesion molecules, E-selectin, sICAM-1, sVCAM-1, and CRP.

Biomarker concentrations were measured using a multiplex bead analysis system (Milliplex X-MAP, EMD Millipore Corp, Billerica, MA). Intra-assay and inter-assay variations were <15% and 18%, respectively. Measurements were done using 25 μl samples. After overnight incubation in 96-well plates, the specific fluorescence corresponding to each biomarker was measured on the Luminex 100 instrument (Luminex, Austin, TX). Quantification was done against 4-parameter logistic regression-generated standard curves using the reference cytokine standards supplied by the kit manufacturer. For statistical analysis, the biomarkers concentrations below the lowest concentration point in the standard curves were given a value of zero.

Definitions

Incidence of overt proteinuria was calculated for patients who had an AER 200 mcg/min, were not on dialysis, and had not received a kidney transplant for diabetic renal failure at baseline and who had an AER>200 mcg/min at the 6-year follow-up. Incidence of renal failure was calculated for patients who had an eGFR 60 mL/min at baseline and who developed renal failure at follow-up: either eGFR<60 mL/min, on dialysis or had a kidney transplant for diabetic renal failure, or were identified in the National Death Index as having died from renal failure.

Patient's age was defined at baseline. MAP was calculated as: diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure). CVD was present if, at baseline, the patient reported: (i) foot or leg amputation for LEAD; (ii) coronary disease or myocardial infarction (heart disease); or (iii) stroke. Baseline CVD was confirmed by review of medical records of hospital admissions. Smoking was defined as ever smoked and alcohol consumption as heavy if the patient reported ever consuming 4 or more alcoholic beverages a day for at least one year.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (v.21; Armonk, NY). Preliminary inspection showed that the distribution of each baseline inflammatory biomarkers, IL-1α, IL-2R, IL-6, IL-10, IL-12p40, IL-12p70, sCD40, TNF-α, IFN-γ, GM-CSF, PDGF, VEGF, eotaxin, fractalkine, GRO-α, IL-8, MCP-1, MCP-3, RANTES, MIP-1α, MIP-1β, IP-10, SDF-1, ENA-78, E-selectin, sICAM-1, sVCAM-1, and CRP, was positively skewed, thus all were transformed two ways prior to analysis: first, each marker was rank transformed for computation of Spearman correlations between biomarkers and known risks for incident

proteinuria; second, each marker was transformed to a quartile scale to examine ORs relating baseline marker levels to incident overt proteinuria or renal failure in logistic regression models. P values <0.05 were considered significant.

Multiple logistic regression analyses were run by first building the best predictive model from the following baseline characteristics: age, age at diagnosis, AER (categorized as normal, micro-albuminuria, or overt proteinuria), eGFR, glycohemoglobin, body mass index, MAP, LEAD, either heart or stroke, blood cholesterol, smoking, and use of ACE or statin medications. In a second step, inflammatory biomarker(s) were allowed to compete for inclusion as additional contributors to the prediction of both outcomes, and to identify confounding between biomarkers and clinical characteristics. In order to limit the inclusion of false positives among the 28 inflammatory markers, only those with univariate p-levels<0.01 competed in the primary multivariate analyses.

Acknowledgments

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Table 1

Baseline characteristics of African Americans with type 1 diabetes (DM) by follow-up renal status

Characteristics	Overt Proteinuria		Renal failure	
	None	Present	None	Present
	(N=213)	(N=51)	(N=293)	(N=63)
Mean (SD)				
Age (years)	24.6 (10.2)	27.1 (9.8)	25.5 (10.2)	32.3 (9.5) [c]
Duration of DM (years)	7.9 (7.9)	9.5 (5.8)	8.4 (8.0)	13.8 (7.5) [c]
Age at diagnosis (years)	16.4 (7.7)	17.1 (7.4)	16.8 (7.6)	17.8 (7.1)
BMI (Kg/m^2)	26.8 (7.9)	28.8 (9.4)	28.0 (8.8)	27.2 (7.2)
MAP (mmHg)	86.0 (11.6)	91.8 (11.2) [c]	87.8 (12.1)	95.2 (13.0) [c]
Glycohemoglobin (%)	13.8 (4.1)	15.2 (4.3) [a]	13.6 (4.2)	15.7 (4.6) [c]
Total cholesterol (mg/dL)	193.8 (45.4)	203.2 (36.7)	196.1 (43.8)	233.6 (77.0) [c]
HDL-C (mg/dL)	54.8 (17.3)	55.6 (19.2)[a]	54.7 (17.5)	55.1 (18.1)
LDL-C (mg/dL)	99.7 (33.4)	110.6 (32.8)	102.8 (34.4)	116.8 (44.7)[b]
eGFR (mL/min)	108.2 (26.8)	116.1 (22.8)[a]	109.4 (26.5)	95.9 (21.2)[c]
%				
Male / Female	39.4 / 60.6	41.2 / 58.8	42.0 / 58.0	36.5 / 63.5
Heart/stroke (no / yes)	96.2 / 3.8	86.3 / 13.7[b]	94.2 / 5.8	86.0 / 14.0[a]
LEAD (no / yes)	99.1 / 0.9	96.1 / 3.9[d]	97.6 / 2.4	91.2 / 8.8[a]
Micro-albuminuria (no / yes)	80.8 / 19.2	58.8 / 41.2 [c]	77.5 / 22.5	44.1 / 55.9[c]
Overt proteinuria (no / yes)		--	93.4 / 6.6	56.7 / 43.3[c]
ACE medication (no / yes)	94.3 / 5.7	96.1 / 3.9	95.2 / 4.8	84.2 / 15.8[b]
Statin medication (no / yes)	99.1 / 0.9	98.0 / 2.0	98.6 / 1.4	95.2 / 4.8

Abbreviations: DM, diabetes mellitus; microalbuminuria, albumin excretion rate (AER), 20–200 mcg/min; overt proteinuria, AER >200 mcg/min; eGFR (estimated glomerular filtration rate); renal failure, eGFR<60 mL/min, dialysis, kidney transplant, or renal failure as cause of death; BMI, body mass index; MAP, mean arterial blood pressure; HDL–C, high-density lipoprotein cholesterol, LDL–C, low-density lipoprotein cholesterol; LEAD, lower extremity arterial disease; ACE, angiotensin converting enzyme inhibitor.

[a] p< 0.05,

[b] $p < 0.01$,

[c] $p < 0.001$,

[d] sample size did not allow for statistical analysis.

Table 2

Associations (expressed as Spearman correlation coefficient) between baseline biomarker levels and known predictors of incident proteinuria[*] in African Americans with type 1 diabetes

	Age	BMI	GlycoHb	AER	eGFR
Cytokines					
IL1α	-0.23^c	-0.17^c	NS	-0.14^b	NS
sIL-2R	-0.21^c	-0.13^a	NS	NS	NS
IL-6	-0.14^b	NS	NS	NS	NS
IFN γ	-0.16^b	-0.11^a	NS	-0.12^a	NS
IL-10	-0.16^b	NS	-0.13^b	NS	NS
IL-12p40	-0.14^b	NS	NS	-0.11^a	NS
IL12p70	-0.22^c	-0.12^a	NS	NS	0.13^b
IP-10	0.11^a	NS	-0.13^b	NS	NS
TNF-α	-0.12^a	NS	NS	0.14^b	NS
Growth factors					
VEGF	-0.14^b	NS	NS	NS	0.13^b
Chemokines					
MCP-1	NS	NS	NS	NS	NS
MIP-1β	NS	NS	0.15^b	NS	NS
ENA-78	NS	NS	0.20^c	NS	NS
Fractalkine	-0.18^b	-0.11^a	NS	NS	0.10^a
GM-CSF	NS	0.18^b	-0.14^b	NS	NS
GRO-α	NS	NS	0.11^a	NS	0.10^a
MCP-3	-0.21^c	-0.16^b	NS	NS	NS
SDF1	-0.15^b	-0.11^a	-0.12^a	-0.16^b	NS
PDGF	NS	NS	0.10^a	NS	NS

	Age	BMI	GlycoHb	AER	eGFR
Eotaxin	NS	-0.22^c	0.12^a	NS	NS
sCD-40L	NS	NS	NS	NS	0.13^b
IL-8	NS	-0.13^a	NS	0.14^b	NS
Soluble Adhesion Molecules					
E-selectin	-0.11^a	NS	0.22^c	0.11^a	0.17^c
sICAM-1	NS	NS	NS	0.11^a	NS
sVCAM-1	-0.12^a	-0.25^c	NS	NS	NS
CRP	0.24^c	0.54^c	NS	NS	NS

Abbreviations: IL-1α, Interleukin-1 α; sIL-2R, Interleukin-2 receptor; IL-6, Interleukin-6; IL-8, Interleukin-8; INF-γ, Interferon-γ; IL-10, Interleukin-10; IL-12p40, Interleukin-12 p40; IL-12p70, Interleukin-12 p70; IP-10, Interferon-inducible protein-10; TNF-α, Tumor necrosis Factor-α; MCP-1, Monocyte chemoattractant protein-1; MIP-1β, Macrophage inflammatory protein-1β; ENA-78, Neutrophil-activating peptide; GM-CSF, Granulocyte macrophage colony stimulating factor; GROα, Growth-regulated oncogene α; MCP-3, Monocyte chemoattractant protein-3; VEGF, Vascular endothelial growth factor; SDF-1, Stromal-derived factor-1α+β; PDGF, Platelet derived growth factor; sCD-40L, Soluble CD40 Ligand; sICAM-1, Soluble intercellular adhesion molecule-1; sVCAM-1, Soluble vascular cell adhesion molecule-1, CRP, C-reactive protein; BMI, body mass index; GlycoHb, glycosylated Hemoglobin; AER, albumin excretion rate.

*
Either AER>200 mcg/min, dialysis, or kidney transplant, or eGFR (estimated glomerular filtration rate) <60 mL/min.

Only significant associations are shown:

a
p< 0.05,

b
p < 0.01,

c
p < 0.001. NS indicates that p 0.05.

Table 3

Relationship between baseline sICAM-1[*] levels and the incidence of overt proteinuria[†] in African Americans with type 1 diabetes: univariate analyses

	Incidence of overt proteinuria[†] OR (95% CI)[‡]
sICAM-1 (pg/mL)[*]	
<122.9	1
122.9–164.2	1.57 (0.58, 4.23)
164.3–232.0	3.39 (1.33, 8.62)[a]
232.1	4.39 (1.55, 12.42)[a]
p for overall model	0.01

[*] sICAM-1, Soluble intercellular adhesion molecule expressed as quartiles;

[†] albumin excretion rate >200mcg/min;

[‡] odds ratio (95% confidence interval), with reference to the first quartile of sICAM-1.

[a] $p < 0.01$.

Table 4

Relationship between baseline inflammatory markers[*] and incidence of overt proteinuria[†] and renal failure[‡] in African Americans with type 1 diabetes: Multivariate analysis

Baseline variables		Overt Proteinuria§		Renal Failure‖	
		OR (95% CI) ¶	p#	OR (95% CI) ¶	p#
sICAM-1 (pg/mL)	Quartile		0.03**		0.007**
	1st	1		1	
	2nd	1.82 (0.64, 5.15)	0.26	3.01 (0.95, 9.50)	0.06
	3rd	3.28 (1.21, 8.88)	0.02	2.87 (0.87, 9.43)	0.08
	4th	4.72 (1.55, 14.41)	0.006	7.66 (2.38, 24.66)	0.001
Eotaxin (pg/mL)	Quartile				
Glycohemoglobin (per 1%)		1.19 (1.09, 1.31)	<0.001		
Proteinuria					
Microalbuminuria vs normal		2.27 (1.12, 4.58)	0.02	4.59 (1.96, 10.73)	<0.001
Overt vs normal		--		19.03 (7.27, 49.79)	<0.001
MAP (per mm Hg)		1.58 (1.15, 2.17)	0.004		
eGFR‡ (per mL/min)				0.98 (0.96, 0.99)	0.006
ACE (if used)				4.65 (1.30, 16.63)	0.02

[*] See abbreviations in table 2;

[†] albumin excretion rate (AER) >200 mcg/min;

[‡] estimated glomerular filtration rate (eGFR) <60 mL/min, renal transplant, dialysis, or renal failure as cause of death;

§baseline variables included in the model: age, age at diagnosis, body mass index, glycosylated hemoglobin, presence of either heart disease or stroke, lower extremity arterial disease, mean arterial blood pressure (MAP), low-density lipoprotein cholesterol (LDL), AER (normal:<20 mcg/min, microalbuminuria: 20–200 mcg/min, overt proteinuria: > 200 mcg/min), eGFR, use of statin or angiotensin converting enzyme (ACE) inhibitor medication, and smoking; for overt proteinuria, only sICAM-1 was modeled;

||for renal failure, competed but not included in the model, were quartiles of sICAM-1 and TNF-α;

¶odds ratio (95% confidence interval);

p value;

** omnibus test. Blank cells indicate that p 0.05.

Table 5

Relationship between baseline biomarker levels[*] and renal failure[†] in African Americans with type 1 diabetes: univariate analyses

	Incidence of renal failure[†] OR(95% CI)[‡]
CYTOKINES	
TNF-α (pg/mL)	
<3.4	1
3.4–5.3	1.31 (0.57, 3.03)
5.4–8.7	3.47 (1.62, 7.46)[b]
8.8	3.54 (1.39, 9.01)[b]
p for overall model	0.002
CHEMOKINES	
Eotaxin (pg/mL)	
<25.5	1
25.5–43.3	2.68 (1.05, 6.81)[a]
43.4–67.5	4.47 (1.78, 11.23)[c]
67.5	7.29 (2.88, 18.43)[c]
p for overall model	<0.001
IL-8 (pg/mL)	
<1.5	1
1.5–3.2	2.90 (1.22, 6.89)[a]
3.3–6.8	3.26 (1.33, 8.0)[b]
6.9	4.04 (1.63, 10.0)[b]
p for overall model	0.02
MCP-1 (pg/mL)	
<141.5	1
141.5–194.4	1.41 (0.58, 3.40)
194.5–281.3	2.28 (0.98, 5.34)
281.4	3.02 (1.27, 7.21)[a]
p for overall model	0.05

	Incidence of renal failure[†] OR(95% CI)[‡]
SOLUBLE ADHESION MOLECULES	
sICAM-1 (pg/mL)	
<122.9	1
122.9–164.2	0.52 (0.21, 1.28)
164.3–232.0	1.78 (0.86, 3.68)
232.1	2.20 (1.02, 4.75)[a]
p for overall model	0.006
sVCAM-1 (pg/mL)	
<724.4	1
724.4–932.1	1.0 (0.42, 2.24)
932.2–1185.9	2.28 (1.09, 4.77)[a]
1186.0	2.02 (0.87, 4.73)
p for overall model	0.05

[*] Please see abbreviations for each biomarker (in pg/mL, expressed as quartiles) in table 2;

[†] estimated glomerular filtration rate <60 ml/min, dialysis, kidney transplant, or renal failure as the cause of death;

[‡] odds ratio (95% confidence interval), with reference to the first quartile of each biomarker.

[a] $p < 0.05$;

[b] $p < 0.01$;

[c] $p < 0.001$.