The Relationship of Serum Soluble Receptor for Advanced Glycation End Products (sRAGE) and Carboxymethyl Lysine (CML) to the Incidence of Diabetic Nephropathy in Persons With Type 1 Diabetes

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We hypothesized that persons with type 1 diabetes with higher serum levels of carboxymethyl lysine (CML) and soluble receptor for advanced glycation end products (sRAGE) are at a higher risk of developing incident diabetic nephropathy (DN). We examined this hypothesis using data from up to four follow-up examinations over a 22-year period in persons with type 1 diabetes participating in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). We also examined the relationship of CML and sRAGE to mortality.

Descriptions of the cohort, participation statistics, and reasons for nonparticipation have appeared elsewhere (1). Of 1,210 persons with type 1 diabetes receiving care in an 11-county area of south central Wisconsin in 1979–1980, 996 were examined in 1980–1982. The individuals were 3–79 years of age with type 1 diabetes duration of 1–59 years. These persons participated in up to six follow-up examinations in 1984–1986 ($n = 903$), 1990–1992 ($n = 816$), 1994–1996 ($n = 667$), 2000–2001 ($n = 567$), 2005–2007 ($n = 520$), and 2012–2014 ($n = 414$).

The tenets of the Declaration of Helsinki were followed, and institutional review board approval was obtained from the University of Wisconsin–Madison. Written informed consent was obtained.

Starting with the third examination in 1990–1992 and continuing with follow-up examinations, serum was collected, processed, and frozen at −80°C. Serum sRAGE and CML levels were measured in 2,480 of these stored frozen samples at the University of Minnesota Advanced Research and Diagnostic Laboratory in Minneapolis in 2013 and 2014. Two batches (sets of reagents) were used. We included quality control samples in the two batches, and a Deming regression was performed to establish a calibration equation between the batches. CML levels were measured using a quantitative competitive ELISA (CircuLex CML/Nε-(carboxymethyl) lysine ELISA Kit; MBL International Corporation, Woburn, MA). Laboratory interassay coefficient of variation was from 12.4 to 15.8%. The sRAGE levels were measured using a quantitative sandwich ELISA (BioVendor, Asheville, NC). Laboratory interassay coefficient of variation was from 4.9 to 11.5%. CML and sRAGE were log transformed for analyses.

Proteinuria was defined as urine protein concentration of ≥30 mg/dL as measured by Labstix (Ames Elkhart, IN). Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate <60 mL/min/1.73m^2 using the CKD Epidemiology Collaboration definition (2). DN was defined by either the presence of proteinuria, CKD, dialysis, or renal transplant. When examining the DN subtypes proteinuria and CKD as distinct end points, participants with renal transplants or dialysis were excluded.

Multistate Markov (MSM) models were used to estimate incidence (3). For these analyses, there were three states: *1*) disease (DN) absent, *2*) disease (DN) present, and *3*) death, which allowed modeling incidence or regression of DN as well as mortality.

There were 676 participants with relevant data who contributed 2,350 person-intervals to the MSM analyses. There were 129 intervals in which a person developed DN; 85 in which a person regressed from DN; 30 in which a person without renal disease died; and 83 in which a person with DN died. The estimated 5-year incidence of DN was 15%. Adjusting only for duration of diabetes, higher levels of sRAGE were significantly associated with the incidence of DN (hazard ratio [HR] 1.12 per 0.2 log pg/mL [95% CI 1.04, 1.20]). A significant association remained while further adjusting for age of diagnosis of diabetes and HbA_{1c} level, as did associations of sRAGE with incidence of CKD and proteinuria (Table 1). CML level was not significantly associated

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Table 1—MSM models of sRAGE and CML relationships to incidence and disappearance of DN, CKD, and proteinuria and to death

	Transitions HR (95% CI)			
Models	Absent to present	Absent to death	Present to absent	Present to death
DN†	$N = 129$	$N = 30$	$N = 85$	$N = 83$
sRAGE (per 0.2 log pg/mL)	1.10 (1.03, 1.18)*	0.73 (0.56, 0.94)*	0.82 (0.75, 0.89)*	1.12 (1.06, 1.19)*
Duration (per 5 years)	1.23 (1.13, 1.35)*	1.48 (1.13, 1.95)*	0.93 (0.81, 1.07)	1.44 (1.32, 1.57)*
Age at diagnosis (per 5 years)	1.15 (1.03, 1.28)*	1.35 (0.85, 2.12)	1.12 (0.95, 1.31)	1.17 (1.03, 1.33)*
HbA_{1c} (per 1%)	1.44 (1.29, 1.62)*	1.01 (0.41, 2.47)	1.04 (0.90, 1.19)	1.23 (1.10, 1.37)*
CKD‡	$N = 102$	$N = 41$	$N = 22$	$N = 45$
sRAGE (per 0.2 log pg/mL)	1.38 (1.25, 1.52)*	0.85 (0.70, 1.04)	1.15 (0.95, 1.40)	1.22 (1.11, 1.34)*
Duration (per 5 years)	1.42 (1.27, 1.59)*	1.43 (1.17, 1.75)*	0.90 (0.69, 1.17)	1.57 (1.38, 1.80)*
Age at diagnosis (per 5 years)	1.39 (1.20, 1.62)*	0.97 (0.72, 1.32)	1.77 (1.25, 2.51)*	1.24 (1.04, 1.48)*
HbA_{1c} (per 1%)	1.52 (1.32, 1.75)*	1.52 (1.16, 1.99)*	1.01 (0.72, 1.41)	1.28 (1.08, 1.53)*
MABP (per 5 mmHg)	1.15 (1.04, 1.26)*	1.03 (0.83, 1.26)	0.87 (0.69, 1.09)	1.01 (0.90, 1.13)
Sex (male)	0.46 (0.29, 0.71)*	1.20 (0.52, 2.77)	0.23 (0.08, 0.68)*	1.62 (0.93, 2.79)
Proteinuria‡	$N = 97$	$N = 43$	$N = 84$	$N = 43$
sRAGE (per 0.2 log pg/mL)	1.07 (0.97, 1.18)	0.98 (0.76, 1.25)	0.90 (0.81, 0.98)*	1.19 (1.06, 1.33)*
Duration (per 5 years)	1.16 (1.03, 1.30)*	1.56 (1.26, 1.93)*	1.05 (0.90, 1.22)	1.70 (1.46, 1.98)*
Age at diagnosis (per 5 years)	0.86 (0.74, 1.00)	1.24 (0.84, 1.82)	0.93 (0.78, 1.11)	1.25 (1.03, 1.51)*
HbA_{1c} (per 1%)	1.43 (1.24, 1.64)*	1.05 (0.57, 1.93)	0.97 (0.81, 1.17)	1.38 (1.10, 1.73)*
MABP (per 5 mmHg)	1.16 (1.04, 1.29)*	0.98 (0.74, 1.30)	0.98 (0.87, 1.10)	0.96 (0.85, 1.09)
Smoking status (current)	2.18 (1.38, 3.43)*	1.95 (0.72, 5.27)	1.38 (0.81, 2.34)	1.26 (0.71, 2.25)
DN†	$N = 129$	$N = 30$	$N = 85$	$N = 83$
CML (per 0.2 log pg/mL)	1.04 (0.97, 1.11)	0.50 (0.34, 0.73)*	1.07 (0.97, 1.17)	1.08 (1.01, 1.16)*
Duration (per 5 years)	1.24 (1.14, 1.35)*	1.45 (1.10, 1.91)*	0.92 (0.80, 1.05)	1.48 (1.36, 1.62)*
Age at diagnosis (per 5 years)	1.15 (1.03, 1.28)*	1.67 (0.98, 2.86)	1.11 (0.94, 1.30)	1.13 (0.99, 1.29)
HbA_{1c} (per 1%)	1.45 (1.30, 1.61)*	0.97 (0.60, 1.56)	1.03 (0.90, 1.18)	1.21 (1.10, 1.34)*
CKD‡	$N = 102$	$N = 41$	$N = 22$	$N = 45$
CML (per 0.2 log pg/mL)	1.04 (0.93, 1.16)	0.72 (0.55, 0.94)*	1.35 (1.10, 1.66)*	1.12 (1.00, 1.25)*
Duration (per 5 years)	1.42 (1.28, 1.58)*	1.38 (1.11, 1.73)*	0.87 (0.67, 1.13)	1.53 (1.35, 1.75)*
Age at diagnosis (per 5 years)	1.44 (1.25, 1.66)*	0.84 (0.58, 1.21)	1.54 (1.07, 2.22)*	1.30 (1.09, 1.55)*
HbA_{1c} (per 1%)	1.41 (1.23, 1.63)*	1.76 (1.31, 2.35)*	0.92 (0.64, 1.32)	1.15 (0.96, 1.37)
MABP (per 5 mmHg)	1.09 (0.99, 1.19)	1.13 (0.92, 1.39)	0.82 (0.66, 1.02)	1.02 (0.92, 1.14)
Sex (male)	0.50 (0.33, 0.75)*	1.36 (0.51, 3.63)	0.51 (0.18, 1.47)	1.34 (0.80, 2.25)
Proteinuria‡	$N = 97$	$N = 43$	$N = 84$	$N = 43$
CML (per 0.2 log pg/mL)	1.00 (0.92, 1.09)	0.59 (0.27, 1.31)	0.98 (0.88, 1.09)	1.05 (0.94, 1.17)
Duration (per 5 years)	1.17 (1.06, 1.30)*	1.48 (1.13, 1.95)*	0.99 (0.86, 1.15)	1.76 (1.51, 2.04)*
Age at diagnosis (per 5 years)	0.87 (0.76, 1.00)	1.56 (0.59, 4.11)	0.93 (0.78, 1.09)	1.17 (0.97, 1.41)
HbA_{1c} (per 1%)	1.44 (1.26, 1.65)*	0.98 (0.47, 2.05)	1.02 (0.86, 1.22)	1.30 (1.09, 1.56)*
MABP (per 5 mmHg)	1.14 (1.03, 1.27)*	0.78 (0.44, 1.38)	0.97 (0.87, 1.08)	1.02 (0.89, 1.16)
Smoking status (current)	2.15 (1.38, 3.33)*	1.84 (0.40, 8.36)	1.43 (0.86, 2.40)	1.31 (0.75, 2.27)

Our MSM models have three states: *1*) disease present, *2*) disease absent, and *3*) death. Transitions between states are simultaneously modeled, resulting in four transitions. MABP, mean arterial blood pressure. *Statistically significant associations; *P* value <0.05. †Defined as either the presence of proteinuria, CKD, dialysis, or renal transplant. ‡Excludes cases with dialysis or renal transplant.

with the development of DN (or with either CKD or proteinuria) (Table 1).

Over the 22 years of follow-up of the cohort, 169 participants (25%) died. Adjusting for other factors, higher levels of sRAGE (HR 1.12 per 0.2 log pg/mL [95% CI 1.06, 1.19]) and CML (HR 1.08 per 0.2 log pg/mL [95% CI 1.01, 1.16]) were significantly associated with an increased hazard of death following DN (Table 1).

sRAGE but not CML was related to the incidence of DN in this cohort, and each was associated with poorer survival. These findings are consistent with those of the Steno and the Epidemiology and Prevention of Diabetes (EURODIAB) studies (4,5), which reported a relationship of sRAGE with the incidence of DN in persons with diabetes. Serum CML was not significantly related to the incidence of DN. In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study, higher skin collagen CML levels were associated with an increased 10-year risk of progression of DN (6).

In summary, we found evidence that serum levels of sRAGE are modestly but significantly associated with the incidence of DN independent of other risk factors studied. There were no significant relationships of serum CML to the incidence of DN. The relationship of sRAGE to incident DN warrants further investigation.

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