Osteopontin Is a Strong Predictor of Incipient Diabetic Nephropathy, Cardiovascular Disease, and All-Cause Mortality in Patients With Type 1 Diabetes

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OBJECTIVE

Osteopontin (OPN) is a multifunctional protein suggested to be a player in the arterial disease of patients with type 2 diabetes. However, its role for complications in patients with type 1 diabetes (T1D) is unknown. We therefore investigated the associations between OPN and diabetic vascular complications and all-cause mortality in patients with T1D.

RESEARCH DESIGN AND METHODS

Serum OPN was measured in 2,145 adults with T1D without end-stage renal disease (ESRD; dialysis or transplantation) as part of the Finnish Diabetic Nephropathy (FinnDiane) Study. Data on renal status, cardiovascular disease (CVD), and all-cause mortality during follow-up were verified from medical files, hospital discharge registries, and the Finnish National Death Registry, respectively. The median follow-up time was 10.5 (interquartile range 8.9–11.8) years.

RESULTS

Serum OPN was higher at baseline in patients who developed incident microalbuminuria (16.0 ± 0.9 vs. 14.1 ± 0.2 μg/L; $P = 0.04$), progressed to ESRD (28.3 ± 1.7 vs. 15.4 ± 0.2 μg/L; $P < 0.001$), suffered an incident CVD event (20.2 ± 1.2 vs. 15.5 ± 0.2 μg/L; $P < 0.001$), or died (23.3 ± 1.4 vs. 15.8 ± 0.2 μg/L; $P < 0.001$) during follow-up. In multivariate Cox regression analysis, OPN was independently associated with the development of incident microalbuminuria, an incident CVD event, and death, after adjustments for associated risk factors. Even after calculating reclassification indexes, OPN was predictive of CVD and all-cause mortality beyond the Framingham risk score covariates and hs-CRP.

CONCLUSIONS

Serum OPN is a strong predictor of incipient diabetic nephropathy, a first-ever CVD event, and all-cause mortality in patients with T1D. Serum OPN may be of clinical significance for the risk prediction of CVD events in patients with T1D.

Osteopontin (OPN) is a multifunctional protein expressed by several different cell types, although the bone is known to be a major source (1). The exact excretion pathway of OPN from the body is not known. OPN is involved in a number of physiological and pathological conditions, including cancer and progression of

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metastases (2), urinary stones (3), wound healing (4), chronic inflammatory and autoimmune diseases (5), obesity-related chronic inflammation, and insulin resistance (6). However, OPN was originally found in bone and shown to regulate the formation and calcification of bone tissue (7). Notably, OPN has also been linked to vascular remodelling and calcification, especially in diabetic arteries (8), and has been shown to associate with diabetic retinopathy (9) and nephropathy (10) in patients with type 2 diabetes (T2D), as well as cardiovascular disease (CVD) events in nondiabetic subjects with a history of coronary artery disease (CAD) (11). However, its role for late complications in patients with type 1 diabetes (T1D) is not known. Furthermore, the risk factor profile for vascular complications differs in T1D from that in T2D. The increased risk for CVD events in T2D is linked to the presence of hypertension, dyslipidemia, overweight, and insulin resistance, whereas the primary determinant of health in T1D is renal disease (12,13).

We therefore explored the association between serum OPN and diabetic vascular complications as well as all-cause mortality in a large well-characterized cohort of patients with T1D.

RESEARCH DESIGN AND METHODS

Study Subjects

The Finnish Diabetic Nephropathy (FinnDiane) Study is an ongoing, nationwide, prospective multicenter study seeking clinical, genetic, biochemical, and environmental risk factors for diabetes complications, with an emphasis on diabetic nephropathy. A detailed description of the follow-up protocol was presented earlier (13). T1D was defined as an onset of diabetes before the age of 40 years and permanent insulin treatment initiated within 1 year of diagnosis. In addition, all patients were C-peptide negative ($\leq$0.3 nmol/L) at the baseline visit. Only patients with T1D are explored in the FinnDiane Study.

The patients were recruited from all university and central hospitals in Finland (N = 21), all district hospitals treating patients with T1D (N = 33), and a substantial number of primary health care centers treating patients with T1D (n = 26; Supplementary Table 1). All adult patients in each center were invited to participate in the study, and the mean response rate was 78%. Thus, no specific groups of patients were excluded from this study. Although, the FinnDiane Study in a strict sense is not a population-based study, the geographical distribution and density well follow that of the Finnish population (13,14).

Serum OPN was estimated in baseline samples (N = 2,145) after excluding patients with end-stage renal disease (ESRD; dialysis or transplantation) at baseline because of the competing risk between ESRD and mortality. The median follow-up time for the study population was 10.5 (interquartile range [IQR] 8.9–11.8) years. The study protocol is in accordance with the Declaration of Helsinki as revised in 2000 and was approved by the local ethics committee in each study center. Written informed consent was obtained from each patient.

Ascertainment of Outcomes

Data on medication, cardiovascular status, and diabetes complications were registered by a standardized questionnaire that was completed by the patient's attending physician and thus immediately verified from the medical files. Blood pressure was measured twice by standard methods with the patient sitting, after the patient had rested for at least 10 min. The average of the two measurements was used in the analysis. Height, weight, and waist-to-hip ratio were recorded.

The baseline urinary albumin excretion rate (AER) was stratified as follows: normal AER was defined as $<$20 μg/min or $<$30 mg/24 h, microalbuminuria as 20 μg/min to $<$200 μg/min or 30 mg/24 h to $<$300 mg/24 h, and macroalbuminuria as AER $\geq$200 μg/min or $\geq$300 mg/24 h, in at least two of three consecutive urine collections. ESRD was defined as patients undergoing dialysis or having received a kidney transplant.

Follow-up data on verified renal status were collected by reexamination of the patients or review of the medical files. Information on CVD events and ESRD until the end of 2010 was obtained from medical files and by linking the FinnDiane data with the Finnish Hospital Discharge Register (HDR) and the Finnish Causes of Death Registry. The HDR is a register listing all discharged hospital patients, on each patient's unique personal identifier, using dates of admission and discharge, up to four diagnoses with the ICD and procedure codes based on the Nordic Classification of Surgical Procedures. The completeness and accuracy of the HDR with regard to vascular disease has been demonstrated to be very high (15).

Severe retinal disease was defined as a history of retinal laser treatment. CVD events were defined as a history of myocardial infarction (MI), a coronary artery procedure (bypass grafting surgery or angioplasty), stroke (ischemic or hemorrhagic), or a peripheral artery procedure (bypass grafting surgery or angioplasty), which were verified on the basis of ICD discharge codes specifying the events. CAD was defined as a history of MI or a coronary artery procedure. Limb amputations were further ascertained on the basis of ICD discharge codes specifying amputation, regardless of the presence or absence of documented peripheral vascular disease (PVD). In the prospective CVD event analysis, we only included patients without any CVD events at baseline. Deaths from any cause through to 18 September 2011 were identified via a search of the Finnish National Death Registry, and center databases. The causes of death in this study were CVD (44%), cancer (7%), infections (9%), and other causes (40%).

Assays

A1C was determined locally by standardized assays, and serum lipid and lipoprotein concentrations were analyzed centrally by automated enzymatic methods (Hoffman-La Roche, Basel, Switzerland). Serum creatinine was determined centrally by an isotope dilution mass spectrometry traceable assay, hs-CRP by a photometric, immunochemical method with an ultrasensitive kit (Orion Diagnostica, Espoo, Finland), and the Chronic Kidney Disease Epidemiology Collaboration formula was used to calculate the estimated glomerular filtration rate (eGFR) (16,17). The presented AER value was assessed from a 24-h urine collection by immunoturbidimetry. Furthermore, serum OPN was measured by an in-house time-resolved immunofluorometric assay using commercially available monoclonal antibodies (DY1433; R&D Systems). The limit detection was 10 ng/L. The intra- and interassay variability (coefficient of variation percentage)

were below 6% and 9%, respectively. The blood and urine samples were drawn at the patient's baseline visit to the study centers, and therefore, the storage time was similar to the follow-up time. Furthermore, the OPN measurements are stable during multiple freeze-thaw cycles.

Statistics

Analyses were performed with PASW Statistics 18 software (SPSS, Chicago, IL). Data for normally distributed and continuous variables are presented as mean ± SEM and data for nonnormally distributed variables as median with IQR. Differences between groups were analyzed with the Student *t* test, ANOVA, or Mann-Whitney *U* test, as appropriate. Categorical variables were analyzed using the Pearson χ^2 test. Longitudinal data were analyzed with Kaplan-Meier survival curves with log-rank tests. Risk factors for the progression of diabetes complications were assessed using Cox proportional hazard survival regression showing results as hazard ratios with 95% CI. The multivariate Cox regression analyses were adjusted for factors associated with serum OPN concentrations as well as other factors independently associated with the studied events by using the multivariate backward conditional option. The *P* value for inclusion of the clinical variables in the models was 0.05 and that for removal was 0.10. Furthermore, a model including the covariates in the Framingham risk score was calculated (18). OPN and hs-CRP were further compared using a receiver operating characteristic (ROC) curve analysis to evaluate the benefit of using OPN alone or on top of hs-CRP, as a clinical predictor for the CVD events.

Net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were used to assess the improvement in discrimination between models. NRI is the difference in percentage moving up and down risk strata among case subjects versus control subjects after the addition of a new biomarker to a known model. Ideally, the predicted probabilities would move up with a category for case subjects and down with a category for control subjects. The IDI is the difference in Yates slopes between the initial model and the initial model plus OPN, where the Yates or discrimination slope is the mean difference in predicted probabilities between case subjects and control subjects. The difference in slopes is a measure of improvement in the model. IDI and NRI are presented as percentage and should be interpreted as the increase of the difference in average predicted probabilities between case subjects and control subjects when OPN was added to the model (19).

With regards to CVD prevention, the 5%, 10%, and 20% cutoff points have been proposed as relevant for clinical decision making (20,21). The same cutoff points were used for both cardiovascular and renal end points. A Fine and Gray competing risk analysis including the same covariates as in the previous Cox regression analysis but also accounting for the competing events of pre-ESRD death and ESRD, as well as pre-CVD death and incident CVD events, was also performed, as earlier described. Stata 11 (2009) software (StataCorp LP, College Station, TX) was used for the discrimination analyses and the competing risk analyses (22).

RESULTS

Baseline Characteristics

Serum OPN was measured in 2,145 patients (52% men) with T1D without ESRD. Patient characteristics according to baseline quartiles of OPN are reported in Table 1. Briefly, the mean age of this cohort was 37.4 ± 0.3 years, and the median duration of diabetes was 20.1 (IQR 11.7–29.0) years. Their mean systolic blood pressure was 133 ± 1 mmHg, diastolic blood pressure (DBP) was 80 ± 1 mmHg, eGFR was 89 ± 1 mL/min per 1.73 m^2, and A1C was 8.5 ± 0.1% (69 ± 1 mmol/mol). At baseline, 1,395 patients had normal AER, 330 had microalbuminuria, and 420 had macroalbuminuria. Altogether, 135 patients had had a CVD event, and 687 patients had diabetic retinopathy requiring laser treatment at baseline.

Serum OPN concentrations were higher in patients with macroalbuminuria (24.1 ± 0.9 vs. 14.3 ± 0.2 μg/L; $P < 0.001$) and microalbuminuria (16.0 ± 0.6 vs. 14.3 ± 0.2 μg/L; $P = 0.001$) compared with those with normal AER. Furthermore, OPN concentrations were higher in patients with macroalbuminuria than in those with microalbuminuria (24.1 ± 0.9 vs. 16.0 ± 0.6 μg/L; $P < 0.001$). After adjustments for eGFR, the difference in OPN in the different albuminuria stages did not change ($P < 0.001$). Similarly, those who had experienced a CVD event (21.1 ± 1.3 vs. 16.1 ± 0.3 μg/L; $P < 0.001$) or had severe retinal disease (19.9 ± 0.6 vs. 14.9 ± 0.2 μg/L; $P < 0.001$) at baseline had higher OPN concentrations than those without, respectively. Patients with severe retinal disease without signs of nephropathy (normal AER) had higher serum OPN concentrations at baseline than those who did not have severe retinal disease (16.1 ± 0.7 μg/L vs. 14.0 ± 0.2 μg/L; $P = 0.001$).

Serum OPN correlated positively with duration of diabetes ($r = 0.14$; $P < 0.001$), waist-to-hip ratio ($r = 0.10$; $P < 0.001$), systolic blood pressure ($r = 0.10$; $P < 0.001$), hs-CRP ($r = 0.09$; $P < 0.001$), and AER ($r = 0.38$; $P < 0.001$) and negatively with eGFR ($r = -0.22$; $P < 0.001$). However, OPN did not correlate with age, BMI, DBP, or A1C at baseline.

OPN and Diabetic Nephropathy in Patients With T1D

During the median follow-up period of 6.1 (IQR 4.3–7.3) years, 313 patients progressed to a higher level of albuminuria or to ESRD (99 to microalbuminuria, 44 to macroalbuminuria, and 170 to ESRD). Serum OPN was higher at baseline in patients who developed microalbuminuria during the follow-up compared with those whose AER remained normal (16.0 ± 0.9 vs. 14.1 ± 0.2 μg/L; $P = 0.04$). Serum OPN did not differ at baseline in patients with microalbuminuria who became macroalbuminuric during follow-up compared with those who did not (16.7 ± 1.4 vs. 15.9 ± 0.6 μg/L; *P* = NS). In patients with macroalbuminuria who developed ESRD, OPN was higher at baseline (28.3 ± 1.7 vs. 15.4 ± 0.2 μg/L; $P < 0.001$). After adjusting for factors associated with serum OPN concentrations, as well as other factors independently associated with diabetic nephropathy, OPN predicted incident microalbuminuria but not progression to macroalbuminuria or ESRD (Table 2). However, in additionally performed models (models 2 and 3, Table 2), OPN was independently associated with the progression to ESRD during follow-up. The results did not change in a competing risk analysis considering pre-ESRD death as a

Table 1—Patient characteristics according to baseline quartiles (range) of serum OPN

	1st (0.5–9.0 μg/L)	2nd (9.0–14.0 μg/L)	3rd (14.0–20.6 μg/L)	4th (20.6–208.9 μg/L)	*P* value
n	533	537	539	536	—
Sex (% men)	41	50	55	61	<0.001
Age (years)	38 ± 1	37 ± 1	38 ± 1	37 ± 1	0.12
Duration of diabetes (years)	19 ± 1	20 ± 1	22 ± 1	23 ± 1	<0.001
Age at onset (years)	19 ± 1	17 ± 1	16 ± 1	14 ± 1	<0.001
BMI (kg/m^2)	25.1 ± 0.2	25.2 ± 0.1	24.9 ± 0.1	25.0 ± 0.2	0.39
Waist-to-hip ratio	0.85 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.001
Women	0.81 ± 0.01	0.80 ± 0.01	0.82 ± 0.01	0.81 ± 0.01	0.15
Men	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.89
Blood pressure (mmHg)					
Systolic	130 ± 1	132 ± 1	133 ± 1	135 ± 1	<0.001
Diastolic	80 ± 1	80 ± 1	79 ± 1	80 ± 1	0.29
A1C (%)	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.6 ± 0.1	0.04
A1C (mmol/mol)	68 ± 1	68 ± 1	68 ± 1	70 ± 1	0.04
Cholesterol (mmol/L)					
Total	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	0.32
HDL	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.01
LDL	3.3 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	<0.001
Triglycerides (mmol/L)	1.01 (0.78–1.43)	1.06 (0.77–1.45)	1.05 (0.78–1.57)	1.12 (0.82–1.59)	0.24
Insulin dose (IU/kg)	0.69 ± 0.01	0.71 ± 0.01	0.71 ± 0.01	0.71 ± 0.01	0.32
eGFR (mL/min per 1.73 m^2)	92 ± 1	93 ± 1	88 ± 1	83 ± 1	<0.001
Urinary AER (mg/24 h)	8.7 (5.9–18.6)	11.1 (6.6–36.9)	13.4 (7.2–64.5)	28.9 (9.0–327.7)	<0.001
Diabetic nephropathy (%)*	9	15	22	37	<0.001
Antihypertensive medication (%)	28	30	36	50	<0.001
History of CVD (%)	4	6	7	10	<0.001
Current smoking (%)	23	24	22	28	0.07
Severe retinal disease (%)	22	27	35	45	<0.001
hs-CRP (mg/L)	1.8 (1.2–3.4)	2.1 (1.3–4.0)	2.0 (1.2–3.8)	2.3 (1.4–5.0)	<0.001
OPN (μg/L)	6.3 ± 0.1	11.4 ± 0.1	16.9 ± 0.1	31.4 ± 0.6	<0.001

Continuous data are presented as mean ± SEM, except for AER, triglycerides, and hs-CRP where median (IQR) is presented. Categorical data are presented as percentages. *Patients with macroalbuminuria.

competing event (data not shown). When we explored the added predictive benefit to our risk factors models with reclassification indexes (NRI and IDI), OPN did not improve the models except for model 3 in patients with macroalbuminuria (Supplementary Tables 2 and 3).

Table 2—Cox regression analysis for the predictive value of serum OPN for incident microalbuminuria or progression to macroalbuminuria or ESRD

	Incident microalbuminuria		Progression to macroalbuminuria		Progression to ESRD	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Model 1	1.03 (1.01–1.05)	0.03	—	NS	—	NS
Model 2	1.03 (1.00–1.05)	0.03	—	NS	1.01 (1.00–1.02)	0.02
Model 3	1.03 (1.01–1.05)	0.009	—	NS	1.02 (1.02–1.03)	<0.001

HR, hazard ratio. Model 1 adjusted for sex, age, waist-to-hip ratio, current smoking, A1C, triglycerides, antihypertensive medication, eGFR, urinary AER, and hs-CRP. Model 2 adjusted for sex, age, duration of diabetes, BMI, waist-to-hip ratio, current smoking, A1C, triglycerides, LDL cholesterol, systolic blood pressure, antihypertensive medication, eGFR, urinary AER, and hs-CRP. Model 3 adjusted for the covariates in the Framingham risk score (sex, age, LDL cholesterol, HDL cholesterol, systolic blood pressure, diabetes, and smoking).

OPN and Incident CVD Events in Patients With T1D

Overall, 191 patients experienced an incident CVD event during the median follow-up period of 10.6 (IQR, 7.1–12.0) years. Serum OPN concentrations at baseline were higher in patients who had an incident CVD event compared with those who did not (20.2 ± 1.2 vs. 15.5 ± 0.2 μg/L; $P < 0.001$). Kaplan-Meier survival curves with quartiles of OPN revealed a clear association between the entire distribution of OPN for an incident CVD event (Fig. 1). After adjusting for the risk factors that were associated with OPN and CVD at baseline, OPN remained significantly associated with an incident CVD event in the multivariate Cox regression analysis (Table 3).

In the analysis of a possible benefit of using OPN on top of hs-CRP for the prediction of an incident CVD event during follow-up, including both markers in the same model, there was no additional predictive value (Supplementary Table 2 and Supplementary Fig. 1). However, after analyzing the added predictive benefit for CVD prediction with reclassification indexes (NRI and IDI), OPN

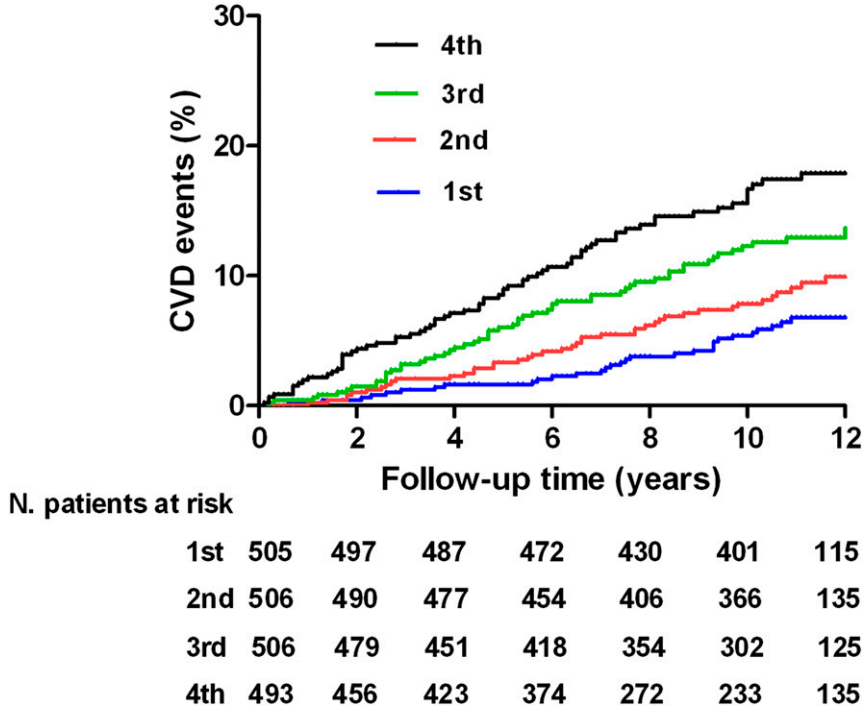


Figure 1—Kaplan-Meier survival curves with log-rank tests for a first-ever CVD event (n = 191) by quartiles of serum OPN concentrations. Log-rank $P < 0.001$. Patients with a history of CVD already at baseline (n = 135) were excluded.

improved model 3 even after adding hs-CRP to the Framingham risk factors (Supplementary Tables 2 and 3).

OPN remained an independent predictor of CVD events in a competing risk analysis considering pre-CVD death and incident CVD event as competing events (data not shown).

Table 3—Cox regression analysis for the predictive value of OPN for a first-ever CVD

	CVD event	
	HR (95% CI)	*P* value
Model 1	1.02 (1.01–1.03)	<0.001
Model 2	1.01 (1.00–1.02)	0.006
Model 3	1.03 (1.02–1.03)	<0.001

HR, hazard ratio. Model 1 adjusted for sex, age, waist-to-hip ratio, current smoking, A1C, total cholesterol, antihypertensive medication, eGFR, microalbuminuria, macroalbuminuria, and hs-CRP. Model 2 adjusted for sex, age, duration of diabetes, BMI, waist-to-hip ratio, current smoking, A1C, LDL cholesterol, systolic blood pressure, antihypertensive medication, eGFR, microalbuminuria, macroalbuminuria, and hs-CRP. Model 3 adjusted for the covariates in the Framingham risk score (sex, age, LDL cholesterol, HDL cholesterol, systolic blood pressure, diabetes, and smoking).

OPN and Incident CAD in Patients With T1D

During a median of 11.4 (IQR 10.3–12.1) years of follow-up, 109 patients experienced an incident CAD event. Serum OPN was increased at baseline in patients who had an incident CAD event at follow-up compared with those who did not (17.5 ± 1.2 vs. 14.5 ± 0.2 μg/L; P = 0.001). Serum OPN was not independently associated with incident CAD at follow-up after correcting for associated covariates in a multivariate Cox regression analysis (Supplementary Table 4). OPN did not yield any predictive benefit for CAD compared with previous risk factors models when analyzed by ROC curve analysis or reclassification indexes (Supplementary Fig. 1 and Supplementary Tables 2 and 3).

OPN and Incident Stroke in Patients With T1D

Sixty-two patients suffered an incident stroke (ischemic or hemorrhagic) during a median of 10.7 (IQR 7.8–12.0) years of follow-up. Serum OPN levels at baseline were higher in patients who had an incident stroke (20.5 ± 1.8 vs. 15.9 ± 0.3 μg/L; P = 0.001). After adjusting for factors associated with serum OPN concentrations, as well as other factors independently associated with stroke at baseline, OPN remained associated with stroke in a multivariate Cox regression analysis (Supplementary Table 4). OPN did not improve the risk factor models for prediction of stroke assessed by ROC curve analysis or reclassification indexes (Supplementary Fig. 1 and Supplementary Tables 2 and 3).

OPN and Incident PVD in Patients With T1D

As a whole, 20 patients underwent a leg revascularization procedure or an amputation (any cause) as their first cardiovascular event during follow-up (10.8 [8.2–12.0] years). Serum OPN concentrations at baseline were higher in patients who underwent an incident leg revascularization procedure or amputation (35.2 ± 9.7 vs. 15.9 ± 0.2 μg/L; P = 0.001). Furthermore, serum OPN independently predicted an incident PVD event in a multivariate Cox analysis (Supplementary Table 4). However, OPN did not add any predictive benefit to our previous models assessed by ROC curve analysis or reclassification indexes (Supplementary Fig. 1 and Supplementary Tables 2 and 3).

OPN and All-Cause Mortality in Patients With T1D

Altogether, 202 patients died during the median follow-up period of 10.5 (IQR 8.3–11.8) years. Serum OPN concentrations at baseline were higher in patients who died during follow-up compared with those who did not (23.3 ± 1.4 vs. 15.8 ± 0.2 μg/L; $P < 0.001$). As with CVD events, serum OPN concentrations were also significantly associated with death during follow-up (Fig. 2). After adjusting for factors associated with serum OPN concentrations, as well as other factors independently associated with all-cause mortality at baseline, OPN was independently associated with death in a multivariate Cox regression analysis (Table 4).

In the assessment of a possible benefit of using OPN on top of hs-CRP for the prediction of death during follow-up, there was no additional predictive value including both markers in the same model (Supplementary Fig. 1 and Supplementary Table 2). However, when reclassification indexes IDI and NRI were used to assess the added predictive benefit of OPN compared with actual models, OPN significantly improved model 3 even after adding CRP to the model (Supplementary Table 3).

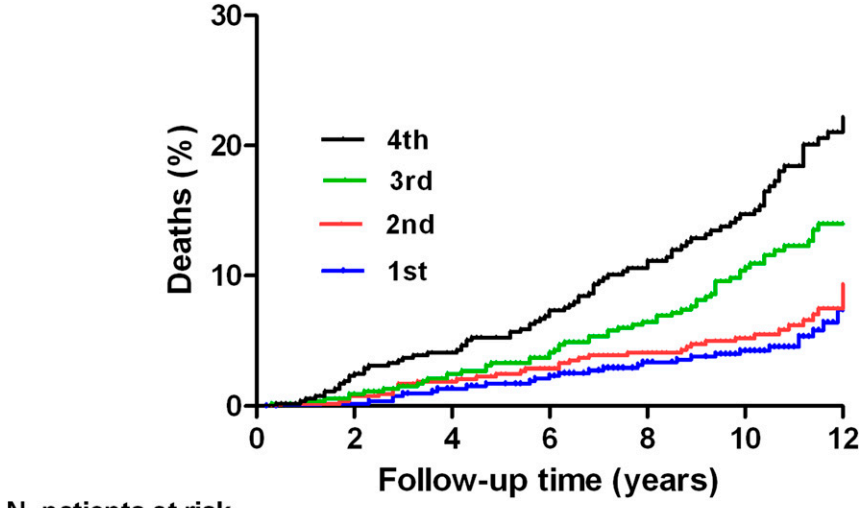
CONCLUSIONS

In this prospective observational study of including 2,145 patients with T1D, serum OPN concentrations predicted incident microalbuminuria, a first-ever CVD event, and all-cause mortality after controlling for traditional risk factors for the subsequent events in multivariate models.

Although serum OPN concentrations have been linked to diabetic vascular disease in vitro (8), the clinical data showing the association between OPN and late vascular complications in patients with diabetes are scarce. In T2D, OPN was related to diabetic retinopathy in 19 patients (9) although the results were not replicated in a larger study with 229 patients (10). However, in the study by Yamaguchi et al. (10), plasma but not urine OPN correlated with the progression of diabetic nephropathy. In our study, serum OPN predicted independently the development of incipient renal disease in patients with T1D.

In the current study, serum OPN was also an independent predictor of CVD events in patients with T1D. The findings are in line with earlier results demonstrating OPN to predict future CVD events in patients with CAD (11). However, the risk factor profile for CVD events differs in T1D from that in the general population. Chronic hyperglycemia and especially renal disease are strong risk factors for cardiovascular morbidity and mortality in this patient group (13). Nevertheless, the results in our study were independent of diabetic nephropathy status and kidney function at baseline, indicating that the association was not entirely driven by renal disease. The size of the study population allowed us to separately analyze the predictive role of OPN for different CVD events. No noticeable differences between the events were observed, suggesting that OPN is possibly involved in more generalized damage to the cardiovascular system.

We have earlier shown that chronic kidney disease strongly predicts all-cause mortality in patients with T1D (13). Most deaths in this study were associated with kidney disease–related CVD. However, because serum OPN concentration was an independent predictor of all-cause mortality, this biomarker may be of clinical value for future risk stratification in patients with T1D. Furthermore, an intriguing question is whether blocking OPN would slow the development of diabetic vascular complications and eventually decrease premature death in patients with T1D.



N. patients at risk

1st	533	522	513	499	459	391	74
2nd	537	524	514	495	446	364	67
3rd	539	516	500	476	405	311	57
4th	536	504	483	438	321	258	52

Figure 2—Kaplan-Meier survival curves with log-rank tests for death (n = 202) by quartiles of serum OPN concentrations. Log-rank $P < 0.001$.

Table 4—Cox regression analysis for the predictive value of OPN for all-cause mortality

	Death	
	HR (95% CI)	*P* value
Model 1	1.01 (1.01–1.02)	0.02
Model 2	1.01 (1.00–1.02)	0.035
Model 3	1.02 (1.02–1.03)	<0.001

HR, hazard ratio. Model 1 adjusted for sex, age, waist-to-hip ratio, current smoking, A1C, total cholesterol, antihypertensive medication, CVD at baseline, eGFR, microalbuminuria, macroalbuminuria, and hs-CRP. Model 2 adjusted for sex, age, duration of diabetes, BMI, waist-to-hip ratio, current smoking, A1C, LDL cholesterol, systolic blood pressure, antihypertensive medication, CVD at baseline, eGFR, microalbuminuria, macroalbuminuria, and hs-CRP. Model 3 adjusted for the covariates in the Framingham risk score (sex, age, LDL cholesterol, HDL cholesterol, systolic blood pressure, diabetes, and smoking).

OPN is a multifunctional protein involved in a number of physiological and pathological conditions (1) and is highly expressed in several chronic inflammatory diseases, such as atherosclerosis (23). Vascular smooth muscle cells, endothelial cells, and macrophages express OPN in atherosclerotic lesions (24). Notably, OPN has also been linked to obesity-related chronic inflammation and insulin resistance that may be one mechanistic player (6).

We also analyzed the predictive value of OPN after adding hs-CRP to the Cox regression models and observed that the results did not change. Results of using ROC curve analysis to evaluate the predictive value of OPN alone or on top of hs-CRP suggested that OPN does not add to the risk prediction beyond hs-CRP. However, when reclassification indexes were calculated, OPN improved the prediction of CVD events and all-cause mortality in the model built from the Framingham risk factors even when hs-CRP was added to the model. This observation suggests that OPN improves the prediction of serious outcomes compared with traditional models.

The search for additional biomarkers as predictors for CVD events has been intense. Brain (b-type) natriuretic peptide (BNP) and cardiac troponins are thoroughly studied cardiac risk markers. BNP is a peptide hormone secreted primarily from the ventricles of the heart in response to cardiomyocyte stretch. BNP has many systemic effects, and this biomarker has been shown to predict CVD in different patient populations as well as in the general population (25–27). Notably, BNP has also been suggested to be an early marker of myocardial disease in young patients with T1D (28). Cardiac troponins T and I, in turn, are components of the cardiomyocytes and established ischemic markers of myocardial necrosis in patients with acute coronary syndromes (29). A small elevation in troponins is associated with an increased risk of an adverse outcome in patients with acute CAD (30). Furthermore, even in men free of CVD, increased troponin concentrations have been associated with increased mortality (31). An interesting question for future studies is whether OPN, acting mostly through different mechanisms, adds to the prediction of CVD events after inclusion of these well-established risk markers.

To summarize, the prevention of diabetic nephropathy and cardiovascular complications in patients with T1D relies on the control of multiple risk factors. Because diabetes confers high risk, every patient should control his or her A1C, blood pressure, lipids, body weight, avoid hypoglycemia, and stop smoking. However, even patients with a strict control of risk factors suffer from complications, and early risk markers to identify the patients at risk are needed. Our observations suggest that one such biomarker could be OPN that may not only reflect the degree of vascular dysfunction but also predict vascular disease. We recognize, however, that further studies are needed to demonstrate the clinical benefit of OPN for the risk stratification.

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