Osteopontin is a strong predictor of diabetic nephropathy, cardiovascular disease and all-cause mortality in patients with type 1 diabetes

Short title: OPN strongly predicts vascular complications in T1D patients

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Abstract

Objective: Osteopontin (OPN) is a multifunctional protein suggested to be a player in the disease of especially diabetic arteries. Therefore, we investigated the associations between OPN and diabetic vascular complications as well as all-cause mortality in patients with type 1 diabetes (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 (ICD codes) and the Finnish National Death Registry, respectively. The median (interquartile range) follow-up time was 10.5 (8.9-11.8) years.

Results: Serum OPN was higher at baseline in patients that developed incident microalbuminuria (16.0 ± 0.9 vs. 14.1 ± 0.2 µg/L; p=0.04), progressed to ESRD (33.9 ± 2.7 vs. 20.9 ± 0.7 µg/L; p<0.001), suffered a first ever 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 a multivariate Cox regression analysis, OPN was independently associated with the development of incident microalbuminuria (hazard ratio 1.03 [95% CI (1.01-1.06)]; P=0.003), ESRD (1.01 [1.00-1.02]; P=0.006), a first ever CVD event (1.02 [1.01-1.03]; P<0.001), and death (1.01 [1.01-1.02]; P=0.002) after adjustments for associated risk factors.

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

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Atherosclerosis and calcification of the arterial wall develops early in patients with type 1 diabetes (T1D), resulting in premature stiffening of the arteries [1]. This process is especially precipitated by diabetic nephropathy, leading eventually to an increased risk of vascular disease [2]. However, the reason for the accelerated vascular disease in these patients is largely unknown.

Osteopontin (OPN) is a multifunctional protein expressed by several different cell types, including epithelial cells, vascular smooth muscle cells, leucosytes, and osteoclasts [3]. OPN is involved in a number of physiological and pathological conditions, such as cancer and progression of metastases [4], urinary stones [5], wound healing[6], chronic inflammatory and autoimmune diseases[7], obesity related chronic inflammation and insulin resistance [8]. However, OPN was originally found in bone and shown to regulate the formation and calcification of bone tissue [9]. Notably, OPN has also been linked to vascular remodelling and calcification especially in diabetic arteries [10], and shown to associate with diabetic retinopathy[11] and nephropathy[12] in patients with type 2 diabetes, as well as coronary artery disease in non-diabetic subjects[13]. However, its role in patients with T1D is not known.

Therefore, we explored the association between serum OPN and cardiovascular outcomes in a large well-characterized cohort of patients with T1D exploring incident diabetic nephropathy, CV events, as well as all-cause mortality.

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 emphasis on diabetic nephropathy. Detailed description of the follow-up protocol has been described earlier [Groop]. T1D was defined as an onset of diabetes before the age of 40 years and permanent insulin treatment initiated within one year of diagnosis. Follow-up data have been collected since year 2004. (7). Serum OPN was estimated on 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 (interquartile range) follow-up time for the study population was 10.5 (8.9-11.8) years. The study protocol is in accordance with the Declaration of Helsinki as revised in 2000, and approved by the local ethics committee in each study centre. Written informed consent was obtained from each patient.

Ascertainment of outcomes

Data on medication, cardiovascular status, and diabetic complications were registered by a standardized questionnaire, which was completed by the patient´s attending physician and thus immediately verified from the medical files. Blood pressure was measured by standard methods. After the patient had rested for at least 10 minutes, blood pressure was measured twice in a sitting position. The average of the measurements were used in the analysis. Height, weight and waist hip ratio were recorded.

Baseline urinary albumin excretion rate (AER) was stratified such that normoalbuminuria was defined as <20 µg/min or <30 mg/24 h, microalbuminuria as 20 µg/min ≤AER< 200 µg/min or 30 mg/24 h ≤AER< 300 mg/24 h, and macroalbuminuria s AER≥200 µg/min or AER ≥300 mg/24 h, in 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 was collected either by re-examination of the patients or review of the medical files. Information on CV-events until the end of 2010, were obtained from medical files and by linking the FinnDiane data with the Hospital Discharge Register (HDR) and the Finnish Cause of Death Registry (CDR). 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 International Classification of Diseases (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 [14]. CV-events were defined as a history of myocardial infarction, a coronary artery procedure (by-pass surgery or angioplasty), stroke, or a peripheral artery procedure (by-pass surgery or angioplasty), which was verified on the basis of ICD discharge codes specifying the events. 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. In the prospective CV analysis, we only included patients without any CV-events at baseline. Deaths from any cause through to the 18th of September 2011 were identified via a search of the Finnish National Death Registry, and center databases.

Assays.

HbA_{1c} was determined locally by standardized assays and serum lipid and lipoprotein concentrations centrally by automated enzymatic methods (Hoffman-LaRoche, Basel, Switzerland). Serum creatinine was determined centrally by the IDMS traceable assay, and GFR estimated using the CKD-EPI formula [15] [16]. AER was assessed from an overnight or a 24-h urine collection by immunoturbidimetry. Furthermore, serum OPN was measured by …, as previously described.

Statistics

Analyses were performed with PASW Statistics 18 (SPSS, Chicago, IL, USA). Data for normally distributed and continuous variables are presented as mean ± SD and data for non-normally distributed variables as median with IQR. Differences between groups were analyzed with Student´s t-test, ANOVA, Mann-Whitney U-test or Kruskal-Wallis test as appropriate. Categorical variables were analyzed using Pearson's χ^2 test. Longitudinal data were analyzed with Kaplan-Meier survival curves with log-rank tests. Risk factors for the progression of diabetic complications were assessed using Cox proportional hazard survival regression showing results as hazard ratios with 95% confidence intervals (HR; 95%CI). The models were adjusted for factors associated with serum OPN concentrations, as well as other factors independently associated with the studied events. A competing risk analysis considering two pairs of (competing) events: (the development of) ESRD and pre-ESRD deaths as well as incident CVD and pre-CVD deaths, were performed as earlier described by Stata statistical software (V11, 2009; College Station, TX, USA) [17]. $P<0.05$ was considered statistically significant.

Results

Baseline characteristics

Serum OPN was measured in 2,145 patients (52% men) with T1D without ESRD. The patient characteristics according to baseline quartiles of OPN are shown in table 1. Briefly, the mean age of this cohort was 37.4 ± 0.3 years and the median (IQR) duration of diabetes 20.1 (11.7-29.0) years. Their mean systolic blood pressure (SBP) was 133 ± 1 mmHg, diastolic blood pressure (DBP) 80 ± 1 mmHg, eGFR 89 ± 1, and HbA_{1c} 8.5 ± 0.1%. At baseline, 1,395 patients had normal albumin excretion rate, 330 microalbuminuria, and 420 macroalbuminuria. Altogether, 143 patients had had a CVD event and 687 patients 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 to those with normal AER. Furthermore, OPN concentrations were higher in patients with macroalbuminuria than those with microalbuminuria (24.1 ± 0.9 vs. 16.0 ± 0.6 µg/L; p<0.001). Similarly, those who had suffered 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 laser-treated diabetic retinopathy without signs of nephropathy (normal AER) had higher serum OPN concentrations (16.1 ± 0.7 µg/L) at baseline, than those who were not laser-treated (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), SBP (r=0.10;p<0.001), hsCRP (r=0.09; p<0.001). 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 HbA_{1c} at baseline.

OPN and diabetic nephropathy in patients with T1D

During the follow-up period of 6.0 (4.0-7.0) years [median (IQR)], 178 patients progressed to a higher level of albuminuria or to ESRD during follow-up (99 to microalbuminuria, 44 to macroalbuminuria, and 95 to ESRD). Serum OPN was higher at baseline in patients that developed microalbuminuria during the follow-up compared to 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 microalbuminuric patients who became macroalbuminuric during follow-up compared to those who did not (16.7 ± 1.4 vs. 15.9 ± 0.6 µg/L; p=ns). In macroalbuminuric patients who developed ESRD, OPN was higher at baseline (33.9 ± 2.7 vs. 20.9 ± 0.7 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 (p=0.003) and ESRD (p=0.006), but not progression to macroalbuminuria (p=NS) (**Table 2A**). Including urinary AER in the models did not change the results. The results did not change in a competing risk analysis considering two (competing) events: (the development of) ESRD and pre-ESRD deaths (data not shown).

OPN and incident CVD in patients with T1D

All in all, 191 patients experienced their first CV event ever during the follow-up period (10.6 [7.1-12.0] years). Serum OPN concentrations at baseline were higher in patients who had an incident CVD event compared to those who did not (20.2 ± 1.2 vs. 15.5 ± 0.2 µg/L; p<0.001). After adjusting for associated risk factors with OPN and CVD, OPN remained significantly associated with incident CVD on multivariate Cox regression analysis (p<0.001; **Table 2B**). The results did not change in a competing risk analysis considering two (competing) events: the development of incident CVD and pre-CVD deaths (data not shown).

OPN and different CV events at follow-up (replaces the 3 forthcoming paragraphs)

When analyzing incident CHD, stroke and PVD separately, we found serum OPN concentrations to predict a first ever stroke (p=0.03; **Supplemental Table 3A**) and leg revascularization procedure or an amputation (any cause) event (p=0.001, **Supplemental Table 3C**) during follow-up. The association between OPN at baseline and an incident CHD event at follow-up was not significant (p=0.09; **Supplemental Table 3A**).

OPN and incident CHD in patients with T1D

During a median (IQR) of 11.4 (10.3-12.1) years of follow-up, 109 patients experienced their first CHD event ever. Serum OPN was increased at baseline in patients who had an incident CHD event at follow-up compared to 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 CHD at follow-up after correcting for associated covariates in a multivariate Cox regression analysis (p=0.09; ***Supplemental Table 3A****)*

OPN and incident stroke in patients with T1D

Sixty-two patients suffered an incident stroke (ischemic or hemorrhagic) during a median (IQR) of 10.7 (7.8-12.0) years of follow-up. Serum OPN 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, OPN remained associated with stroke in a multivariate Cox regression analysis (p=0.03; ***Supplemental Table 3B****).*

OPN and incident peripheral vascular disease (PVD) in patients with T1D

As a whole, 20 patients had 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 had 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 (p=0.001, ***Supplemental Table 3C****).*

OPN and all-cause mortality in patients with T1D

Altogether, 202 patients died during the follow-up period of 10.5 (8.3-11.8) years median (IQR). Serum OPN concentrations at baseline were higher in patients who died during follow-up compared to those who did not (23.3 ± 1.4 vs. 15.8 ± 0.2 µg/L; p<0.001). After adjusting for factors associated with serum OPN concentrations, as well as other factors independently associated with all-cause mortality, OPN was independently associated with death in a multivariate Cox regression analysis (p=0.002; **Table 2C**).

Discussion

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

OPN and diabetic nephropathy in T1D

Although serum OPN concentrations have been linked to diabetic vascular disease *in vitro* [Takemoto], the clinical data showing the association between OPN and vascular complications in patients with diabetes is scarce. In type 2 diabetes, OPN was related to diabetic retinopathy in nineteen patients [Kase] although the results were not replicated in a larger patient material (N=229) [Yamaguchi]. However, in the study by Yamaguchi et al. plasma and urine OPN correlated with the progression of diabetic nephropathy. In our study serum OPN predicted independently the development and progression of incipient and overt renal disease in patients with T1D.

OPN and CVD in T1D

In the present study serum OPN was an independent predictor of CVD events in patients with T1D. The findings are in line with earlier results demonstrating OPN to be associated with the presence and severity of coronary artery disease [Tousoulis]. 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 CV-morbidity and -mortality in this patient group [Groop]. 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 in different CVD events. No noticeable differences between the events were observed, suggesting OPN to possibly be involved in a more generalized damage to the cardiovascular system.

OPN and all-cause mortality in T1D

We have earlier shown that chronic kidney disease strongly predicts all-cause mortality in patients with T1D [Groop]. Most deaths were associated with kidney disease related CVD in our cohort. It is thus likely that the increased all-cause mortality associated with OPN is mostly of vascular origin as part of a generalized arterial disease related to calcifications. However, as serum OPN concentration was an independent predictor of all-cause mortality, it may be of clinical value in future risk stratification in patients with T1D.

Mechanisms

OPN is a multifunctional protein involved in a number of physiological and pathological conditions [Mazzali]. It has been shown to be highly expressed in several chronic inflammatory diseases, such as atherosclerosis [18]. Vascular smooth muscle cells (VSMC), endothelial cells and macrophages express OPN in atherosclerotic lesions [19]. Experimental data show that OPN regulates migration, proliferation, and accumulation of VSMCs in the intimal layer, especially after vascular injury such as stenosis [20]. Besides inflammatory functions, OPN seems to be involved in vascular calcification through mineralizing arteries possibly through inorganic pyrophosphate signaling [21 22].

As early vascular calcification and stiffening of the arteries appear to be especially important in diabetic vascular disease, an intriguing finding was made by Takemoto et al. showing OPN antibodies in the arteries of diabetic patients but not in non-diabetic controls [Takemoto]. Similar findings have also been reported in diabetic animal models [23 24]. Two pathways linking hyperglycemia to diabetic complications[25], the hexosamine and the protein kinase C-dependent pathways, were shown to increase OPN concentrations in cultured rat aortic VSMCs [26]. Furthermore, hypoxia, another player in diabetic vascular disease[27], stimulated OPN expression in cultured VSMCs. This effect was potentiated by hyperglycemia[28]. Again, the protein kinase C-dependent pathway was shown to be activated in the process. In summary, hypoxia enhances the effect on proliferation of VSMCs in a hyperglycemic environment partly through OPN, and may thus play an important role in the development of diabetic vascular disease.

Genetic and experimental studies have shown OPN to correlate with albuminuria and glomerular disease [29 30]. Notably, diabetes-induced albuminuria and mesangial expansion did not develop in OPN knockout mice [Lorenzen]. The mechanisms for the findings are not known, but OPN as an activator of the nuclear factor-κB pathway, may disturb podocyte signaling and motility resulting in albuminuria. Further

data supporting a role of OPN in diabetic kidney disease was recently published by Nicholas et al. showing protective effects of thiazolidinediones (even compared to insulin) on the expression of OPN and albuminuria in diabetic mice [31] [32]. In contrast to earlier reports, this study focused on mesangial cells and suggested the damage to be mediated through transforming growth factor (TGF)-beta signaling contributing to diabetic nephropathy. OPN may thus serve as a potential target for early treatment of diabetic kidney disease. Although, OPN neutralizing antibodies have been reported to decrease tubulointerstitial macrophage infiltration in glomerulonephritis in rats, no studies on diabetic animal models have been performed as far as we are concerned [33].

Obesity-induced inflammation and insulin resistance are well-known risk factors for vascular disease, especially in patients with type 2 diabetes. However, these entities often co-exist with the metabolic syndrome, also shown to be common in patients with T1D [34]. Notably, it has been shown that antibody mediated neutralization of OPN decreased insulin resistance partly by decreasing obesity-related inflammation both in the liver and the adipose tissue [35]. Furthermore, OPN was recently demonstrated to be involved in the incretin hormone GIP (glucose-dependent insulinotropic polypeptide) action promoting pancreatic β-cell function by potentiating insulin secretion and b-cell proliferation [36]. Whether these mechanisms are involved in the origin of vascular disease in patients with T1D is unknown.

Conclusion

Serum osteopontin is a strong predictor of incident and progression of diabetic nephropathy, first ever CVD event, and all-cause mortality in patients with T1D. This is most likely due to hyperglycemia causing an inflammatory process resulting in atherosclerosis and calcification of the arterial wall. Intriguingly, hypoxia seems to enhance the effects of high blood glucose. High serum OPN concentrations may thus reflect the degree of vascular disease and therefore predict vascular disease. Serum OPN may in the future be of clinical use in risk the prediction of vascular events in patients with T1D.

Table 1. Patient characteristics according to baseline quartiles of serum OPN.

	1st	2nd	3rd	4th	P-value
Patients (N)	533	537	539	536	-
Gender (% men)	41	50	55	61	<0.001
Age (yr)	38 ± 1	37 ± 1	38 ± 1	37 ± 1	0.12
Duration (yr)	19 ± 1	20 ± 1	22 ± 1	23 ± 1	<0.001
Age at onset (yr)	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/Hip ratio	0.85 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.001
SBP (mmHg)	130 ± 1	132 ± 1	133 ± 1	135 ± 1	<0.001
DBP (mmHg)	80 ± 1	80 ± 1	79 ± 1	80 ± 1	0.29
HbA_{1c} (%)	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.6 ± 0.1	0.04
Total cholesterol (mmol/l)	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	0.32
HDL cholesterol (mmol/l)	1.3 ± 0.1	1.3 ± 0.1	1.3 ±0.1	1.3 ± 0.1	0.01
LDL cholesterol (mmol/l)	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
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
Smoking (%)	23	24	22	28	0.07
Laser treated diabetic retinopathy (%)	22	27	35	45	<0.001
Osteopontin (OPN) µg/L	6.3 ± 0.1	11.4 ± 0.1	16.9 ± 0.1	31.4 ± 0.6	<0.001

SBP; systolic blood pressure, DBP; diastolic blood pressure, AER; urinary albumin excretion rate, eGFR; estimated glomerular filtration rate, CVD; established cardiovascular disease. Data is presented as mean±SEM, and percentages except for AER, Triglycerides, and serum creatinine where median and interquartile range is presented

Figure 1. Kaplan-Meier survival curves for;

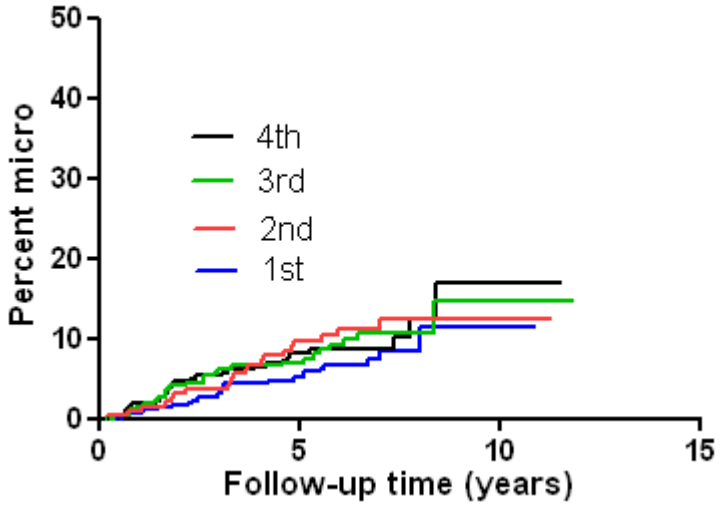
A: incident microalbuminuria by quartiles of serum OPN concentrations

B: progession to ESRD by quartiles of serum OPN concentrations

C: a first ever CV-event by quartiles of serum OPN concentrations

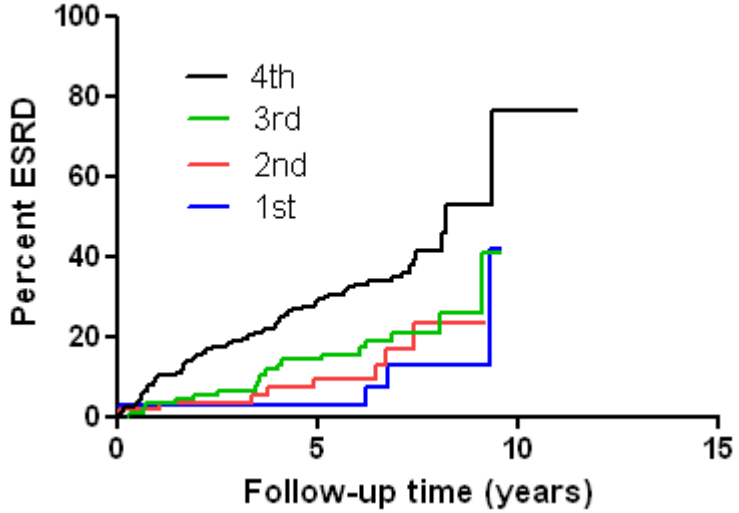
D: death by quartiles of serum OPN concentrations

A



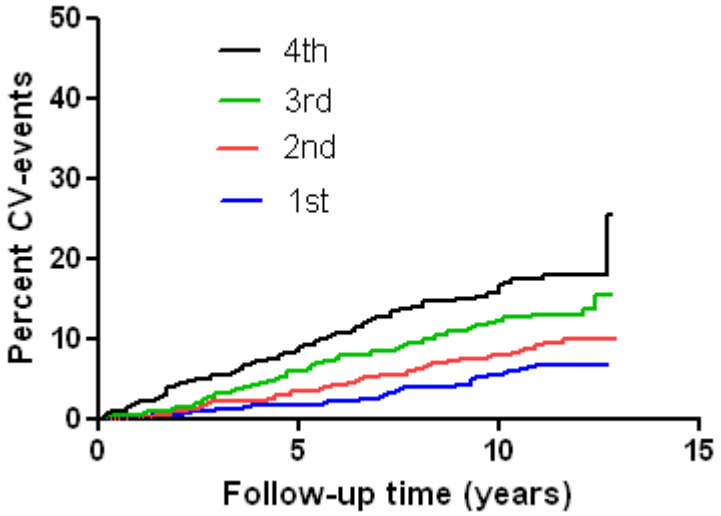
p=NS

B


100
80
60
40
20
0
Percent ESRD
4th
3rd
2nd
1st
0
5
10
15
Follow-up time (years)

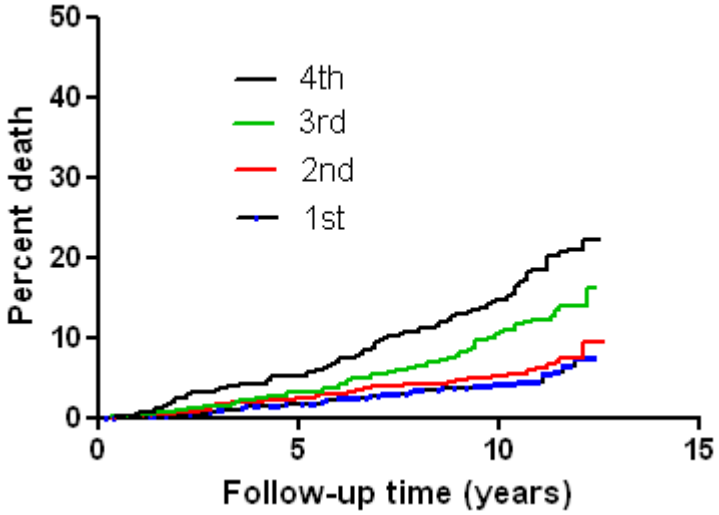
p<0.001

C


50
40
30
20
10
0
Percent CV-events
4th
3rd
2nd
1st
0
5
10
15
Follow-up time (years)

p<0.001

D


50
40
30
20
10
0
Percent death
4th
3rd
2nd
1st
0
5
10
15
Follow-up time (years)

p<0.001

Table 2. Cox regression analysis for the predictive value of serum OPN for;

(A) incident microalbuminuria, or progression to macroalbuminuria or ESRD
(B) first ever CVD
(C) all-cause mortality

after adjusting for factors associated with serum OPN concentrations, as well as other factors independently associated with the studied events. HR (95% CI); Hazard ratio (95% confidens interval)

2A. Cox regression analysis for the predictive value of OPN for diabetic nephropathy.

	Incident microalbuminuria		**Progression to macroalbuminuria**		**Progression to ESRD**	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex (male)	1.06 (0.62-1.81)	0.84	1.33 (0.53-3.36)	0.54	1.95 (1.13-3.35)	0.02
Age (years)	0.99 (0.97-1.02)	0.81	0.99 (0.95-1.04)	0.76	0.94 (0.91-0.97)	<0.001
Waist to hip-ratio (cm/cm)	15.7 (0.43-571.9)	0.13	96.4 (1-18394)	0.09	0.68 (0.09-44.1)	0.68
Current smoking	1.32 (0.78-1.50)	0.27	0.76 (0.48-1.62)	0.44	1.24 (0.78-2.03)	0.40
HbA_{1C} (%)	1.60 (1.41-1.81)	<0.001	1.25 (1.06-1.47)	0.008	1.27 (0.12-1.44)	<0.001
Triglycerides (mmol/l)	1.35 (1.11-1.65)	0.003	1.27 (1.03-1.59)	0.03	1.13 (0.97-1.31)	0.11
Antihypertensive medication (%)	1.28 (0.63-2.60)	0.49	1.58 (1.08-1.81)	0.03	1.57 (0.56-4.39)	0.39
eGFR (ml/min per 1.73 m^2)	1.00 (0.98-1.01)	0.61	1.01 (0.98-1.03)	0.79	0.93 (0.92-0.94)	<0.001
OPN (µg/L)	1.03 (1.01-1.06)	0.003	1.01 (0.98-1.04)	0.63	1.01 (1.00-1.02)	0.006

2A. Cox regression analysis for the predictive value of OPN for a first ever CV-event.

	CV-event	
	HR (95% CI)	p-value
Sex (male)	1.16 (0.62-1.47)	0.74
Age (years)	1.07 (1.05-1.08)	<0.001
Waist to hip-ratio (cm/cm)	6.8 (0.7-70.1)	0.11
Current smoking	1.42 (1.20-1.58)	0.001
HbA_{1C} (%)	1.17 (1.05-1.30)	0.003
Total cholesterol (mmol/l)	1.14 (0.99-1.32)	0.06
Antihypertensive medication (%)	1.12 (0.64-1.43)	0.57
eGFR (ml/min per 1.73 m^2)	0.99 (0.98-0.99)	<0.001
Microalbuminuria	1.58 (1.28-1.76)	0.002
Macroalbuminuria	1.26 (0.81-1.53)	0.22
OPN (µg/L)	1.02 (1.01-1.03)	<0.001

2D. Cox regression analysis for the predictive value of OPN for all-cause mortality.

	Death	
	HR (95% CI)	p-value
Sex (male)	1.25 (0.77-1.42)	0.39
Age (years)	1.06 (1.04-1.07)	<0.001
Waist to hip-ratio (cm/cm)	13.3 (1.5-121.3)	0.02
Current smoking	1.37 (1.15-1.33)	0.001
HbA_{1C} (%)	1.22 (1.10-1.36)	<0.001
Total cholesterol (mmol/l)	1.05 (0.91-1.22)	0.53
Antihypertensive medication (%)	1.26 (0.85-1.52))	0.18
CVD at baseline	1.55 (1.35-1.69)	<0.001
eGFR (ml/min per 1.73 m^2)	0.99 (0.98-0.99)	0.02
Microalbuminuria	1.63 (1.40-1.78)	<0.001
Macroalbuminuria	1.68 (0.88-1.53)	0.14
OPN (µg/L)	1.01 (1.01-1.02)	0.002

Supplemental Table to be deleted 2C. Cox regression analysis for the predictive value of OPN for incident CV-events.

	Incident CHD		**Incident stroke**		**Incident PVD**	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Sex (male)						
Age (years)						
Waist to hip-ratio (cm/cm)						
Current smoking						
HbA_{1C} (%)						
OPN						

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