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Elevated osteoprotegerin predicts declining renal function in elderly women: a 10-year prospective cohort study

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*Co-first authors, contributed equally to work

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Running title: OPG and renal disease

Word count: Abstract 243, Body 3,252

Tables: 2  Figures: 4

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**Background:** Elevated osteoprotegerin (OPG) levels are inversely correlated with creatinine clearance and end stage renal disease in patients with diabetes. However its role in predicting decline in renal function and progression to more advanced stage disease in the elderly general population is unknown.

**Methods:** This was a prospective cohort study of 1,157 elderly women with serum osteoprotegerin measured in 1998 and renal function estimated using serum creatinine and cystatin C-based estimated glomerular filtration rate (eGFR) at 5-yearly intervals. The primary objective of the study was to determine the relationship of circulating OPG levels with 5 and 10-year renal decline.

**Results:** At baseline, participants with elevated OPG above the median (≥ 2.2 ng/mL) had a 5.0% lower CKD-EPI-creatinine and cystatin C eGFR compared to participants with lower OPG levels. In multivariable-adjusted linear regression models, elevated OPG levels at baseline was associated with greater 5 and 10-year decline in CKD-EPI-creatinine and cystatin C eGFR [-0.105; P = 0.002 and -0.104; P = 0.010 respectively]. Elevated OPG at baseline was associated with increased 5 and 10-year risk of rapid renal decline (OR 2.13, 95% CI, 1.33-3.43, P = 0.002 and OR, 4.10, 95% CI, 1.49-11.27, P = 0.006 respectively) and renal disease hospitalizations or deaths [HR 1.99 95% CI 1.31-3.03, P = 0.001] after adjusting for known risk factors.

**Conclusion:** Elevated OPG levels are associated with long-term renal dysfunction and may be provide a useful biomarker to predict the trajectory of renal decline in older women.
**Introduction**

Chronic kidney disease (CKD) is common in older people and is an independent risk factor for cardiovascular disease and all-cause mortality [1,2]. It has been shown that renal decline over time is associated with an increased risk of all-cause mortality in older individuals [3], but the pathophysiological factors contributing to renal function decline in the general population remains largely unknown.

Osteoprotegerin (OPG) is a soluble member of the tumor necrosis factor receptor superfamily and binds to receptor activator of nuclear factor-κB ligand (RANKL), acting as a decoy receptor to competitively inhibit the interaction between RANKL with its receptor, RANK. [4] OPG inhibits osteoclastic bone resorption [5,6] and recent studies have demonstrated a strong association between OPG and vascular inflammation, endothelial dysfunction [7] and vascular calcification [8,9]. OPG is produced in a variety of tissues including the endothelium, intestines, lungs, kidneys and bones, therefore OPG may have an important role in pathogenesis of disease processes affecting these tissues [10].

In a cross-sectional study of patients with type 2 diabetes, an inverse correlation between log-transformed OPG and creatinine clearance (R = -0.20, P = 0.01) was observed [11] while a second study reported a nine-fold increased risk of end stage renal disease (ESRD) requiring dialysis or transplantation in patients with type 2 diabetes and elevated OPG levels compared to low OPG levels [12]. These studies have suggested OPG may be a biomarker for CKD progression but it remains unclear whether a similar association is present in the non-diabetic population.

The aim of this study was to examine the relationship between serum OPG levels and change in renal function in a large longitudinal unselected cohort of elderly women.
METHODS

Study Population

The participants were recruited in 1998 to a 5-year prospective, randomised, controlled trial of oral calcium supplements to prevent osteoporotic fractures, the Calcium Intake Fracture Outcome study (CAIFOS) [13]. Women were recruited from the Western Australian general population aged over 70 years by mail using the electoral roll. Of the 5,586 women approached, 1,500 were recruited into the study. All participants were ambulant with an expected survival beyond 5 years and were not receiving any medications (including hormone replacement therapy) known to affect bone metabolism. Baseline disease burden and medications were comparable between these participants and the general population of similar age although these participants were more likely to be from higher socio-economic groups [13]. In the subsequent 5 years following randomization, participants received 1.2 g of daily elemental calcium or matching placebo. At the conclusion of CAIFOS, participants were followed-up for a further 5 years. This study reports on 1,157 participants with serum OPG, creatinine and cystatin C measured at baseline. The Human Ethics Committee of the University of Western Australia approved the study and written informed consents were obtained from all participants.

Baseline Risk Factors

Baseline medical history including the presence of diabetes, hypertension, smoking history (current smokers/former smokers or non-smokers) and anti-hypertensive medications, were obtained from all participants. Participants’ medical histories and medications were verified by their General Practitioners where possible. Weight was obtained using digital scales with participants wearing light clothes and no shoes. Height was measured using a stadiometer and body mass index (BMI) was calculated for each participant. Blood pressure
was measured in 1,122 participants on the right arm with a mercury column manometer using an adult cuff after the participants have been seated in an upright position and had rested for 5 minutes. An average of three blood pressure readings was recorded.

**Biochemistry**

Fasting blood samples were collected at baseline (1998), 5 years (2003) and 10 years (2008). Baseline free OPG was measured by a validated enzyme immunoassay using commercially available matched antibodies (R&D Systems, Minneapolis, MN, USA) as previously described [14,15]. The intra- and interassay coefficients of variation were 3.6% and 10.6% respectively [15]. Baseline creatinine was measured using an isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay on a Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim Germany), whereas the 5 and 10-year creatinine were measured on the Architect ci16200 analyzer (Abbott, Illinois, U.S.A). The correlation coefficient (r²) between the machines was 0.998 with a Passing and Bablok slope of 0.966 and a Passing and Bablok intercept of 6.16 (n = 37). Baseline serum cystatin C was measured using a fully automated particle-enhanced immunoturbidimetric assay with Sentinel Diagnostics reagents (Sentinel CH, Milan, Italy) on the Architect ci 16200 System (Abbott Laboratories, Illinois, USA) according to manufacturer instructions. Estimated glomerular filtration rate (eGFR) creatinine and cystatin C (CKD-EPI-creatinine and cystatin C) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [16]. Rapid renal decline was defined as participants with an average of >3 mL/min/1.73m² annual decline in eGFR [17-19].

**Renal disease-related hospitalizations and deaths**

Prevalent renal disease-related events between 1980 and 1998 and incident events between 1998 and 2008 were retrieved from the Western Australian Data Linkage System
(WADLS) using the diagnoses codes from the International Classification of Diseases, Injuries and Causes of Death Clinical Modification (ICD-9-CM) [20] and the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10-AM). [21] WADLS is a comprehensive, population-based linkage system connecting 40 years of data from over 30 health-related datasets for Western Australian residents using ICD codes [22]. The coded discharge diagnosis data included all public and private inpatient hospitalizations within WA and provides a complete validated record of every participant’s primary diagnosis and up to 21 additional diagnoses. Renal disease-related hospitalization codes collected for this study included glomerular diseases (ICD-9-CM codes 580 – 583, ICD-10-AM codes N00-08); renal tubulo-interstitial diseases (ICD-9-CM codes 593.3 – 593.5, 593.7 and 590-591, ICD-10-AM codes N09-16); renal failure (ICD-9-CM codes 584 – 586, ICD-10-AM codes N17-19); and hypertensive renal disease (ICD-9-CM code 403, ICD-10-AM codes I12) derived from either the primary discharge or additional discharge diagnoses. These renal disease-related outcomes were further categorized into acute renal failure (ICD-9-CM code 584, ICD-10-AM code N17), chronic renal failure (ICD-9-CM code 585.0 - 585.6, ICD-10-AM code N18), diabetes-related renal disease (ICD-9-CM code 250.4, ICD-10-AM code E11.2) and others/unspecified renal disease (all other abovementioned renal disease related ICD codes). The search for renal disease-related death ICD codes included all available diagnostic information that comprised Parts 1 and 2 of the death certificate. All diagnosis text fields from the death certificate were used to ascertain the cause(s) of deaths where these data were not yet available from the WADLS.

Statistical Analysis
Statistical analyses were conducted using SPSS (version 18; SPSS Inc, Chicago, IL) and SAS (Version 9, SAS Institute Inc., Chicago, IL) programs. Baseline characteristics stratified by above and below the median OPG were expressed as mean and standard deviation (SD) for continuous variables or as proportion for categorical variables. Unadjusted and adjusted linear regression models were used to test the association between baseline OPG, either as a continuous variable (ng/mL) or dichotomised above or below the median OPG (2.2 ng/mL) with 5 and 10-year change in eGFR. Multivariable-adjusted analysis of covariance (ANCOVA) was used to compare the 5-year change in eGFR (adjusted for baseline eGFR) between participants above and below the median OPG levels while a multivariable-adjusted general linear model (GLM) repeated-measures analysis was used to compare the change in eGFR over the 10 years. If the GLM repeated-measures analysis indicated a significant OPG and time interaction over the 10 years, the OPG effects were analyzed with Analysis of Covariance (ANCOVA). To visualize this, annual change in eGFR was used. We applied the multivariable-adjusted logistic regression models to examine the association between above the median OPG levels and rapid renal decline (defined using CKD-EPI equation). Results were expressed as odds ratio (OR) and 95% confidence interval (CI). For renal hospitalizations and deaths multivariable-adjusted (same as above) Cox regression analysis was used to examine the association of above the median OPG with clinical events. No violations of the Cox proportional hazards assumptions were detected and results were presented as a hazard ratio (HR) and 95% CI. Interaction tests were used to assess whether effects of OPG levels above or below the median were different for participants with renal disease and diabetes or CKD. No interactions were detected (P > 0.05). All multivariable models included; age, body mass index, systolic blood pressure, baseline CKD-EPI eGFR, anti-hypertensive medications, smoking history, treatment code (adjustment for the calcium or placebo treatment), diabetes and previous renal
hospitalizations. In all analyses p-values of less than 0.05 in the two tailed testing were considered as statistically significant.
RESULTS

Baseline characteristics

A total of 1,157 participants (77.1%) from the baseline cohort had available data on baseline serum OPG, creatinine and cystatin C levels and were included in the final analyses. The baseline characteristics of those excluded were similar to participants included in the study. Less than 2% of participants had prevalent renal disease while over 50% of participants were maintained on anti-hypertensive medication(s) or had a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Baseline circulating OPG levels were separated into deciles to assess the relationship with 5 and 10 year renal decline (Figure 1). Renal decline over 5 and 10 years in participants with elevated OPG in deciles 6-10 (above the median) was significantly higher than deciles 1-5. OPG levels were then dichotomized by baseline above and below the median (2.2 ng/ml) for further analyses (Table 1). Participants with above the median OPG levels were older, had higher systolic blood pressure and were more likely to have diabetes or taking anti-hypertensive medications. The proportion of participants with prior renal disease and those allocated to calcium supplementation were similar in the two OPG categories. CKD-EPI-creatine and cystatin C measurements were available for 816 (70.5%) participants at 5 years and 588 (50.8%) participants at 10 years (Figure 2).

Associations between OPG and eGFR CKD-EPI-creatine and cystatin C (Table 2)

Participants with serum OPG above the median levels had lower estimated eGFR by the CKD-EPI-creatine and cystatin equation at baseline. In multivariable-adjusted analysis participants with serum OPG above the median levels had a greater decline in eGFR at 5 years (-1.040 ± 0.089 vs. -0.647 ± 0.091 mL/min/1.73m², P = 0.002) and 10-years (-1.084 ± 0.112 vs. -0.630 ± 0.111 mL/min/1.73m², P = 0.005). In the GLM repeated-measures analysis
participants with above the median OPG levels had a more rapid 10-year decline in renal function compared to those below the median OPG level (Figure 3). A similar inverse relationship between OPG with 5-year and 10-year eGFR CKD-EPI-creatinine and cystatin C decline was observed if OPG was considered as a continuous variable of nanograms per ml (multivariable-adjusted standardized β coefficient -0.081, P = 0.019 and standardized β coefficient -0.084, P = 0.039 respectively).

**Associations between OPG and rapid renal decline**

At 5 years there were a total of 98 / 816 (12.0%) participants that experienced rapid renal decline, defined as an average reduction in eGFR of ≥ 3 ml/min/1.73m² per year using the CKD-EPI-creatinine-cystatin C equation. Participants with above the median OPG had a significantly increased risk of rapid renal decline (multivariable-adjusted OR, 2.13, 95% CI, 1.33-3.43, P = 0.002).

At 10 years, a total of 26 / 588 (4.4%) participants experienced rapid renal decline and participants with above the median OPG levels had a significantly increased risk of rapid renal decline (multivariable-adjusted OR, 4.10, 95% CI, 1.49-11.27, P = 0.006).

**Renal disease-related hospitalizations and deaths**

Using Cox regression, participants with above the median OPG had a two-fold increased risk of renal disease-related hospitalization or death in the age-adjusted analysis; HR 2.47, 95%CI, 1.64-3.72, P < 0.001, which remained significant after adjustment (Figure 4). When renal disease hospitalizations were stratified into acute renal failure (n = 25), chronic renal failure (n = 66), diabetes-related renal disease (n = 17) and other or unspecified renal disease (n = 17), participants with serum OPG above the median levels had a higher incidence of chronic renal failure (8.5% vs. 2.9%, P < 0.001), diabetes-related renal disease (2.4% vs. 0.5%, P = 0.012) and other or unspecified renal disease (2.2% vs. 0.7%, P = 0.047)
but not acute renal failure (2.6% vs. 1.7%, P = 0.419). Similarly, when renal failure deaths (n = 27) were stratified into acute renal failure deaths (n = 11) and chronic renal failure deaths (n = 16), participants with serum OPG above the median levels had a higher incidence of all renal failure deaths (3.6% vs. 1.0%, P = 0.005), chronic renal failure deaths (2.2% vs. 0.5%, P = 0.020) but not acute renal failure deaths (1.5% vs. 0.5%, P = 0.224).

Subgroup and sensitivity analyses

Detailed subgroup and sensitivity analyses are provided in the online supplementary data.
DISCUSSION

We have identified a robust association between elevated OPG levels and renal function decline, independent of traditional risk factors such as age, diabetes and blood pressure in an unselected cohort of ambulatory elderly women. The association between elevated OPG and renal function decline was similar for both 5 and 10-year change in eGFR CKD-EPI-creatinine and cystatin C equations, which further strengthens our findings. Furthermore, elevated OPG was associated with rapid renal decline, which has been identified as a strong predictor of cardiovascular events and all-cause mortality [3,17-19] as well as renal disease-related hospitalization or deaths. These data further support the concept that OPG may be a sensitive biomarker of renal dysfunction and the trajectory of future renal decline. These finding also support our previous findings [23] in this cohort of elderly women that OPG above the median is associated with increased 8.5 year risk of cardiovascular and all-cause mortality and may help explain a portion of the shared comorbidity of chronic renal disease and cardiovascular disease.

It is well established that eGFR of less than 60 ml/min/1.73m$^2$ is a strong predictor of cardiovascular and all-cause mortality in the general population [24]. While there are many other shared risk factors such as smoking and hypertension for both cardiovascular disease and CKD, it is plausible that the association between these risk factors and cardiovascular disease is mediated by the presence of CKD. Therefore biomarkers such as OPG that independently predict the decline in renal function over time may provide clinical utility for identifying patients with both poorer renal and cardiovascular clinical outcomes. The robust associations observed in our study with OPG and renal function at baseline, long-term renal decline at 5 and 10 years, rapid renal decline and renal disease-related hospitalizations and deaths provides strong observational evidence that elevated OPG is an independent risk factor for chronic kidney disease and may help explain the previous observations of others that
elevated OPG is associated with cardiovascular disease[23,25,26]. As a biomarker OPG has several advantages, such as low intra-individual variation, it is present at relatively high serum levels and the detection is unaffected by freeze thaw cycles [27].

Our findings are consistent with previous studies in patients with diabetes and CKD that reported elevated serum OPG levels were associated with increased risk of rapid renal decline. In the study by Altinova et al, an inverse correlation between log-transformed OPG and creatinine clearance (r = - 0.20, p = 0.01) was observed [11] while Jorsal et al, [28] reported a ninefold increased risk of progression to end stage kidney disease requiring dialysis or transplantation in patients with high OPG levels compared to patients with low levels (adjusted HR 4.32, 95%CI 1.45–12.87) in patients with type 1 diabetes. Matsubara et al, [29] reported that OPG was strongly associated with surrogate markers of inflammation and endothelial dysfunction suggesting that the association between OPG and mortality may be mediated through inflammation [29]. Other studies of stage 5 CKD patients on maintenance dialysis have reported plasma OPG is an independent risk factor for arterial stiffness and/or all-cause mortality [12,30,31]. In addition, several studies have also demonstrated similar associations between OPG and CVD and all-cause mortality in the general population without prevalent CVD [23,25,26]. Thus it appears that elevated OPG levels may have clinical utility as a prognostic marker for both CKD and CVD in the general elderly populations as well as in diabetic and CKD populations.

It remains unclear whether circulating OPG is causally linked to the pathophysiology of the observed renal decline or merely represents an epiphenomenon. OPG is known to derive locally from bone mesenchymal and vascular smooth muscle cells and is present in high concentrations throughout all layers of normal and atherosclerotic blood vessel walls [32]. It is plausible that high circulating levels of OPG may reflect injuries to these vessel walls resulting in the release of OPG from within vessel wall into the circulation [33].
Because of the pleiotropic role of OPG in a variety of cellular systems and its known deleterious associations with vascular disease [8] it may be that elevated OPG and age-related renal decline and are in fact due to age-related renal-vascular deterioration. This concept is supported by the association with cardiovascular risk factors including hypertension and smoking history seen in Table 1. However following successful kidney transplantation, there is a significant reduction in circulating OPG levels corresponding with an improvement in renal function, [34,35] suggesting the elevated OPG levels in CKD patients may be attributed to reduced renal clearance [36] although it remains unclear whether the elevated OPG levels in these patients are due to reduced renal clearance and/or an increased production of OPG by tissues.

Limitations of this study include the observational nature of the study and the lack of data regarding the temporal sequence of the potential changes in OPG levels over time. In particular as baseline eGFR was lower in participants with an elevated OPG we cannot exclude that this may have influenced our findings despite adjusting for baseline eGFR in the multivariable-adjusted model. A further limitation of the study was the lack of measured GFR or albuminuria at the three time points during the study. We also cannot rule out the potential for unmeasured residual confounders. Finally the study was limited to elderly Caucasian women and may not be generalizable to younger populations or elderly men. However as the associations between OPG and age or renal function have been observed equally for both males and females by others [11,37], we believe that our finding of an inverse association between serum OPG levels and renal decline may be equally applicable to elderly men. Despite these limitations, to our knowledge this is the largest study to report the association of OPG with long-term renal decline and our findings suggest that OPG may be a novel risk factor for poorer long-term prognosis in people with CKD.
Strengths of this study include the complete and accurate data and sample collection with eGFR CKD-EPI-creatinine and cystatin C measured at 3 time points over a 10-year period. Cystatin C is an endogenous marker of GFR and unlike creatinine is unaffected by weight or muscle mass, and has been demonstrated to be a better predictor of all-cause mortality in elderly populations [38-41]. The eGFR CKD-EPI-creatinine and cystatin C is also considered a better reflector of measured GFR compared to the eGFR creatinine or cystatin C individually [16]. The use of linked hospital and mortality records independent of self-report allowed complete follow-up in a large cohort of elderly women for renal disease-related events.

In conclusion, further investigations into this novel biomarker are needed in younger populations and elderly men to elucidate whether OPG is a causative agent of poorer clinical outcomes or is merely a sensitive biomarker of renal dysfunction. Our findings support the hypothesis that OPG related mechanisms may play an important role in age-related renal dysfunction and predicting the trajectory of long-term renal decline.
Acknowledgements The authors wish to thank the staff at the Data Linkage Branch, Hospital Morbidity Data Collection and Registry of Births, Deaths and Marriages for their work on providing the data for this study.

Conflict of interest statement None declared.

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Table 1. Baseline characteristics of the study population stratified by median OPG (2.2 ng/mL)

<table>
<thead>
<tr>
<th></th>
<th>OPG below median (&lt; 2.2 ng/mL)</th>
<th>OPG above median (≥ 2.2 ng/mL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>578</td>
<td>579</td>
<td></td>
</tr>
<tr>
<td>Osteoprotegerin, mean ± SD, ng/mL</td>
<td>1.7 ± 0.3</td>
<td>2.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>74.9 ± 2.6</td>
<td>75.6 ± 2.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index, mean ± SD, kg/m²</td>
<td>27.2 ± 4.4</td>
<td>27.2 ± 5.0</td>
<td>0.875</td>
</tr>
<tr>
<td>Systolic blood pressure, mean ± SD, mmHg</td>
<td>137.0 ± 18.0</td>
<td>139.5 ± 18.0</td>
<td>0.020</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean ± SD, mmHg</td>
<td>72.8 ± 10.9</td>
<td>73.5 ± 11.1</td>
<td>0.298</td>
</tr>
<tr>
<td>Baseline creatinine, mean ± SD, mg/dL</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline cystatin C, mean ± SD, mg/dL</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>eGFR CKD-EPI-creatinine and cystatin C, mean ± SD, mL/min/1.73m²</td>
<td>67.4 ± 12.2</td>
<td>64.0 ± 13.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcium treatment, No. (%)</td>
<td>292 (50.5)</td>
<td>320 (55.3)</td>
<td>0.106</td>
</tr>
<tr>
<td>Smoked ever, No. (%)</td>
<td>214 (37.2)</td>
<td>211 (36.7)</td>
<td>0.872</td>
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<tr>
<td>Anti-hypertensive medications, No. (%)</td>
<td>226 (39.1)</td>
<td>267 (46.1)</td>
<td>0.016</td>
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<tr>
<td>Diabetes at baseline, No. (%)</td>
<td>29 (5.0)</td>
<td>49 (8.5)</td>
<td>0.019</td>
</tr>
<tr>
<td>Renal disease at baseline, No. (%)</td>
<td>5 (0.9)</td>
<td>12 (2.1)</td>
<td>0.088</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD or number and (%). P values calculated by ANOVA or chi squared test where appropriate. OPG – osteoprotegerin, mmHg - millimeters mercury and CKD-EPI eGFR - Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate.
Table 2. Multivariable-adjusted clinical correlates of annual change in eGFR CKD-EPI-creatinine and cystatin C (ml/min/1.73m²) over 5 (n = 816) and 10-years (n = 588)

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized β coefficient*</th>
<th>Standard error</th>
<th>Standardized β coefficient*</th>
<th>P value</th>
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<tbody>
<tr>
<td>5-year annual change in eGFR CKD-EPI-creatinine and cystatin C</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (per year)</td>
<td>-0.057</td>
<td>0.025</td>
<td>-0.081</td>
<td>0.022</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.016</td>
<td>0.015</td>
<td>-0.039</td>
<td>0.279</td>
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<td>Systolic blood pressure (mm/Hg)</td>
<td>-0.012</td>
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<td>-0.114</td>
<td>0.002</td>
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<td>eGFR CKD-EPI-creatinine and cystatin C (ml/min/1.73m²)</td>
<td>-0.038</td>
<td>0.005</td>
<td>-0.258</td>
<td>&lt; 0.001</td>
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<td>0.137</td>
<td>-0.052</td>
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<tr>
<td>Smoked ever (yes/no)</td>
<td>-0.376</td>
<td>0.133</td>
<td>0.096</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>-1.010</td>
<td>0.278</td>
<td>-0.126</td>
<td>&lt; 0.001</td>
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<td>Previous renal hospitalization (yes/no)</td>
<td>-0.291</td>
<td>0.640</td>
<td>-0.016</td>
<td>0.649</td>
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<td>OPG above median (yes/no)</td>
<td>-0.393</td>
<td>0.129</td>
<td>-0.105</td>
<td>0.002</td>
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<tr>
<td>10-year annual change in eGFR CKD-EPI-creatinine and cystatin C</td>
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</tr>
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<td>Age (year)</td>
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<td>-0.079</td>
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<td>Body mass index (kg/m²)</td>
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<td>-0.103</td>
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<td>Systolic blood pressure (mm/Hg)</td>
<td>-0.009</td>
<td>0.003</td>
<td>-0.121</td>
<td>0.005</td>
</tr>
<tr>
<td>eGFR CKD-EPI-creatinine and cystatin C (ml/min/1.73m²)</td>
<td>-0.021</td>
<td>0.004</td>
<td>-0.208</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anti-hypertensive medications (yes/no)</td>
<td>-0.187</td>
<td>0.105</td>
<td>-0.076</td>
<td>0.076</td>
</tr>
<tr>
<td>Smoked ever (yes/no)</td>
<td>0.046</td>
<td>0.103</td>
<td>0.018</td>
<td>0.652</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>-0.841</td>
<td>0.232</td>
<td>-0.148</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Previous renal hospitalization (yes/no)</td>
<td>0.019</td>
<td>0.517</td>
<td>0.001</td>
<td>0.971</td>
</tr>
<tr>
<td>OPG above median (yes/no)</td>
<td>-0.251</td>
<td>0.098</td>
<td>-0.104</td>
<td>0.010</td>
</tr>
</tbody>
</table>

For continuous variables the standardized regression coefficients indicates the effect of change per unit increment on the 10-year change in eGFR CKD-EPI-creatinine or CKD-EPI-creatinine and cystatin C; for binary traits, this corresponds to the effect of the presence of the trait. OPG – osteoprotegerin, mmHg - millimeters mercury and CKD-EPI eGFR - Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate. Standardized β coefficients and P values are multivariable-adjusted values.
**Figure legends**

**Fig. 1** Annual rate of change (Mean and 95% confidence intervals) of estimated glomerular filtration rate measured over 5 years (n = 816) or 10 years (n = 588) by deciles of circulating OPG. a) 5-year annual change in eGFR using serum creatinine and cystatin; b) 10-year annual change in eGFR using serum creatinine and cystatin C.
Fig 2 Overview of the study participants and follow up.
Fig. 3 Multivariable-adjusted GLM repeated-measures analysis of 5-year (n = 816) and 10-year (n = 588) change in eGFR dichotomized by OPG above (black solid line) and below (red dotted line) the median (≥ 2.2 ng/mL). Multivariable GLM repeated-measures analysis indicated participants with above the median OPG had significantly greater renal decline, P = 0.037. Multivariable model includes age, body mass index, systolic blood pressure, baseline CKD-EPI eGFR, anti-hypertensive medications, smoking history, treatment code, diabetes and previous renal disease-related hospitalizations.
**Fig. 4** Multivariable adjusted Cox proportional Hazard ratio and 95% confidence interval for 10-year renal disease hospitalizations or deaths (n = 112) dichotomized by OPG below the median (≥ 2.2 ng/mL, dashed black line, referent) and above the median (solid black line), multivariable-adjusted HR; 1.99, 95% CI, 1.31-3.03, P = 0.001. Multivariable-adjusted model includes age, body mass index, systolic blood pressure, baseline CKD-EPI eGFR creatinine and cystatin C, anti-hypertensive medications, smoking history, treatment code, diabetes and previous renal hospitalization.
References


