ORIGINAL ARTICLE

Association between serum osteoprotegerin level and mortality in kidney transplant recipients - a prospective observational cohort study

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ABSTRACT

Paradoxically, higher serum levels of osteoprotegerin (OPG: a vascular calcification inhibitor) have been associated with increased arterial stiffness, risk of cardiovascular disease and all-cause mortality. A few studies reported that post-transplant OPG levels are associated with mortality in kidney transplant (KT) recipients. In this study, this association was assessed in a cohort of prevalent KT recipients, adjusting for previously untested potential confounders, including fibroblast growth factor 23 (FGF23) and interleukin 6 (IL-6). Socio-demographic and clinical parameters, medical and transplant history, and laboratory data were collected from 982 prevalent KT recipients. The association between serum OPG and all-cause mortality over a 6-year follow-up period was examined using Kaplan–Meier survival curves and multivariable-adjusted Cox regression models. Participants with high serum OPG were more likely female, older, deceased donor KT recipients and have more comorbidity, lower eGFR, higher FGF23, higher IL-6, and longer dialysis vintage. Each 1 pmol/l higher serum OPG level was associated with a 49% higher risk of mortality (hazard ratio (HR) [95% confidence interval (CI)]: 1.49 [1.40–1.61]). This association persisted after adjusting for confounders (HR [95% CI]: 1.20 [1.10–1.30]). In conclusion, serum OPG was associated with all-cause mortality independent of several novel confounders in prevalent KT recipients.

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Key words
chronic kidney disease—mineral and bone disorder, kidney transplant, osteoprotegerin, outcomes, vascular calcification, cardiovascular disease

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Introduction

End-stage kidney disease affects close to 2 million individuals worldwide, and its prevalence is increasing [1,2]. Kidney transplant (KT) is the preferred renal replacement modality because it improves quality of life and reduces mortality, morbidity and healthcare costs compared to maintenance dialysis [3-6].

The leading cause of mortality in KT recipients is cardiovascular disease. Vascular calcification is a marker

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and contributor to the pathophysiology of cardiovascular disease in patients with chronic kidney disease (CKD) and end-stage kidney disease. Vascular calcification is also a defining component of CKD-bone mineral disorder (CKD-BMD). Consequently, markers of bone and mineral metabolism, vascular calcification, and their association with clinical outcomes in patients with end-stage kidney disease have been the focus of intensive research [7,8].

Osteoprotegerin (OPG) is a potent inhibitor of vascular calcification [9]. It is a member of the superfamily of tumor necrosis factor receptors and is a soluble decoy receptor for receptor activators of nuclear factor kappa-B ligand (RANKL) [10]. OPG prevents vascular calcification through its strong inhibition of bone resorption [11]. Paradoxically, in the general population, elevated OPG concentration is associated with an increased risk of cardiovascular disease, ischemic stroke, all-cause mortality, and vascular calcification [12,13].

Serum OPG levels are elevated in patients with CKD and end-stage kidney disease, potentially in response to the strongly pro-calcific milieu [8,14]. Studies in dialyzed and nondialyzed patients with CKD have found that, like the general population, high serum OPG levels are associated with vascular calcification and all-cause and cardiovascular mortality [7,15-18]. Short-term studies have found that serum OPG declines immediately after KT [19,20]. These studies used relatively small sample sizes but found that early post-transplant levels of OPG are associated with patient mortality [20]. These findings have been confirmed in one larger study [21]. As well, we have demonstrated that OPG is associated with pulse pressure, a marker of vascular stiffness and calcification that is a predictor of cardiovascular morbidity and mortality [15].

The precise relationship between OPG and mortality is difficult to ascertain because of the multidirectional interplay between various components of CKD-MBD, which include OPG itself, vascular calcification, and inflammation [8,22]. Markers of these processes such as interleukin 6 (IL-6) and fibroblast growth factor 23 (FGF23) are associated with clinical outcomes. Elevated levels of FGF23 and IL-6 are associated with increased morbidity and mortality in CKD and KT recipients [23-26]. FGF23 has a complex association with inflammation and is also associated with a higher pulse pressure [8,27-29].

In this study, we wanted to assess if higher serum OPG levels are associated with all-cause mortality in stable prevalent KT recipients. In our complex analyses, frequently used socio-demographic and clinical covariables were used, and in addition, our models were also adjusted with biomarkers of inflammation and CKD-MBD (such as serum FGF23, IL-6 levels) that have not been considered previously. Whether pulse pressure mediates the association between serum OPG and mortality was also assessed. It was hypothesized that higher OPG levels are associated with increased all-cause mortality in this patient population.

Materials and methods

Study design

The study design was a prospective observational (cohort) study (Malnutrition – Inflammation in Transplant – Hungary [MINIT-HU] Study). It was approved by the Ethics Committee of Semmelweis University (49/2006). Before enrollment, patients received detailed written and verbal information regarding the aims and protocol of the study and provided written consent to participate. All research activities were performed in accordance with relevant guidelines and regulations.

Setting

Recruitment took place at the outpatient transplant clinic at Semmelweis University in Budapest, Hungary, between February 2007 and August 2007 (MINIT-HU Study). Participants were followed up for an average of five years from recruitment. The study end date was September 24, 2013.

Participants

All prevalent KT recipients 18 years of age or older (n = 1,214) followed at the outpatient transplant clinic at Semmelweis University in Budapest, Hungary, were invited to participate in this observational cohort study. Exclusion criteria were acute rejection within the last four weeks, current hospitalization, transplantation in the previous three months, and acute infection or active bleeding. The study methods have been described [30-34].

Data sources/measurement

Routine laboratory data were measured at the baseline visit in a fasting state and were extracted from the patients' charts and from the hospital's electronic laboratory database. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney

Disease Epidemiology Collaboration equation [23]. The following laboratory parameters were tabulated: serum alkaline phosphatase, serum albumin, serum cholesterol, serum calcium, serum phosphate, blood hemoglobin, random blood glucose, and serum C-reactive protein. Additional clinical information extracted from medical records and self-reported includes the following: smoking status, diabetes status, tacrolimus, phosphate binder and statin use, donor type, transplant vintage, and delayed graft function and rejection.

Serum samples were collected at the time of the baseline assessment and stored at –70°C for future use. Serum intact parathyroid hormone (PTH) (pg/ml) was determined by second-generation electrochemiluminescence assay (iPTH Elecsys System; Roche, Mannheim, Germany). Serum osteoprotegerin was measured with immunoassay kits based on a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Biomedica, Vienna, Austria). FGF23, IL-6, and tumor necrosis factor (TNF) were also measured using ELISA, while high-performance liquid chromatography was used to measure plasma 25(OH) vitamin D levels. Serum adiponectin and leptin concentrations were measured using immunoassay kits based on solid-phase sandwich ELISA (R&D Systems, Minneapolis, MN).

The modified Charlson Comorbidity Index [30] was used to assess comorbidity; the Index is a predictor of survival in KT recipients [35]. Blood pressure was recorded at baseline using a calibrated mercury sphygmomanometer as the average of three readings after ten minutes of rest.

Standard maintenance immunosuppressive therapy included prednisolone, either cyclosporine A microemulsion formulation (CsA) or tacrolimus, combined with mycophenolate-mofetil (MMF) or azathioprine or sirolimus.

Exposure and outcome

The primary exposure variable was serum OPG level as a continuous variable (1 pmol/l increase); the secondary exposure was tertiles of serum OPG (lowest tertile as referent). The primary outcome of interest was death with a functioning graft (censored for graft loss).

Statistical analysis

Categorical variables were described using frequencies and percentages, while continuous variables were presented using mean (standard deviation, SD) for normally distributed data and median (interquartile range) for skewed variables. The associations between exposure and patient characteristics were assessed using the chi-square test and ANOVA as appropriate; and a log transformation applied to skewed continuous variables.

Restricted cubic splines, with and without adjusting for co-variates, were used to model potential nonlinear association between serum OPG levels and the log hazard ratio of mortality. Knots were placed at equally spaced percentiles along the OPG continuum.

The cumulative probabilities of the study endpoint were assessed graphically using the Kaplan–Meier product limit method, and differences examined across survival functions using the log-rank test. Univariable and multivariable-adjusted associations between the exposure and outcome in Cox proportional hazards models were explored. Proportional hazards assumptions were tested using scaled Schoenfeld residuals. The variables entered in the multivariable-adjusted models were selected based on theoretical considerations; independent covariables in the models were included which were known to be associated both with serum OPG levels and mortality based on external evidence and clinical experience, and which were available in our database.

Models were fitted that contained an expanding set of covariables sequentially. Six models were examined: (0) unadjusted model; (1) adjusted for age, sex, and body mass index; (2) model 1 plus Charlson Comorbidity Index, smoking habit, presence of hypertension and diabetes mellitus, and duration of end-stage kidney disease; (3) model 2 plus eGFR, hemoglobin, and serum albumin; (4) model 3 plus markers of bone metabolism: serum phosphate, FGF23, and iPTH levels; (5) model 4 plus IL-6 as a marker of inflammation. Mediation analysis was used to determine if the pulse pressure of participants served as a direct or indirect mediator of the effect of OPG levels on survival. We used the four-way decomposition method by adding pulse pressure to model 5 (model 6) and assessed the change in coefficient [36]. This method determines the level at which a covariant affects the ability of the exposure variable to predict the outcome variable.

In a sensitivity analysis, propensity score adjustment was used instead of adjusting our regression models for individual confounders (model 7). The propensity score of having low ($<$4.2 pmol/l) and high ($\geq$4.2 pmol/l) serum OPG (cut point between highest and mid tertile) was calculated to incorporate information from multiple confounding factors in a single variable. The propensity score was calculated in a logistic regression model, using 27 variables potentially associated with the exposure variable that satisfied balancing criteria (Table S1).

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Variance inflation factors (VIF) were used to assess collinearity between independent variables. Patients were censored for graft loss. Missingness was found to be less than 3% across variables. Multiple imputation using chained equations for the multivariable models was carried out using 10 iterations of imputation, replacing missing values with a set of imputed values based on the joint distribution of the existing variables.

Subgroup analysis was performed to assess the impact of several covariables and is presented by a forest plot. Potential interaction between serum OPG levels was assessed, and the subgroup variables were selected based on their potential association with mortality. Variables included were age, Charlson Comorbidity Index, diabetes status, use of phosphate binders, tertiles of serum level of FGF23 and iPTH. Multivariable-adjusted hazard ratios from the fully adjusted models are shown with corresponding confidence intervals, and interaction values are presented.

All statistical analyses were performed using Stata 15.0 (StataCorp, College Station, TX). A 2-sided *P* value less than 0.05 was considered statistically significant.

Results

Of the 1,214 patients invited to participate, 205 (17%) declined. Sixteen (1%) patients were excluded based on exclusion criteria. Thus, 993 patients were recruited into the study. Baseline OPG level was missing for 11 patients, resulting in a cohort of 982 patients (Fig. 1).

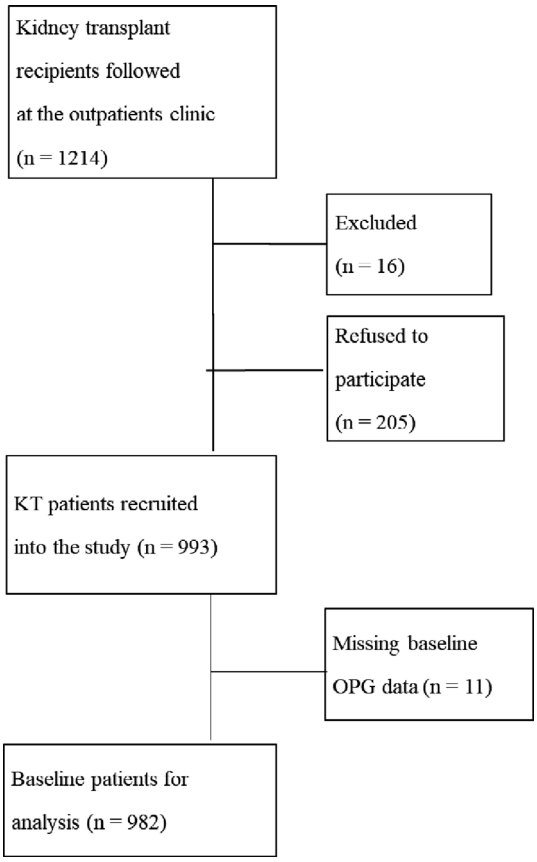

Figure 1 Study Flow Chart. A total of 1214 participants were followed in the outpatient transplant clinic. 16 patients did not meet the inclusion criteria, while 205 declined consent to be included in the study. Of the 993 participants recruited into the study, 11 were missing baseline OPG data, and thus, analysis was complete for only 982 patients.

Baseline patient characteristics are shown in Table 1. Mean (SD) age was 51(13) years, and age increased across the serum OPG tertiles from low to high. More males than females were in the cohort; the proportion of males decreased with increasing OPG levels. The median (interquartile range) duration of dialysis before transplant was 20 (9–38) months. Patients with lower serum OPG had shorter pretransplant dialysis and shorter total end-stage kidney disease vintage (Table 1). Participants in the lower versus (vs) higher OPG tertile had lower Charlson Comorbidity Index. Hemoglobin and estimated eGFR were lower among participants in the lower vs the highest tertile (Table 1).

Of the 982 patients, 239 died during the follow-up and none were lost to follow-up, with a median (interquartile range) follow-up time of 76(5) months. The crude mortality rate was 43.3/1000 patient-years (95% confidence interval [CI]: 38.2–49.1). Compared to patients in the lowest OPG tertile (18.9/1000 patient-years, 95% CI: 13.8–26.0), patients in the highest tertile (83.5/1000 patient-years, 95% CI: 70.6–98.7) and the middle tertile (33.4/1000 patient-years, 95% CI: 26.2–42.7) had a higher cumulative probability of mortality during the study (Fig. 2). Higher baseline serum OPG levels were associated with higher mortality, as demonstrated by a uniformly increasing curve when mortality risk was modeled as a continuous variable using restricted cubic splines (Fig. 3).

Variance inflation factors (VIF) were used to assess collinearity between independent variables used in the proportional hazards models. Table S2 shows the VIFs and tolerance (1/VIF) of the independent variables. The VIFs ranged from 1.06 to 2.05 with a mean of 1.44, while the tolerance values ranged from 0.52 to 0.94. VIF values less than 5 and tolerance values greater than 0.1 indicate the absence of significant multicollinearity among the independent variables, indicating the fitness of these variables for the models used in the analysis.

Table 2 shows the association of all-cause mortality with serum OPG levels. In unadjusted Cox proportional

Table 1. Baseline characteristics of participants by OPG tertiles

Characteristics	OPG Tertiles (pmol/l)				
	Low (<3.20) *n* = 328	Med (3.20–4.39) *n* = 327	High (>4.39) *n* = 327	Total *n* = 982	*P* value
Male – *n* (%)	208 (63%)	186 (57%)	171 (52%)	565 (58%)	0.015
Age – mean (SD)	44 (13)	51 (12)	57 (10)	51 (13)	<0.001
BMI (kg/m^2) mean (SD)	28 (5)	27 (5)	27 (5)	27 (5)	0.050
Charlson Comorbidity Index—*n* (%)					<0.001
2 or less	220 (67%)	188 (57%)	142 (44%)	550 (56%)	
3 and 4	87 (27%)	87 (27%)	109 (33%)	283 (29%)	
5 or more	21 (6%)	52 (16%)	76 (23%)	149 (15%)	
Current Smoking Habits—*n* (%)					0.100
Smoker	59 (18%)	73 (22%)	52 (16%)	184 (19%)	
Nonsmoker	269 (82%)	254 (78%)	275 (84%)	798 (81%)	
Hypertension—*n* (%)					0.115
Hypertensive	300 (91%)	307 (94%)	312 (95%)	919 (94%)	
Nonhypertensive	28 (9%)	20 (6%)	15 (5%)	63 (6%)	
Pulse Pressure (mm/Hg)-mean (SD)	53 (14)	58 (17)	64 (18)	58 (17)	<0.001
eGFR (ml/kg/1.73m2)-mean (SD)	57 (19)	52 (20)	44 (22)	51 (20)	<0.001
Serum ALP (iu/l)-mean (SD)	85 (31)	86 (35)	94 (50)	88 (40)	0.001
Serum Albumin (g/l)-mean (SD)	41 (4)	40 (4)	39 (4)	40 (4)	<0.001
Serum Cholesterol (mmol/l)-mean (SD)	5.4 (1.1)	5.6 (1.3)	5.5 (1.3)	5.5 (1.3)	0.024
Serum Calcium (mmol/l)-mean (SD)	2.4 (0.1)	2.4 (0.1)	2.3 (0.2)	2.4 (0.2)	0.081
Serum Phosphate (mmol/l)-mean (SD)	1.0 (0.3)	1.0 (0.2)	1.1 (0.3)	1.1 (0.3)	<0.001
Serum Hemoglobin (g/l)-mean (SD)	138 (17)	135 (16)	132 (18)	135 (17)	<0.001
Serum PTH (pg/mL)-median [IQR]	62 [47]	69 [54]	75 [75]	68[56]	0.003
Serum Random Blood Glucose (mmol/l)	5.8 [1.5]	6.0 [2.4]	5.9 [2.1]	5.9 [1.9]	0.146
Plasma 25-OH Vit D (ng/mL)-median [IQR]	10.8 [8.4]	10.3 [9.1]	8.6 [8.7]	9.8 [9.0]	0.001
Serum FGF23 (ug/l)-median [IQR]	25 [17]	27 [20]	38 [38]	28 [23]	0.001
Serum CRP (mg/ml)-median [IQR]	2.9 [4.7]	3.4 [5.3]	3.3 [5.7]	3.1 [5.3]	0.271
Serum IL-6 (iu/l)-median [IQR]	1.9 [1.9]	2.1 [2.6]	2.3 [3.0]	2.1 [2.3]	0.001
Tacrolimus Use—*n* (%)					0.001
Used	152 (46%)	127 (29%)	103 (32%)	382 (39%)	
Not Used	176 (54%)	200 (61%)	224 (68%)	600 (61%)	
Phosphate Binder Use—*n* (%)					0.012
Used	11 (3%)	16 (5%)	28 (9%)	55 (6%)	
Not Used	317 (97%)	311 (95%)	299 (91%)	927 (94%)	
Statin Use—*n* (%)					0.021
Used	82 (25%)	114 (35%)	102 (31%)	298 (30%)	
Not Used	246 (75%)	213 (65%)	225 (69%)	684 (70%)	
Donor Type—*n* (%)					0.004
Living Donor	22 (7%)	10 (3%)	6 (2%)	38 (4%)	
Deceased Donor	299 (93%)	310 (97%)	313 (98%)	922 (96%)	
Dialysis vintage (months)-median [IQR]	18 [29]	20 [28]	25 [37]	20 [29]	<0.001
Transplant vintage (months)-median [IQR]	72 [84]	80 [81]	79 [76]	77 [79]	0.212
Total ESKD vintage (months)-median [IQR]	101 [90]	108 [89]	113 [93]	108 [87]	0.011

BMI, body mass index; eGFR, estimated glomerular filtration rate; ALP, alkaline phosphatase; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; CRP, C-reactive protein; IL-6, interleukin 6; ESKD, end-stage kidney disease.

regression analysis, a 1 pmol/l higher serum OPG level was associated with significantly higher mortality [$HR_{1\ increase}$ = 1.50; 95% CI: 1.40–1.61]. This association remained significant after multivariable adjustment, with HR of 1.20 in the fully adjusted model (Table 2). This association was examined when the study group was divided into tertiles of equal weights based on serum OPG levels. The lower tertile consisted of participants with serum OPG levels of less than 3.20 pmol/l (328 participants), the middle tertile consisted of participants with serum OPG levels between 3.20 pmol/l and 4.39 pmol/l (327 participants) while the upper tertile

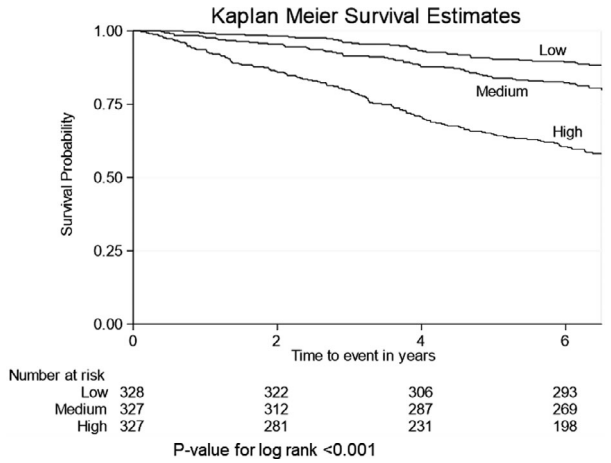

Figure 2 Kaplan–Meier Survival Estimates. We defined three groups of participants based on tertiles of OPG. Survival probability decreased across the study period. This probability was highest in the participants in the low OPG tertile and lowest in the high OPG tertile range at the end of the study period. This pattern was statistically significant with a log rank of < 0.001.

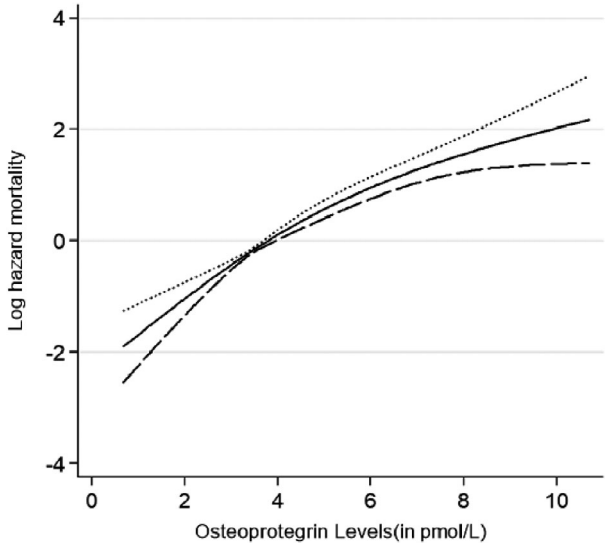

Figure 3 Restricted Cubic Splines Showing Association between OPG level and Mortality, without adjustment. Mortality is represented as log hazard mortality. There is a strong relationship between log hazard mortality and Osteoprotegerin with higher OPG levels associated with higher risk of mortality. The correlation has steeper slopes at the lower range of Osteoprotegerin levels than the middle and upper parts. The confidence intervals around the cubic spline are wider at the lower and upper ends of the sample than at the middle.

consisted of participants with serum OPG levels of greater than 4.39 pmol/l (327 participants). In this situation, the upper OPG tertile was associated with significantly higher mortality [HR_{upper} = 4.48; 95% CI: 3.12–6.41] compared to the middle and lower tertiles in an unadjusted Cox proportional regression analysis. This association remained significant after multivariable adjustment, with an HR of 1.80 in the fully adjusted model Importantly, the association remained unchanged after including pulse pressure in the multivariable model [HR_{upper} = 1.79; 95% CI: 1.21–2.66].

The association between serum OPG and mortality remained qualitatively similar in our sensitivity analysis, i.e. in the models using a propensity score to adjust imbalances in the exposure groups: HR (95% CI) 1.24 (1.08–1.41) for 1 pmol/l higher serum OPG and 1.77 (1.21–2.59) for the highest OPG tertile, respectively.

To assess if the association between serum OPG and mortality was mediated by pulse pressure, a simple mediation analysis was performed [36]. Adding pulse pressure to model 5 did not change the HR substantially (Table 2). The HR (95% CI) due to mediated interaction by pulse pressure with serum OPG was 0.002761 (−0.0000165 to 0.0055384), while the HR (95% CI) due to pure indirect effect of pulse pressure on survival was −0.00796 (−0.033 to 0.0176). All observed effects were not statistically significant.

In subgroup analyses, the association between serum OPG and mortality was similar across the subgroups examined (Fig. 4). No statistically significant interactions were found in our analysis. Specifically, no interaction was found with markers of inflammation or CKD-MBD, including iPTH, FGF23, and IL-6.

Discussion

In this large dataset of prevalent KT recipients, serum OPG levels were found to be independently associated with mortality, even after adjusting for novel and relevant confounders, such as FGF23 and IL-6. To extend previous reports further, a parsimonious propensity score adjustment approach was also used; our propensity score incorporated several additional potentially relevant inflammatory biomarkers, such as serum resistin and adiponectin, and additional biomarkers of bone metabolism, such as serum vitamin D levels and osteocalcin, in addition to the confounders analyzed in papers and in our primary models [13,19-21]. The association between serum OPG and mortality remained significant even in this propensity score-adjusted model. Pulse pressure, a marker of vascular stiffness, was also excluded as a potential mediator on the association between serum OPG and mortality.

In our study, age was correlated with serum OPG. Two studies of healthy adults found that serum OPG levels increased with age [37,38], which may reflect an insufficient paracrine mechanism of bone cells to

Table 2. Association of OPG with mortality in participants using Cox Regression Models

	OPG (1 pmol/l increase) HR (95% CI)	OPG (Middle Tertile) HR (95% CI)	OPG (Upper Tertile) HR (95% CI)
Unadjusted	1.50 (1.40–1.60)	1.77 (1.19–2.65)	4.48 (3.12–6.41)
Model 1	1.32 (1.22–1.43)	1.27 (0.85–1.92)	2.44 (1.66–3.59)
Model 2	1.30 (1.19 –1.41)	1.16 (0.77–1.76)	2.21 (1.49–3.26)
Model 3	1.22 (1.12–1.33)	1.12 (0.74–1.69)	1.92 (1.30–2.84)
Model 4	1.21 (1.11–1.31)	1.10 (0.73–1.66)	1.86 (1.24–2.75)
Model 5	1.21 (1.11–1.31)	1.10 (0.73–1.66)	1.86 (1.26–2.75)
Model 6	1.20 (1.10–1.30)	1.09 (0.72–1.64)	1.79 (1.21–2.66)
Model 7	1.20 (1.10–1.31)	1.09 (0.72–1.64)	1.80 (1.21–2.67)

Unadjusted: Osteoprotegerin Levels; Model 1: OPG + age, sex, BMI; Model 2: Model 1 + CCI Group, Smoking habit, Hypertension, Diabetes Mellitus and Duration of ESKD; Model 3: Model 2 + serum Albumin, Baseline eGFR, Hemoglobin; Model 4: Model 3 + serum Phosphate, FGF23, PTH; Model 5: Model 4 + IL-6; Model 6: (Mediation Analysis) Model 5 + pulse pressure; Model 7 (Propensity Score-Adjusted Model): Model 5 + Propensity Score: adjusted for propensity score based on proportional hazards regression model presented in Table S1.

compensate for bones loss in older age [39]. Although our study included more males than females, a significantly higher proportion of females were found in the highest vs the lowest OPG tertile. A similar trend was observed by Svensson *et al* [21]. Importantly, serum OPG levels were associated with increased risk of osteoporosis in predialysis females with CKD but not in males [40].

Two studies have investigated the relationship between OPG and mortality among KT recipients. In a study with 8 years of follow-up, Hjelmesæth *et al*. found that high OPG level at baseline post-transplantation is associated with all-cause mortality [20]. However, the incident cohort included only 173 KT recipients, and the authors only adjusted for four confounding variables [20]. Svensson *et al*. found a similar association in a larger cohort of 1,889 incident KT recipients [21]. Although Svensson *et al*. adjusted for a larger number of potential confounding variables, these only included traditional cardiovascular risk factors, socio-demographic characteristics, and PTH. The potential confounding effect of important CKD-MBD biomarkers, such as FGF23, and inflammation has not yet been considered. Our findings are consistent with other studies that found an association between OPG and mortality in various patient populations, including the general adult population [12,13,17,21]. In addition, several small studies found that increased OPG levels are associated with mortality in patients with various stages of CKD [7,8,16]. These studies were relatively short-term, and only adjusted for baseline cardiovascular risk, renal function, and some markers of mineral metabolism.

Elevated FGF23 is a robust predictor of mortality in multiple populations, including patients with CKD and KT recipients [25,41]. Although most reports support a role for FGF23 in congestive heart failure [42-45], some reports suggested an involvement in cardiovascular calcification [46]. Furthermore, FGF23 may modulate OPG expression [47]. Given the robust association between FGF23 and mortality, and the complex relationship between FGF23 and OPG, we felt that adjusting for FGF23 was an important novel contribution of our analyses when considering an independent association between OPG and mortality.

Inflammation is linked to clinical outcomes in patients with CKD. Serum IL-6 is reportedly associated with mortality in KT recipients [30,48]. Inflammation is an important component of CKD-MBD and has been shown repeatedly to contribute to cardiovascular calcification and OPG levels [49,50]. Interestingly, adjusting for FGF23 and IL-6 (Table 2) did not significantly change the hazard ratio associated with OPG. This suggests that the potential mechanisms through which OPG is associated with mortality are likely independent from FGF23 and IL-6 in KT recipients.

In our primary set of multivariable analyses, a model was built that included serum FGF23 and IL-6 levels to represent these domains. However, a propensity score was also computed that included additional potential confounders known to be associated with both OPG and mortality, including other biomarkers of CKD-MBD (osteocalcin and 25(OH) vitamin D), cytokines (such as tumor necrosis factor-alpha), and adipokines (adiponectin and resistin) (Table S1). Higher serum OPG remained significantly associated with mortality after adjusting for this propensity score. Our results are important in this regard since no study of similar size in KT recipients has reported an

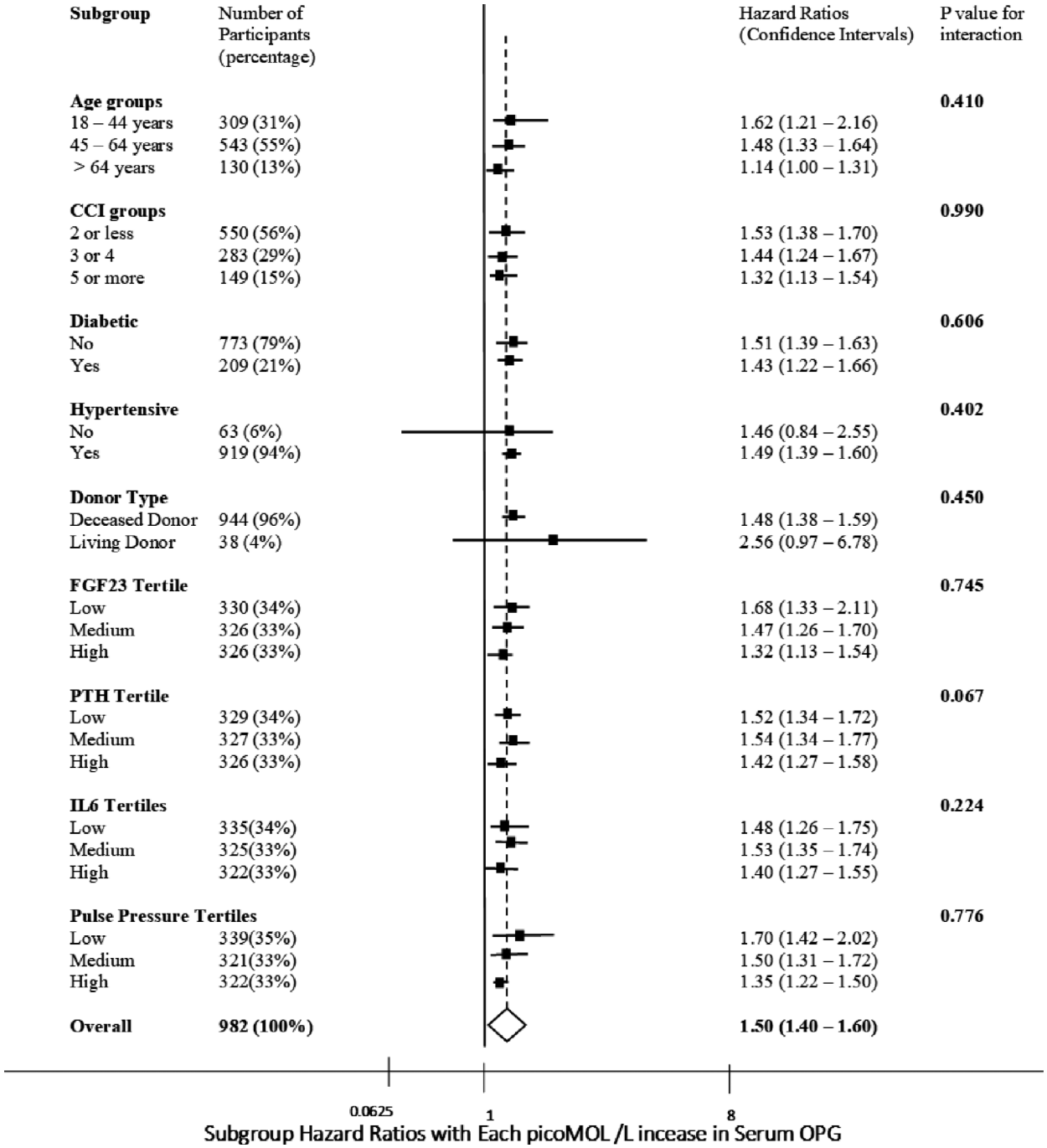

Figure 4 The association between serum OPG and death in different subgroups, showing Hazard Ratios for each 1 pmol/l increase in serum OPG (fully adjusted model—Model 6.) This forest plot shows the hazard ratios and confidence intervals for different subgroups of the population, assessing the interaction between OPG and the subgroup characteristics using Cox proportional hazard regression. The subgroups analyzed include age group, Charlson Comorbidity Index, presence of diabetes mellitus, hypertension, type of donor, and serum levels of FGF23, PTH and IL-6, and participant pulse pressure at baseline. There were no statistically significant interactions noted, with all *P* values greater than 0.05.

analysis considering these novel and potentially relevant biomarkers.

A hallmark of CKD-MBD and a potential link to mortality is arterial stiffness, secondary to arterial calcification. In previous work, we showed that OPG is associated with pulse pressure, a marker of arterial stiffness [15]. In this analysis, it was possible to determine if the association between OPG and mortality is mediated by pulse pressure. In our analysis, adding pulse pressure to the model did not change the association

between OPG and mortality, suggesting that this association is likely mediated by different mechanisms (Table 2).

Limitations of this study will have to be noted when interpreting our results. This was a prevalent cohort of patients enrolled at a single center, and all participants were Caucasian. This may limit the generalizability of our findings. Variables of interest were only assessed at a single time point, at baseline. Several variables have been shown to vary in level with time, such as FGF23, which decreases drastically over the course of a year post-transplant [51]. Furthermore, arterial stiffness and vascular calcification were not measured but would have provided additional information about vascular characteristics. Also, we did not have accurate data regarding cause of death.

A recent paper did not confirm the association between OPG and all-cause mortality in patients on maintenance dialysis in a multiethnic hemodialysis population after adjusting for several clinical, socio-demographic, and biomarker confounders, including FGF23 [52]. That study, however, was relatively small, had shorter follow-up and fewer events than ours [52]. Still, OPG was associated with all-cause mortality in unadjusted analysis in that dataset, and even after adjustment, a trend could be suspected [52]. Future studies will need to confirm the relationship between OPG and mortality in well-powered studies in various settings and populations.

Although the mechanism mediating the association between serum OPG and clinical outcomes remains unknown, the strong association between OPG and mortality demonstrated indicates that OPG may be considered for use in prognostication to advance patient care.

Conclusion

We have shown that serum OPG was associated with mortality in KT patients, even after extensive adjustment for traditional cardiovascular risk factors and uremia-related factors. Importantly, the association remained significant even after adjusting for biomarkers of inflammation and CKD-MBD. This adds to existing evidence that supports the potential role of OPG as an important, independent biomarker of clinical outcomes in KT recipients. Further research needs to define the mechanisms underlying these findings. However, serum OPG may be considered for use in clinical risk assessment, given the strong independent association with outcomes.

Authorship

V.G. contributed to analysis of data, interpretation of data, and writing of the manuscript; O.E. contributed to analysis of data, interpretation of data, and writing of the manuscript; Z.K.N. contributed to data collection, interpretation of results and writing of the manuscript; Y.Y. contributed to analysis of data and writing of the manuscript; A.C. contributed to analysis and interpretation of data and writing of the manuscript; Z.M. contributed to analysis and interpretation of data and writing of the manuscript; C.P.K. contributed to the design of the study, analysis of data, and writing of the manuscript; M.Z.M. contributed to the design of the study and analysis of data, organization and supervision of data collection, and writing of the manuscript; I.M. conceived the plan for the study and for current analysis, design of the study, organization and supervision of data collection, interpretation of data and writing of the manuscript.

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Conflict of interest

The authors have nothing to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Logistic regression used to calculate propensity score (reference: OPG $\geq$ 4.2pmol/l as high, OPG $<$ 4.2pmol/l as low).

Table S2 Collinearity Diagnostics.

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