

Arterial Calcifications and Osteoprotegerin in Chronic Hemodialysis Patients – Impact on 6 Years Survival

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Abstract

The study *goal* was to assess the impact of each type of arterial intimal calcifications (AIC) and arterial medial calcifications (AMC) identified on ultrasound, of osteoprotegerin (OPG), mineral metabolism markers and other features on all-cause and cardiovascular mortality in chronic hemodialysis patients. Ultrasound was performed in 87 patients on the carotid and femoral arteries, and the severity of AIC and AMC was assessed calculating a score according to the extension of calcification. The cut-off value for OPG determined using ROC was 4.9 pmol/L for all-cause and cardiovascular mortality. Patients with higher serum OPG levels presented higher mortality rates. Our results revealed that AIC, high OPG, low ankle-arm index, presence of diabetes, smoking status, and lack of arteriovenous fistula are associated with all-cause and cardiovascular mortality in univariate regression analysis. Multivariate analysis identified AIC scoring based on the segmentation method as an independent predictor of all-cause and cardiovascular mortality, along with increased OPG levels. AMC scoring was not a predictor of mortality. Identifying and scoring AIC on ultrasound and measuring OPG levels, as a basis of the HD patient assessment may become valuable tools in clinical work, as these have an impact on death toll.

Introduction

End-stage renal disease (ESRD) is the image of a successful story of survival for an end-stage organ damage. No other internal medicine derived specialty can report such long-life span when the function of a vital organ is irreversible lost. The death rate of our end-stage renal disease patients remains still high, when comparing to general population, even in young adults¹. Uremic syndrome does not lead to death anymore, but cardiovascular complications are major risk factors. In our effort to keep our patients alive, we are interested in factors that impede a good evolution.

Mineral metabolism is commonly disturbed due to progression of chronic kidney disease and loss of kidney functions. This can lead to cardiovascular diseases, especially related to the high rate of vascular calcifications. Vascular calcification is a consequence of calcium phosphate deposition into arteries, either in their intima or media layers. But vascular calcification is not just a simple mechanical process, it is a multifactorial phenomenon which induces a phenotype switch of vascular smooth muscle cells to osteoblast-like cells; this represents a subject of great interest for medical research². A disequilibrium between pro-calcification and anti-calcification factors contribute highly to this process³.

One of the most important anti-calcification factors is osteoprotegerin (OPG). OPG is produced by osteoblasts and can inhibit osteoclast activation. It is a soluble receptor of receptor activator of nuclear factor- κ B ligand (RANKL). OPG raised the interest of many research groups, but there is no consensus yet regarding its impact on hemodialysis (HD) patients' outcome. Although OPG is an anti-calcification factor, the results of clinical studies indicated poor outcomes. Some papers linked OPG with the pathogenesis of vascular calcification and atherosclerosis. Patients with coronary artery disease or heart failure and high OPG had an increased morbidity and mortality⁴. An association between OPG levels and mortality is present in the general population was the conclusion of a relatively recent meta-analysis⁵.

There is no consensus about the relationship between OPG and cardiovascular morbidity and mortality in HD patients, still raising questions regarding its role^{3,6}.

The association between ESRD and cardiovascular mortality seems to be influenced through vascular alterations, in particular atherosclerosis and vascular calcification⁷.

We *aimed* to study the influence of both types of vascular calcifications (medial and intimal), of OPG serum levels and other specific factors on the survival time to all-cause and cardiovascular death in chronic HD patients.

Patients And Methods

The study was designed as longitudinal, prospective, and analytical. It was carried on a randomly selected population of chronic HD patients. The patients were dialyzed in three sessions of 4–5 h every week, with synthetic high-flux dialyzers. The dialysate respected the standards, bicarbonate was the buffer, and heparin was the anticoagulant agent. We included all prevalent, adult HD patients, who agreed to participate to this study, and we excluded the patients with severe infections, acute illness, neoplasia, parathyroidectomy, previous renal transplant. Evaluation at baseline comprised clinical data, as well as laboratory assessment. We recorded data regarding patients' characteristics, as age, gender, HD vintage, presence of diabetes, medical history, dialysate calcium, HD prescription and medication. Treatment prescriptions were made according to guidelines. HD adequacy was assessed through spKt/V and urea reduction ratio (URR). We calculated body mass index (BMI), pulse pressure (PP) and ankle – arm index (AAI). Osteoprotegerin (OPG) (human-OPG ELISA, Biomedica, Wien, Austria) serum levels were measured. Testing of serum calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH), pre- and post-HD urea, creatinine, albumin, C-reactive protein (CRP), bicarbonate, hemoglobin (Hb), ferritin, cholesterol, HDL-cholesterol, and triglycerides levels, was performed.

Vascular calcifications were detected in carotid and femoral arteries. The arteries walls were examined using a 5–10 MHz linear transducer and real-time B mode and Doppler functions were used. The operator was unaware of patient's clinical and biochemical data, to avoid biases. Arterial intima calcifications (AIC) referred to calcified atheroma plaques recognized as areas of focal intima thickening, with hyperechoic protrusion in the vascular lumen and posterior shadows. Multiple punctiform hyperechoic images in the vascular wall, not protruding in the lumen represented the aspect of the arterial media calcifications (AMC). Examination was done bilateral on common carotid artery, bifurcation, internal carotid artery, common and superficial femoral arteries, and calcification scores were calculated summarizing the presence of the typical image on each examined site, so the AIC and AMC scores ranged from 0 to 10.

Eighty-seven patients, with 47 males (53.87%) were included. Eleven patients were smokers (12.6%) and 21 patients (24.13%) had diabetes. Arteriovenous fistula (AVF) was the vascular access for 62 patients and 25 patients had a central venous catheter. Mean age was 62.74 ± 12.95 years, mean HD vintage was

47.96 ± 49.36 months and a mean spKt/V of 1.46 ± 0.22 was obtained. Vascular calcifications were identified in 71 patients, 68% had AIC, 68% had AMC and 54% had both. More about the baseline characteristics and descriptive statistics are depicted in our previous cross-sectional study⁸.

Evolution, fatal events, death date and cause were registered. Patients were prospectively followed up for 6 years (72 months). All-cause and cardiovascular mortality were analyzed. Cardiovascular mortality was defined as death due to pulmonary edema, heart failure, arrhythmia, ischemic heart disease, peripheral artery disease and stroke. Their frequencies were calculated. Impact of AIC, AMC, OPG and different other factors on mortality was analyzed.

The statistical analysis was realized in IBM SPSS Statistics 25.0 program. Data were expressed as mean ± standard deviation (SD), percentage, or median (25th–75th percentile) for the follow-up period. To compare the means of independent characteristics of two groups, we used Student's t-test or the Mann–Whitney according to the variable distribution. For comparison of categorical variables, Chi-square test or Fisher's exact test was applied. Multiple regression was applied between all-cause and cardiovascular mortality and the potentially associated factors. Significant variables in univariate analysis were entered into multivariate analysis. Survival analysis was performed with Cox regression. The hazard ratios and their 95% confidence intervals for all-cause and CDV deaths were calculated. When the parameter was found significant by Cox's hazard model, Kaplan–Meier analysis was applied to compare two groups stratified by a cut-off. According to the maximum of the Youden Index, a cut-off value of OPG, AIC and AMC that best predicted the all-cause and cardiovascular deaths was identified using a receiver-operating characteristic (ROC) curve. Survival analysis was performed with log-rank test, survival curves were represented with Kaplan-Meier curve. Statistical significance threshold was considered $p < 0.05$.

Ethical standards. This study was approved by the Ethics Committee for Scientific Research of the University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania; the study respected the ethical standards of the Declaration of Helsinki. All included patients agreed to participate to the study and signed the informed consent.

Results

All-cause mortality was analyzed in our research group. Among the 87 patients, 43 patients died (49.4%) due to different causes, as cardiovascular events, or other causes, which indicate to 8.23% annual all-cause mortality. Eight patients died due to infections, 3 due to cancer, 1 due to hemorrhage and 5 of unknown cause. Among all patients included in the study, 26 patients died due to cardiovascular causes (29.88%). Cardiovascular death was produced by pulmonary edema and heart failure in 8 patients, ischemic heart disease and myocardial infarction in 7 patients, arrhythmia in 5 patients, stroke in 4 patients and peripheral artery disease in 2 patients.

Comparison between the deceased and survivor groups is presented in table 1. We found positive significant association between all-cause mortality and age, male gender, current smoking, coexistence of

DM, presence of AIC and AMC and a negative association with the treatment with Ca salts (Table 1).

The results of univariate Cox proportional hazards regression analysis for all-cause and cardiovascular mortality in all study patients are shown in Table 2. Univariate regression analysis showed that old age ($p<0.0001$), male gender ($p=0.03$), diabetes ($p=0.008$), smoking ($p=0.03$), AIC ($p=0.002$), decreased AAI <1 ($p=0.01$), and low usage of treatment with Ca salts ($p=0.01$) were associated with increased all-cause mortality; there was a trend of association between catheters as vascular access due to lack of AVF and death ($p=0.05$). The relation between OPG levels and survival time until all-cause death was tested, and no significant association was found ($p=0.07$) (Table 2). Instead, elevated OPG levels were associated with the decreased survival time in patients who died due to cardiovascular events ($p=0.03$). In univariate analysis, cardiovascular mortality was also associated with older age ($p<0.001$), with diabetes ($p=0.03$), increased AIC score ($p=0.001$), lack of AVF as vascular access ($p=0.03$), low AAI ($p=0.04$) and low prescription of treatments with Ca salts ($p=0.02$).

The cut-off value of OPG that best predicted the all-cause and cardiovascular deaths was 4.9 pmol/ml with an area under the ROC curve of 0.642 and sensitivity 69.76% and specificity 59.1% (95%CI=0.525-0.759, $p=0.022$). The group with OPG <4.9 pmol/ml consisted in 39 patients and 13 deaths (survival rate=66.7%) and the group with OPG ≥ 4.9 pmol/ml consisted in 47 patients (54.7%) with 30 deaths (survival 37.5%) (Figure 1). In 17 patients from survivors' group (39.5%) and in 30 patients from deceased group (69.8%), the OPG levels were ≥ 4.9 pmol/l ($p=0.005$). The Kaplan-Meier analysis showed that, from the group of 39 patients with OPG <4.9 pmol/ml 25.4% died due to cardiovascular causes and from the group of 48 patients with OPG ≥ 4.9 pmol/ml 41.7% died due to cardiovascular causes ($p=0.004$) (Figure 2).

Regarding associations between AIC and AMC with outcomes, we considered 3 categories of arterial calcifications for each type, according to the number of sites affected. A score was calculated, and patients were included in one group with no AIC or AMC, the second with a score from 1 to 4 and the third with a score from 5 to 10. There were no significant differences at AMC analysis; from survivors' group, 22 patients (51.2%) had no calcification, 5 patients (11.6%) had an AMC score of 1-4 and 16 patients (37.2%) had an AMC score ≥ 5 ; from the deceased group, 19 patients (44.2%) had no calcification, 13 patients (30.2%) had an AMC score of 1-4 and 11 patients (25.6%) had an AMC score ≥ 5 ($p=0.095$). Regarding AIC, there were highly significant differences; from survivors: 21 patients (48.8%) had no calcification, 13 patients (30.2%) had an AIC score of 1-4 and 9 patients (20.9%) had an AIC score ≥ 5 ; from deceased: 6 patients (14%) had no calcification, 9 patients (20.9%) had an AIC score of 1-4 and 28 patients (65.1%) had an AMC score ≥ 5 ($p<0.0001$).

In Kaplan-Meier analysis, survival was 78.6% in no AIC group (6 deaths in 28 patients), 59.1% in the group with AIC score of 1-4 (9 deaths in 22 patients) and 24.3% in the group with AIC ≥ 5 (28 deaths from all 37 patients) ($p<0.0001$) (Figure 3). Kaplan-Meier analysis demonstrated a significant association between AIC and cardiovascular mortality ($p=0.001$). Cardiovascular mortality was 10.7 % in the group with no AIC, 22.7% in the group with an AIC score of 1-4 and 48.6% in those with AIC score 5-10 (Figure 4).

Multivariate Cox regression tested the impact of OPG and arterial calcifications on mortality. Covariates were DM, smoking, the vascular access, AAI<1 and treatments with Ca-salts. It demonstrated a significant association between all-cause mortality and increased age, male gender, smoking, presence of diabetes, lack of AVF, increased AIC, AAI<1, low use of treatment with Ca salts. As the age and gender are non-modifiable factors, they were removed from the model; afterwards, OPG and AIC, but not AMC, entered in the model as risk factor for all-cause mortality. The factors influencing cardiovascular mortality were also analyzed in multivariate Cox regression multivariate analysis. After removal of age and gender, OPG and AIC remained in the model, as their high levels are significant risk factors for cardiovascular mortality (Table 3).

Discussion

The quality of the arteries is a very important prognostic factor for chronic HD patients; it has a great impact on their health, disease, and survival. An increasing interest on arterial structure and functions' assessment can be observed in everyday practice as a consequence of positive research results. The main strength of our study is the prospective data of HD patients over a follow-up period of 72 months, in a group known to be at high risk of death.

In our study, the presence of arterial calcification was identified as belonging to the atheroma plaques (AIC) or being situated in the media of the arteries (AMC). These are two different categories of vascular calcifications with different consequences on arterial functions, broadly speaking, vessels occlusion after AIC and stiffness in case of AMC⁹. We demonstrated that severity of AIC, stratified on 3 categories, was associated with all-cause and cardiovascular mortality; AMC had no impact on outcome. The AIC were significant important factors influencing the outcome in our HD patients. Other recent studies evaluated this relation. Some authors used CT and radiograph to examine of the arteries. The presence and extension of vascular calcifications, detected on coronary arteries and abdominal aorta, predicted risk of all-cause death in patients starting hemodialysis¹⁰. The aortic arch calcification was measured on chest radiographs and its progression was associated with mortality in HD patients¹¹. Ultrasound, as one of the most valuable and, also available tool, can easily detect morphological abnormalities of the vascular walls. Discrete modifications of carotid intima media thickness increase cardiovascular mortality in peritoneal dialysis patients¹². Different scoring of AMC realized on ultrasound exam of the lower limbs was associated with chronic complications of diabetes mellitus, especially the nephropathy¹³. There is a constant concern related to possible intervention strategies to prevent and regress the vascular calcification in dialysis patients^{14,15}.

Biomarkers may be essential elements influencing the cardiovascular outcome of ESRD patients^{16,17}. We analyzed, also, other factors impacting the mortality in HD patients. One important biomarker in patients with chronic kidney disease is OPG. Increased levels are present in ESRD patients and indicate a crosstalk between the bone and vessels in chronic HD patients¹⁶. In one of our previous studies, we demonstrated that increased circulating OPG levels are associated with vascular calcifications⁸. The present research

identified a significant relationship between high OPG serum levels and all-cause mortality in chronic HD patients, after 6 years of follow-up. Cardiovascular mortality was also significantly associated with increased OPG levels. A literature-based meta-analysis involving a high number of participants analyzed possible links between OPG and cardiovascular outcomes in the general population; it concluded that high OPG is associated with an increased risk of incident cardiovascular disease⁵. In predialysis chronic kidney disease patients, OPG was declared a marker of cardiovascular events and mortality^{18,19,20}. Hemodialysis patients were also studied and OPG was associated with mortality^{21,22}. A recent research on HD patients demonstrated no significant link between OPG and mortality²³. All these results may seem a medical paradox, as it was demonstrated that OPG is an anti-calcification factor²⁴ and low OPG maybe associated with poor prognosis in some HD patients²⁵. Studies have also demonstrated that denosumab, an endogenous RANKL inhibitor which mimics the natural action of OPG, may suppress the progression of arterial calcifications^{26,27}. Further studies are warranted before a sound hypothesis for this seeming contradiction can be set.

We obtained other important results related to impact on outcome. Our patients with no AVF, using a central venous catheter as the vascular access for HD had an elevated all-cause and cardiovascular mortality; this is a result consistent some studies²⁸, but not with others²⁹. Best survival belonged to patients with AVF.

The relationship between smoking and outcomes in HD patients is not well understood. In our study, smokers and diabetes had higher mortality rate. A retrospective cohort study analyzed death at 2 years in HD patients and reported that incidence rate of mortality for active smokers with diabetes was high and that death probabilities increased³⁰ with greater exposure to smoking³⁰.

We demonstrated also that AAI<1 is associated with all-cause and cardiovascular mortality. A low ankle-arm index is a marker of atherosclerosis and it can suggest stenosis or thrombosis of the arteries in the lower limbs. So, this result indicates that atherosclerotic peripheral arterial disease is a risk factor for death in ESRD patients. Studies were generally inconsistent regarding this issue, but a newer publication showed promising and similar results³¹.

Treatment with Ca-salts offered protection against death in our HD patients. This is a controversial area, some authors detected an increased risk of cardiovascular death³², but our results may be explained by a possible trend to better control of mineral metabolism due to this treatment, even thou the specific markers were not significantly correlated with mortality.

There are also several *limitations in this study*. First, including patients with both AIC and AMC could be misleading. Second, relatively reduced number of patients impede us to make a general statement about these results. Finally, a single measurement of OPG, AIC, AMC and AAI do not offer the possibility to assess their progression in relation with mortality. Further studies are necessary to address this issue.

In conclusion, we assessed AIC and AMC on ultrasound, OPG and other data with regards to predicting survival in HD patients. Univariate analysis showed that high AIC and OPG, age, smoking, diabetes, low

AAI and lack of AVF are associated with all-cause and cardiovascular mortality in all study patients. In multivariate analysis, AIC scoring and OPG predicted all-cause and cardiovascular mortality. To improve the survival of dialysis dependent patients, it is critical to understand the contributions of potentially modifiable risk factors. Hence, identifying AIC on ultrasound and measuring OPG may provide benefit in survival prediction in HD patients.

Declarations

Data availability. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests

The author(s) declare no competing interests.

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Tables

Table 1. Comparison between the deceased and survivor groups.

	HD deceased (43 patients)	HD survivors (44 patients)	p
OPG (pmol/ml)	5.9 (4.60-8.00)	4.5 (3.15-6.50)	0.103
Age (years)	68 (62-74)	57 (52.50-68.50)	<0.0001
HD vintage (months)	44.26±39.06	51.58±58.62	0.497
Gender 46 males (53.5%)	28 (65.1%)	18 (41.9%)	0.031
Smoker 10 pts (11.6%)	8 (18.6%)	2 (4.7%)	0.044
DM 21 pts (24.4%)	16 (37.2%)	5 (11.6%)	0.006
AVF 61 pts (70.9%)	27 (62.8%)	34 (79.1%)	0.096
BMI (kg/m ²)	27.12 (24.43-29.94)	26.67 (26.79-31.65)	0.855
PP (mmHg)	60 (50-65)	50 (50-60)	0.970
AAI	0.54 (0.38-0.74)	0.75 (0.18-1)	0.375
AIC	6 (3.5-9)	1 (0-4)	<0.0001
AMC	4 (1-6)	4 (0-4)	0.037
Ca (mg/dl)	8.38±0.44	8.31±0.55	0.527
P (mg/dl)	5.07±1.67	5.18±1.72	0.762
ALP (U/l)	66 (53-85)	65 (52-90)	0.653
iPTH (pg/ml)	231.85 (147.90-421.70)	203.80 (107.80-353.70)	0.472
Bicarbonate	17.56±3.67	17.07±3.00	0.499
Hb (g/dl)	11.58±1.11	11.53±1.43	0.847
Ferritin (ng/ml)	772.05 (477-897.80)	772.50 (673.30-937.95)	0.339
CRP (mg/dl)	0.90 (0.39-2.27)	0.88 (0.40-1.72)	0.839
Albumin (g/dl)	4.20 (4.04-4.42)	4.11 (3.97-4.32)	0.578
Cholesterol (mg/dl)	159 (144.50-203.50)	161 (143-189)	0.276
HDL- Cholesterol (mg/dl)	45.81±12.06	49.91±13.50	0.170
Trygliceride (mg/dl)	139 (107.50-211.50)	133 (84-173)	0.260
Urea (mg/dl)	133.01±32.08	136.74±30.42	0.582
Creatinine (mg/dl)	8.60±2.22	8.43±2.38	0.740
URR	70.57 (64.03-76.11)	73.12 (68.59-77.19)	0.379

spKt/V	1.42 (1.26-1.55)	1.48 (1.37-1.58)	0.379
Ca in HD solution	1.51±0.14	1.53±0.13	0.432
Treatment with Ca salts - 71 patients (82.6%)	32 (74.4%)	39 (90.7%)	0.047
Treatment with Vitamin D - 30 patients (34.9%)	16 (37.2%)	14 (32.6%)	0.651
Sevelamer - 21 patients (24.4%)	11 (25.6%)	10 (23.3%)	0.802
ACEI - 29 patients (33.7%)	16 (37.2%)	13 (30.2%)	0.494
Betablockers – 59 patients (68.6%)	32 (74.4%)	27 (62.8%)	0.245
CCB - 39 patients (45.3%)	19 (44.2%)	20 (46.5%)	0.829
Statins - 11 patients (12.8%)	4 (9.3%)	7 (16.3%)	0.333

Legend: OPG=osteoprotegerin; HD=hemodialysis; DM=diabetes mellitus; AVF=arteriovenous fistula; BMI=body mass index; PP=pulse pressure; AAI=ankle-arm index; AIC=arterial intima calcification; AMC=arterial media calcification; Ca=calcium; P=phosphorus; ALP=alkaline phosphatase; iPTH=intact parathyroid hormone; Hb=hemoglobin; CRP=C-reactive protein; URR=urea reduction ratio; spKt/V=dialysis adequacy; ACEI=angiotensin converting enzyme inhibitors, CCB= Calcium channel blockers. Data are expressed as mean ±standard deviation and as median (25th-75th percentiles) or percentages. Statistically significance is marked with bold characters.

Table 2. Results of univariate Cox regression analysis for all-cause and cardiovascular mortality.

	All-cause mortality		Cardiovascular mortality	
	HR (95%CI)	p	HR (95%CI)	p
OPG (pmol/ml)	1.07 (0.99-1.16)	0.098	1.10 (1.01-1.21)	p=0.033
OPG \geq 4.9 (pmol/ml)	2.45 (1.28-4.71)	0.005	3.53 (1.42-8.81)	0.007
Age (years)	1.07 (1.03-1.10)	<0.0001	1.12 (1.06-1.17)	<0.001
HD vintage (months)	1.00 (0.99-1.00)	0.588	1.00 (0.99-1.01)	0.54
Gender (males)	1.99 (1.06-3.73)	0.03	1.24 (0.57-2.68)	0.58
Smoker	2.27 (1.05-4.90)	0.03	1.79 (0.62-5.21)	0.285
DM	2.26 (1.21-4.20)	0.008	2.34 (1.06-5.18)	0.03
AVF	0.54 (0.29-1.00)	0.05	0.44 (0.20-0.95)	0.03
BMI	0.99 (0.94-1.04)	0.757	1.00 (0.94-1.07)	0.937
PP	1.00 (0.98-1.02)	0.546	1.00 (0.97-1.03)	0.926
AAI<1	0.35 (0.16-0.79)	0.01	0.33 (0.11-0.96)	0.04
AIC score	1.18 (1.09-1.27)	0.002	1.20 (1.08-1.34)	0.001
AMC score	1.13 (1.03-1.24)	0.013	1.13 (1.00-1.28)	0.056
Ca (mg/dl)	1.18 (0.67-2.09)	0.570	0.85 (0.41-1.80)	0.679
P (mg/dl)	0.95 (0.79-1.13)	0.546	1.02 (0.81-1.28)	0.888
ALP (U/l)	1.00 (0.99-1.01)	0.614	1.00 (0.99-1.01)	0.496
iPTH (pg/ml)	1.00 (1.00-1.00)	0.642	1.00 (0.999-1.002)	0.346
Bicarbonate (mmol/l)	1.04 (0.94-1.15)	0.421	1.07 (0.94-1.21)	
Hb (g/dl)	1.03 (0.82-1.28)	0.823	1.04 (0.78-1.38)	0.803
CRP (mg/dl)	1.00 (0.999-1.001)	0.618	1.00 (0.998-1.001)	0.773
Albumin (g/dl)	1.03 (0.91-1.17)	0.688	1.03 (0.86-1.22)	0.671
Cholesterol (mg/dl)	1.22 (0.46-3.28)	0.168	0.77 (0.23-2.60)	0.240
HDL- Cholesterol (mg/dl)	1.00 (1.00-1.01)	0.183	1.01 (1.00-1.01)	0.523
Trygliceride (mg/dl)	1.00 (0.999-1.004)	0.176	1.00 (1.00-1.01)	0.688
Urea	1.00 (0.99-1.01)	0.407	0.745 (0.586-1.144)	0.255
Creatinine (mg/dl)	1.01 (0.89-1.15)	0.891	1.09 (0.92-1.28)	0.325
URR	1.00 (0.96-1.02)	0.472	0.97 (0.93-1.01)	0.189

spKt/V	0.61 (0.16-2.37)	0.472	0.30 (0.05-1.80)	0.189
Ca in HD solution	2.78 (0.31-25.11)	0.364	5.73 (0.34-96.91)	0.226
Treatment with Ca salts	0.40 (0.20-0.80)	0.01	0.38 (0.16-0.91)	0.029
Treatment with Vitamin D	1.18 (0.64-2.20)	0.593	1.47 (0.67-3.20)	0.333
Sevelamer	1.10 (0.56-2.19)	0.779	0.96 (0.39-2.39)	0.929
ACEI	1.18 (0.64-2.19)	0.600	0.88 (0.38-2.03)	0.765
Betablockers	1.53 (0.77-3.04)	0.223	1.19 (0.52-2.74)	0.681
CCB	0.91 (0.50-1.66)	0.757	0.84 (0.39-1.84)	0.668
Statins	0.65 (0.23-1.83)	0.416	0.84 (0.25-2.80)	0.775

Legend: OPG=osteoprotegerin; HD=hemodialysis; DM=diabetes mellitus; AVF=arteriovenous fistula; BMI=body mass index; PP=pulse pressure; AAI=ankle-arm index; AIC=arterial intima calcification; AMC=arterial media calcification; Ca=calcium; P=phosphorus; ALP=alkaline phosphatase; iPTH=intact parathyroid hormone; Hb=hemoglobin; CRP=C-reactive protein; URR=urea reduction ratio; spKt/V=dialysis adequacy; ACEI=angiotensin converting enzyme inhibitors, CCB= Calcium channel blockers. Statistically significance is marked with bold characters.

Table 3. Multivariate Cox regression analysis for all-cause and for cardiovascular mortality.

	All-cause mortality		Cardiovascular mortality	
	HR (95%CI)	p	HR (95%CI)	p
OPG \geq 4.9 (pmol/ml)	2.976 (1.380-6.418)	0.005	5.838 (1.943-17.542)	0.002
AIC score	1.797 (1.148-2.813)	0.01	2.078 (1.145-3.771)	0.016
AMC score	1.331 (0.878-2.019)	0.178	1.476 (0.881-2.474)	0.139

Legend OPG=osteoprotegerin, AIC=arterial intima calcification; AMC=arterial media calcification. Covariates were DM, smoking, vascular access, AAI, treatments with Ca salts. Statistically significance is marked with bold characters.

Figures

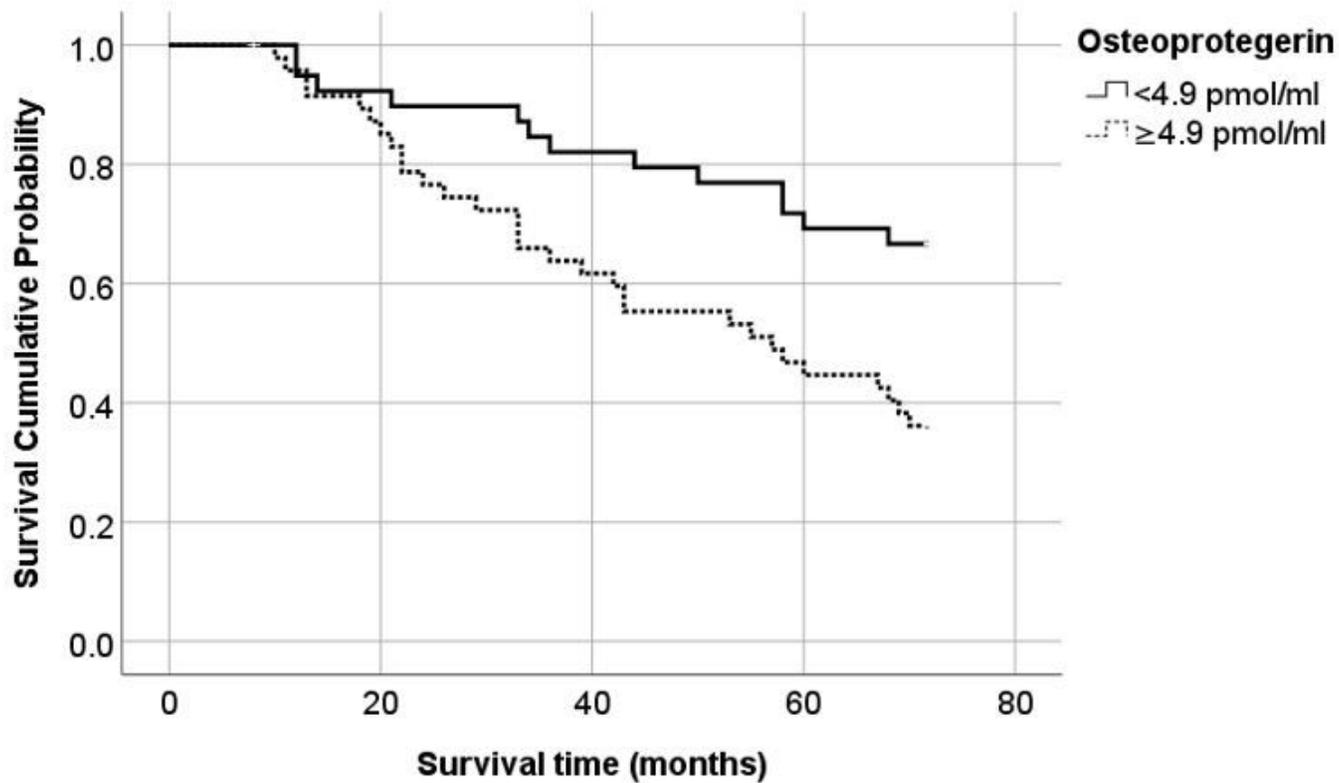


Figure 1

All-cause mortality comparison according to OPG. Kaplan–Meier curve illustrates 72-month survival. Survival rate was 66.7% in the group with $OPG < 4.9$ pmol/ml and 37.5% in the group with $OPG \geq 4.9$ pmol/ml ($p=0.005$).

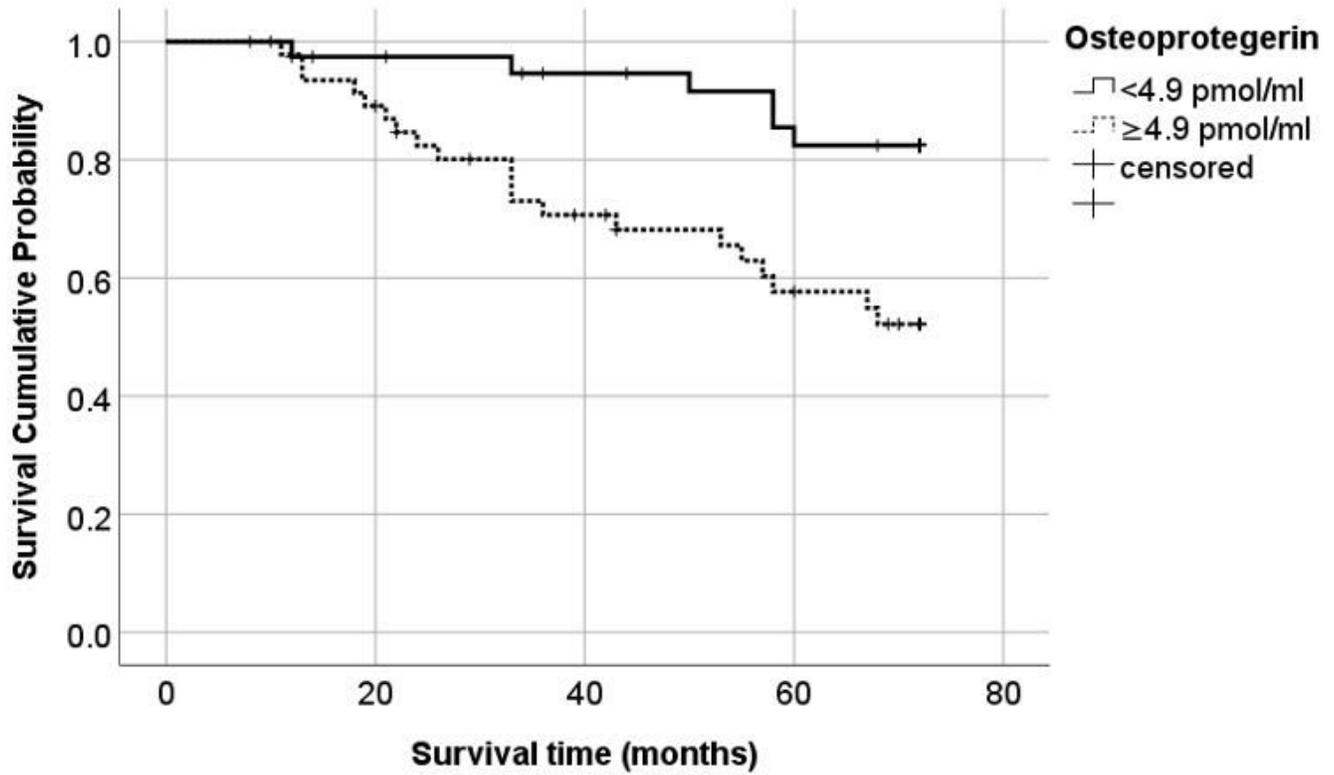


Figure 2

Kaplan–Meier curve illustrates 72-month survival according to OPG. Cardiovascular mortality was 25.4% in the group with OPG<4.9 pmol/ml and 41.7% in the group with OPG≥4.9 pmol/ml (p=0.004).

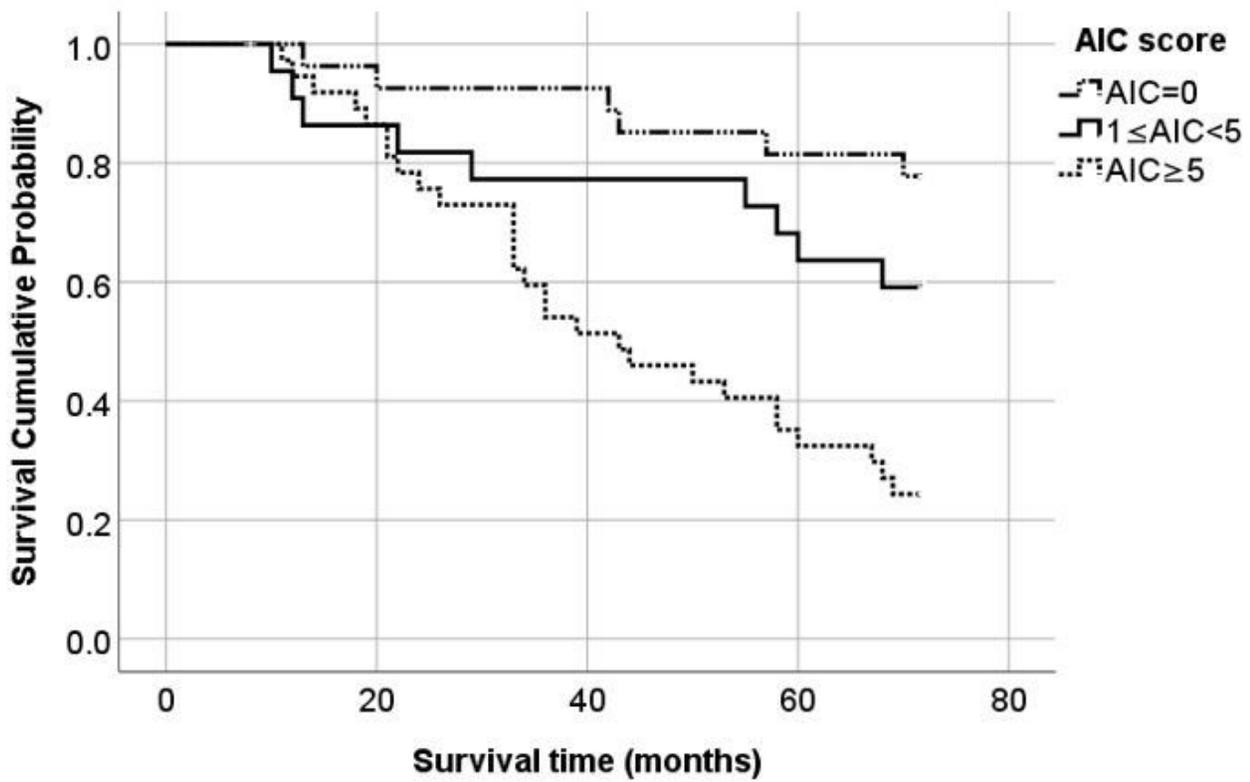


Figure 3

All-cause mortality comparison between groups according to AIC score. Survival in no AIC group was 78.6%, in AIC=1-4 group was 59.1% and in AIC=5-10 was 24.3% ($p < 0.0001$).

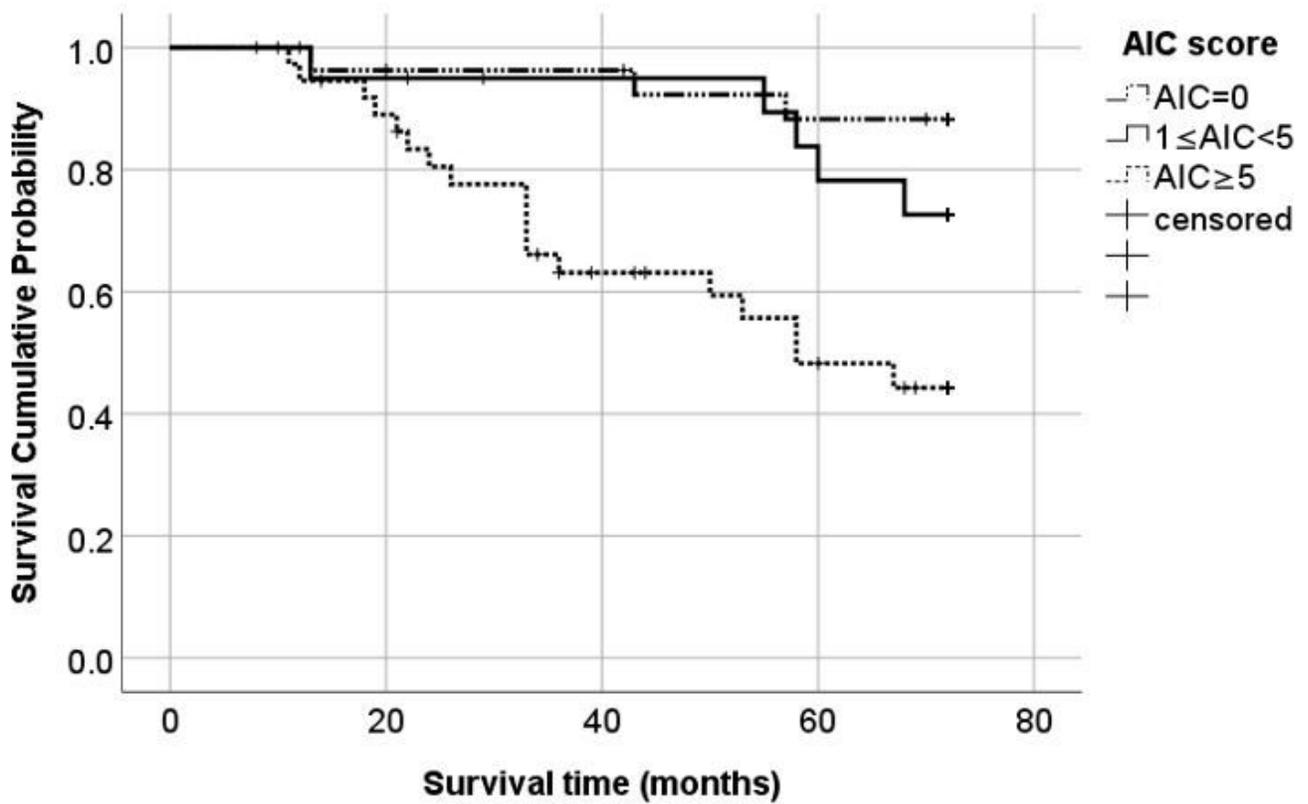


Figure 4

Kaplan–Meier curve illustrates 72-month survival according to AIC score. Cardiovascular mortality rate was 10.7% in the group with no AIC, 22.7% in the group with AIC=1-4 and 48.6% in the group with AIC=5-10 ($p=0.01$).