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Elevated osteoprotegerin is associated with all-cause mortality in CKD stage 4 and 5 patients in addition to vascular calcification

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Abstract

Background. Cardiovascular disease is the leading cause of death in the chronic kidney disease (CKD) population. The mechanisms of vascular damage are not fully understood. The objective of this study was to prospectively investigate the importance of novel mediators of vascular damage, in conjunction with vascular calcification (VC), on survival.

Methods. A total of 134 subjects [60 haemodialysis (HD), 28 peritoneal dialysis (PD) and 46 CKD stage 4] were studied. All survivors completed 40 months of follow-up. VC was measured using multi-slice spiral CT of the superficial femoral artery. Circulating osteoprotegerin (OPG), Fetuin-A and high sensitivity C-reactive protein (hs-CRP) were measured in addition to standard clinical biochemical analysis.

Results. After a 40-month follow-up, 31 patients had died (27 men and 4 women). Of 31 subjects, 31 had evidence of significant VC. The majority of deaths were in the HD group (48%), 36% were PD subjects and 16% were CKD subjects. The outcome of interest was survival at the end of follow-up. Multivariate logistical regression analysis revealed male gender [OR 8.06 (1.34–48.450) $P = 0.02$], OPG >25 pmol/L [OR 5.31(1.35–20.88) $P = 0.02$] and hypoalbuminaemia [OR 0.26 (0.12–0.56) $P < 0.01$], were associated with increased odds of death.

Conclusion. We have previously reported that VC and low albumin predict death in CKD stages 4 and 5 over a 2-year follow-up period. These data show that OPG, independent of CRP, is also associated with a negative outcome. The mechanisms remain to be elucidated; however, it is likely that they are associated with vascular damage through mechanisms in addition to VC.

Keywords: CKD; mortality; osteoprotegerin; vascular calcification

Introduction

Cardiovascular disease is the leading cause of death in the chronic kidney disease (CKD) population [1]. The mechanisms of vascular damage in this population are not fully explained by traditional cardiovascular risk factors [2]. We have previously demonstrated that rapid progression of vascular calcification (VC) occurs in a heterogeneous population of CKD stage 4 and 5 patients [3]. In addition, progressive VC has direct detrimental effects on vascular function and mortality in this population. The progression of VC in this longitudinal follow-up study was not however associated with a variety of factors (such as circulating markers of inflammation and Fetuin-A) described previously in cross-sectional studies [4].

OPG is a key cytokine that belongs to the tumour necrosis factor (TNF) receptor superfamily, which has a range of pleiotropic effects on bone metabolism, endocrine function and the immune system [5]. OPG inhibits osteoclastic bone resorption by binding to the receptor activator of nuclear factor-κB ligand (RANKL), acting as a decoy receptor to competitively inhibit RANKL interaction with its receptor, RANK [6]. Recently, the OPG/RANKL/RANK axis has been implicated in various inflammatory responses and has also been linked to atherogenesis and endothelial dysfunction [7–9]. In addition, OPG has been associated with the presence of VC in both CKD and diabetic populations [8,10].

OPG is associated with increased mortality in diabetes, coronary artery disease and acute coronary syndrome [8,11,12]. In addition, the association between OPG and mortality has recently been demonstrated in haemodialysis patients and kidney transplant recipients [13,14]. The association between OPG and mortality in CKD stage 4 and 5 patients, receiving differing dialysis modalities, has not previously been investigated.

The objective of this study was to prospectively investigate the importance of novel mediators of vascular damage, in conjunction with VC, on long-term patient survival.

Method

Subjects

A total of 134 subjects (60 HD, 28 PD and 46 CKD stage 4) were recruited from Derby City General Hospital (DCGH). All HD, PD and CKD stage 4 patients were eligible to enter the study unless they had been previously transplanted, or had an amputated limb. Patients with malignancies were not excluded from the study; however, only one patient had a known active malignancy on entry to the study. All eligible patients registered under the care of the renal consultants were approached to take part in this study (total population of 158 HD, 71 PD and 62 CKD stage 4 in May 2003, at the start of recruitment). CKD stage 4 patients were staged on four variable MDRD estimated glomerular filtration rate (eGFR) with values between 15 and 29 mL/min/1.73 m^2. Agents that can modulate OPG including bisphosphonates and oestrogens were not exclusions to the study; however, only one patient was receiving each agent. The Local Regional Ethics Committee granted project approval, and written consent was obtained from participants.

Study design

Multi-slice computerized tomography (MSCT) was used to quantify VC (CaSc) using the GE Medical Systems lightspeed16® multi-slice spiral CT scanner. Images were acquired when the patients were supine, and no contrast was used. A standardized section of the superficial femoral artery (SFA), 20 cm above the tibial plateau, 5 cm in length, was imaged in 2.5 mm slices, and care was taken to ensure that none of the slices overlapped. This image allowed accurate, reproducible quantification of calcium load in this section of artery. Each slice was scored individually and a CaSc was generated. For each individual area of calcification in the SFA, a 'region of interest' was drawn. The software then calculated the area and the density of any lesion ≥130 HU. A score was determined based on the maximal CT density in the following manner: 1 = 130–199, 2 = 200–299, 3 = 300–399 and 4 = ≥400 HU. A score for each lesion was then calculated by multiplying the density score with the area. A total CaSc for each image was calculated by totalling all scores from each of the 20 slices acquired. As the CaSc is a composite of other measures, it has no specific units. Validation studies confirmed that the scoring technique is highly reproducible. Interobserver reproducibility between the investigator and a consultant radiologist was assessed in a 1 in 20 sample. The intra-class correlation was 1 [confidence interval (CI) 1–1], and the coefficient of variation (CoV) was 3.9%. Repeatedly scored scans showed an intra-observer intra-class correlation of 1 (CI 1–1), and a CoV of 2.4%. Pulse wave velocity (PWV) was performed using SphygmoCor® (AtCor Medical Pty Ltd, Australia) by a single observer. Electrocardiogram-gated PWV was assessed at the carotid and radial pulses. Three blood pressure (BP) recordings were taken using an automated AND® UA-767 oscillometric device.

Biochemistry

Monthly blood samples were collected for HD patients and at regular clinic visits for PD and CKD stage 4 patients. Serum phosphate (PO_4), Ca, albumin corrected calcium (CCa), albumin, intact parathyroid hormone (PTH), lipid profile and alkaline phosphatase (ALP) were analysed using standard autoanalyser techniques (Roche diagnostics modular IIP®). Serum PTH was measured using the immunometric, Immulite® 2000 assay. A time-averaged baseline value is given for biochemical variables (results averaged over the 6 months prior to the study). Commercially available ELISA kits were used to assess OPG, Fetuin-A and high-sensitivity C-reactive protein (hs-CRP) from samples obtained at baseline. The OPG ELISA kit measures free OPG and complexed OPG–sRANKL concentration in the sample, the monomeric as well as the dimeric form of OPG (Immunodiagnostic Systems, UK). The OPG ELISA has a detection limit of 0.14 pmol/L; intra- and inter-assay CoV 10–4% and 7–8%. The Fetuin-A ELISA (BioVendor, Czech Republic) had a detection limit of 0.35 ng/mL, intra- and inter-assay CoV 4.8–3% and 2.5–5.4%.The hs-CRP (DRG instruments, Germany) detection limit was 0.1 mg/L, intra-assay CoV of 7–2%.

Statistical analysis

Results are reported as mean ± SD for normally distributed data and median (range) otherwise. Pearson chi-square, one-way ANOVA and Kruskal–Wallis tests were used to compare each baseline variable among modality groups, where appropriate. The outcome of interest was defined as death or alive at the end of the follow-up. Univariate associations between the outcome and each of the baseline variables were investigated using logistic regression analysis, adjusted for age and gender. Variables that were univariately associated with the outcome ($P < 0.15$) (diabetes, cardiovascular comorbidity, total calcium, serum albumin, dichotomized OPG (>25, ≤25 pmol/L), dichotomized CRP (>2, ≤2 mg/L) and CaSc (0, 1–400, >400) were entered simultaneously into a multivariate logistics regression model. Using a stepwise selection method, significant predictors of mortality were identified. The odds ratio (OR) (95%CI) and statistical

Table 1. Cohort characteristics at baseline by dialysis modality

	Total ($n = 134$)	CKD ($n = 46$)	PD ($n = 28$)	HD ($n = 60$)	P-value
Age (years)	60 ± 14	60 ± 14	61 ± 14	60 ± 15	0.98
Male (%)	64	56	60	70	0.25
Diabetes (%)	27	21	29	27	0.57
Cardiovascular comorbidity[a] (%)	26	24	43	20	0.07
Non-calcium-based phosphate binders (%)	32	2	57	43	<0.001
Calcium-based phosphate binders[b] (%)	50	37	46	61	0.04
1-alpha calcidol (%)	50	37	61	55	0.34
Calcification score	49 [0–4596]	2 [0–1159]	26 [0–4596]	121 [0–2279]	0.016
PWV (min/sec)	10.3±3.4	9.4±3.1	10.8±2.4	10.8±3.8	0.91
Corrected calcium (mmol/L)	2.45 ± 0.13	2.38 ± 0.12	2.51 ± 0.11	2.46 ± 0.12	<0.001
Phosphate (mmol/L)	1.59 ± 0.35	1.49 ± 0.28	1.59 ± 0.27	1.69 ± 0.42	0.013
PTH (pg/mL)	195 [15–1876]	180 [34–428]	207 [72–1446]	228 [15–1876]	0.18
Albumin (g/L)	34 [18–40]	35 [28–40]	29 [20 -34]	34 [18–39]	<0.001
Bicarbonate (mmol/L)	24 ± 4	23 ± 4	26 ± 3	23 ± 3	<0.001
Alkaline phosphatase (IU/L)	216 ± 151	165 ± 106	308 ± 204	212 ± 132	<0.001
Total cholesterol (mmol/L)	4.61 ± 1.03	5.09 ± 0.91	4.51 ± 1.02	4.29 ± 1.00	<0.001
Osteoprotegerin (pmol/L)	24.5 [9.3–58.6]	21.9 [9.3–47.6]	28.3 [17.1–58.6]	28.9 [12.2–48.0]	<0.001
C-reactive protein (mg/L)	3.9 [0.1–147.9]	2.3 [0.2–44.3]	3.4 [0.3–147.9]	6.88 [0.1–45.9]	0.028
Fetuin-A (ng/mL)	0.24 (0.01–1.16)	0.24 (0.03–0.63)	0.23 (0.05–0.38)	0.24 (0.01–1.16)	0.61

Results are expressed as mean ± SD for normally distributed data, median [range] for data not normally distributed or number of observations (percentage of total) otherwise.
[a]CV comorbidities are defined as any previous description of ischaemic heart disease, heart failure, cerebral vascular disease or peripheral vascular disease recorded in the patients medical notes.
[b]Some patients were prescribed both calcium-containing and non-calcium-containing phosphate binders.

significance (P) are reported. The interaction between OPG and CRP was examined using exact logistic regression due to zero death count observed in low OPG and low CRP group. The computation of exact logistic regression is quite intensive. To reduce the computation intensity, age was dichotomized into >60 versus ≤60 years, and albumin into >34 versus ≤34 g/L in this model. Two-sided P-values <0.05 were considered significant unless otherwise stated. All analyses were performed with the SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

Results

The clinical characteristics of the 134 study subjects are summarized in Table 1. The CKD stage 4 and 5 patients in each group were well matched for age, gender and diabetic status. The patients were characterized by good mineral control (mean CCa 2.45 ± 0.13 mmol/L, mean PO_4 1.59 ± 0.35 mmol/L) and low levels of systemic inflammation (mean hs-CRP 3.9, range 0.1–147.9 mg/L).

After a 40-month follow-up, 31 patients had died (27 men and 4 women). Of 31 subjects, 30 had evidence of significant VC. A total of 42% of deaths were due to defined cardiovascular causes. The majority of deaths were in the HD group (48%), 36% were on PD and 16% were CKD subjects. The univariate factors associated with all-cause mortality, adjusting for age and gender, were diabetes, cardiovascular comorbidities, hypoalbuminaemia, OPG >25 pmol/L and CaSc >400 (summarized in Table 2). Serum phosphate, CRP, PTH, ALP, Fetuin-A, PWV, phosphate binders or vitamin D analogues were not associated with patient survival. Figure 1 illustrates the significant predictors of mortality from the multivariate logistic regression model (gender, hypoalbuminaemia and OPG >25 pmol/L).

Univariate analysis showed OPG to be strongly correlated with PWV ($P = 0.001$, $r = 0.33$) and less so with CaSc ($P = 0.02$, $r = 0.21$). We found no correlation between hs-CRP and OPG ($r = 0.16$, $P = 0.11$). We then investigated the additive impact of CRP and OPG on mortality; Figure 2 depicts this (high and low/normal values of OPG and hs-CRP are defined as >25 pmol/L and >2 mg/L, respectively). Although there was a trend towards a lower mortality in individuals with low OPG and CRP, statistical analysis did not show a significant interaction between OPG and CRP on mortality ($P = 0.16$). Table 3 shows the differing characteristics between those individuals with high and low OPG levels. Those individuals with OPG >25 pmol/L were found to be older, have higher CV mortality and higher CaSc, and also to have less diabetes and lower serum total cholesterol levels.

Table 2. Logistic regression analysis of factors that univariately predict all-cause mortality in CKD stage 4 and 5 patients, adjusting for age and gender

Variable	Odds ratio	95% Confidence interval	P-value
Diabetes	0.33	0.13–0.84	0.020
Cardiovascular comorbidities	3.76	1.51–9.34	0.005
Albumin (g/L) (per 5-unit increase)	0.23	0.12–0.44	<0.001
OPG >25 (pmol/L)	5.28	1.68–16.57	0.004
CaSc = 0	0.04	0.005–0.36	0.006
CaSc 1–400	0.38	0.15–0.97	
CaSc >400	Ref	Ref	

OPG = circulating osteoprotegerin, CaSc = calcification score.

Discussion

This is the first study to show an association between OPG and mortality, in addition to VC, in both CKD stage 4

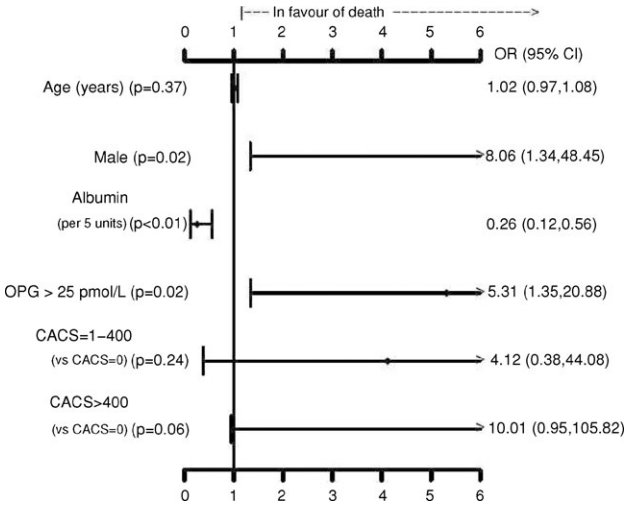

Fig. 1. Multivariate logistic regression model of factors that predict all-cause mortality in CKD stage 4 and 5 patients.

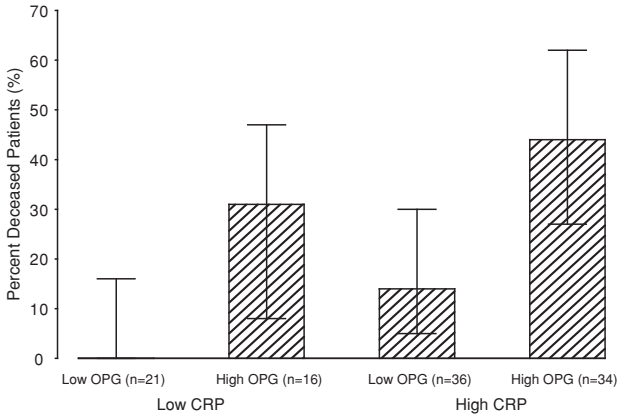

Fig. 2. Mortality by the combination of hs-CRP and OPG categories. Bars represent the 95% confidence interval (*P*-value for the interaction of OPG and CRP = 0.16).

and 5 patients. This prospective observational study has demonstrated that individuals with both a high OPG and a high CRP experience a higher incidence of death (although not ultimately statistically significant). This non-significant finding may be due to small numbers included in this analysis. These findings are consistent with three other studies investigating OPG and mortality in patients with renal disease. Hjelmesaeth *et al*. followed 173 patients for 8 years post-renal transplant [13]. These authors found that post-transplant OPG was independently associated with a 6-fold increase in all-cause mortality, and an 8-fold increase in CV mortality, in a multivariate model adjusted for known risk factors. Morena *et al*. investigated 2-year survival in 185 HD patients [14]. This group also found that elevated OPG predicted all-cause mortality, but only in those individuals with a raised CRP (≥12.52 mg/L). Speer *et al*. investigated mortality risk in a population of 98 HD patients with known OPG and PWV. OPG was a strong predictor of survival in the multi-variate analysis (HR 7.189, 1.89–27.25), independent of PWV. This study found a strong correlation between PWV and OPG, consistent with our

Table 3. Characteristics of those individuals with a low (<25 pmol/L) and high (>25 pmol/L) OPG levels

	OPG ≤25 pmol/L	OPG >25 pmol/L	*P*-value
Age (years)	56 ± 15	66 ± 13	<0.001
Male (%)	63	70	0.47
Diabetes (%)	83	65	0.03
Cardiovascular comorbidity (%)	17	32	0.05
Calcification score	2 [0–2279]	164 [0–4596]	0.001
Corrected calcium (mmol/L)	2.44 ± 0.13	2.45 ± 0.13	0.66
Phosphate (mmol/L)	1.60 ± 0.35	1.59 ± 0.37	0.92
PTH (pg/mL)	195 [15–1876]	196 [34–773]	0.54
Albumin (g/L)	34 [21–40]	34 [20–39]	0.17
Bicarbonate (mmol/L)	23 ± 4	24 ± 3	0.15
Total cholesterol (mmol/L)	4.75 ± 1.10	4.34 ± 0.90	0.03
Alkaline phosphatase (IU/L)	225 ± 162	207 ± 124	0.51
Osteoprotegerin (pmol/L)	20.1 [9.3–24.8]	32.7 [25.3–58.6]	–
C-reactive protein (mg/L)	3.4 [0.04–45.8]	6.2 [0.3–147.9]	0.12

OPG = circulating osteoprotegerin, PTH = parathyroid hormone.
Results are expressed as mean ± SD for normally distributed data, median [range] for data not normally distributed or number of observations (percentage of total) otherwise.

findings. None of these studies included any assessment of VC.

The possible mechanisms to explain the negative prognostic effect of OPG are not yet fully understood. VC and arterial stiffening are associated with mortality in both general population and in individuals with CKD [3,15,16]. OPG has been implicated in the pathogenesis of VC, particularly in HD patients, in a number of studies [10,17,18]. VC is thought to be predominantly an active, cell-mediated process secondary to vascular smooth muscle cell transdifferentiation into osteoblast like cells (although passive processes may also play a role in the pathogenesis of VC). OPG and RANKL have been shown to be localized within calcified vessels [19]. VC results in stiffened blood vessels (increased PWV). Consistent with this, we found that OPG is associated with both PWV and CaSc at baseline. Our previous findings showed that OPG was not associated with the progression of VC over 2 years; thus, OPG may be associated with the presence rather than the progression of VC.

Our findings imply that OPG is associated with mortality in addition to its established role in VC. This theory is supported by several epidemiological studies which suggest that elevated levels of OPG are associated with vascular risk in the general population, not normally at risk of developing VC [20,21]. Clinical studies have identified OPG as a novel biomarker for incident heart failure in patients with acute coronary syndrome and silent myocardial ischaemia [12,22]. Omland *et al*. postulated that OPG could play a direct pathological role in the development of left ventricular function and systolic dysfunction [23]. In addition, Jono *et al*. found an association between rising OPG level and coronary artery stenosis identified by coronary angiography [11]. It is possible that OPG has multiple roles in

vascular damage. One potential mechanism is via its inflammatory role. Despite the lack of association with hs-CRP in this study, as a member of the TNF receptor superfamily, OPG could be involved in inflammatory pathways. The association between raised CRP and OPG is inconsistent in the literature. Hjelmesaeth *et al*. and Kiechl *et al*. found a strong and independent correlation between these two factors, whereas Morena *et al*. and Browner *et al*. found no correlation [7,13,14,20,21]. Consistent with our findings, Morena *et al*. found that CRP attenuated the effect of high CRP with OPG on mortality.

OPG may be directly involved in the development of atherosclerosis via its second ligand TNF-related apoptosis inducing ligand (TRAIL). TRAIL is a potent activator of apoptosis. OPG could influence vascular disease by inhibiting TRAIL-induced apoptosis of vascular cells. Another mechanism whereby OPG could be associated with vascular damage independent of VC could be via its association with endothelial dysfunction. OPG has been related to endothelial cell survival, apoptosis and modulation of endothelial inflammatory response [24–26]. This association between OPG and endothelial dysfunction has been demonstrated clinically in type II diabetes [7]. The dialysis outcomes and practice patterns study (DOPPS) classified non-cardiovascular causes of mortality as infection or gastrointestinal and liver disease with over of 20% of mortality being defined as 'other' or 'unknown' causes [27]. It is not clear from the current definitions how much the pleiotropic effects of the RANKL/OPG complex could be contributing to mortality via its non-cardiovascular associations such as mineral bone disease and its deleterious consequences.

In this study, we found no association between circulating Fetuin-A and PWV or VC, additionally we found no association between CRP and Fetuin-A. Despite strong associations between Fetuin-A and mortality in the literature [28], a direct association between circulating levels of Fetuin-A and PWV or VC has not been constantly shown in adults [29]. However, a recent study of children on dialysis found that Fetuin-A independently predicted PWV, and OPG and Fetuin-A together predicted cardiac calcification. It is possible that in older populations with multiple comorbidities and vascular disease, the inhibitory effects of Fetuin-A upon VC are diluted. In this study, subjects with OPG levels <25 pmol/L show a higher percentage of diabetes mellitus compared to subjects with OPG levels >25 pmol/L; this is in contrast to previous reports of circulating OPG in diabetic patients. The reasons for this are unclear at present, although it may reflect better medical management, glycaemic control and/or vascular health in this group than other diabetic populations studied.

Limitations of the present study include the relatively small patient population studied and the small number of events in this group. The observed mortality rate in this population is lower than previous reports; however, this is representative of DCGH patient population; the 2007 UK renal registry data shows that our prevalent dialysis patients have 90% 1-year survival, plus this group also includes a number of well-managed CKD stage 4 patients [30]. Another potential limitation of the study is the measurement of circulating OPG, as this is a relatively novel molecule, and issues around clinical relevance of the OPG assay (e.g. potential fragmentation) are unexplored at present. An additional limitation of this study is the absence of determination of RANKL, although it is generally assumed that OPG levels accounted for the activation of the RANK/RANKL/OPG axis.

We have previously reported that VC and low serum albumin are predictive of death in CKD stages 4 and 5 over a 2-year follow-up period. These data show that hs-CRP and OPG are also associated with a negative outcome, but that OPG alone or in combination with elevated hs-CRP is associated with worse outcomes. The mechanisms remain to be elucidated; however, it is likely that they are associated with vascular damage through mechanisms independent of VC.

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Conflict of interest statement. None declared.

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Thrice weekly warfarin administration in haemodialysis patients

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Abstract

Background. Medication adherence in haemodialysis patients is often challenging due to a high pill burden, complex and dynamic medication regimens and limited patient self-interest in care. The purpose of this study was to investigate the time within target INR and safety profile of thrice weekly warfarin administration in haemodialysis patients with a clinical indication for anticoagulation and documented nonadherence to medications.

Methods. Thirty-seven patients from two haemodialysis units in Winnipeg, Manitoba, Canada, were recruited, and 17 patients were treated with thrice weekly warfarin and compared to 20 patients treated with daily warfarin therapy. The patients were followed for 1 year with weekly international normalized ratio (INR), dosage and adverse events recorded. The primary outcome was percentage of time with INR in target and sub (<1.5)- and supra (>4)-therapeutic INR. Adverse events were recorded in the two groups.

Results. The thrice weekly group had a higher burden of comorbidity (Charlson comorbidity index of 6.35 ± 1.77 versus 4.55 ± 1.64, $P = 0.003$) compared to the daily dosage group. In the thrice weekly dosage group, time within target INR was higher (56.9 versus 49.3%, $P = 0.008$), and time