

# Denosumab Recovers Aortic Arch Calcification During Long-Term Hemodialysis



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**Introduction:** Aortic arch calcification (AoAC) is related closely to mortality risk in patients undergoing maintenance dialysis. Recent experimentally obtained data suggest that osteoprotegerin/receptor activator for nuclear factor κB ligand signal transmission plays a role in *de novo* chondrogenic transition of vascular cells leading to calcification that is unrelated to bone metabolism. This study investigated the long-term effects of denosumab, an osteoprotegerin mimic peptide, on AoAC.

**Methods:** This study examined 58 patients with an 8 year vintage of dialysis at 1 center for observational study during 2009 to 2020. Denosumab was administered to 28 patients every 6 months. Blood chemical data were used. AoAC proportions were measured using a simple but computed tomography-equivalent computer-based chest X-ray analysis (calcified pieces of areas around the aorta).

**Results:** Blood chemical data of the control and denosumab groups that did not differ at the start showed differences of mineral metabolism after 30 months of observation. Remarkably, the AoAC proportion increased from 29.4% to 46.25% in the control group but decreased significantly from 25.0% to 20.0% ( $P < 0.01$ ) in the denosumab group. Denosumab effects on decalcification were not observed 12 months after initiation.

**Conclusion:** We conclude that long-term use of denosumab is effective to reverse or treat AoAC in patients undergoing hemodialysis.

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**KEYWORDS:** hyperphosphatemia; monoclonal antibody; osteoprotegerin; parathyroid hormone; receptor activator for nuclear factor κB ligand; vascular calcification

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The high prevalence of arterial calcification in patients undergoing hemodialysis is recognized beyond mere incidence deriving from common cardiovascular risk factors, such as aging, diabetes, hypertension, and dyslipidemia. For the maintenance of appropriate dry weight of patients undergoing hemodialysis, monthly examinations of chest X-ray images is commonly done. Chest roentgenography can reveal the aortic arch, where calcification is frequently observed. Numerous studies have examined aortic arch calcification (AoAC) and mortality risk in patients undergoing maintenance dialysis.<sup>1,2</sup> A meta-analysis investigating the association between the presence of AoAC and cardiovascular or all-cause mortality risk in patients undergoing maintenance dialysis has revealed

significant correlation of AoAC and mortality.<sup>3</sup> Therefore, treatment or amelioration of AoAC is quite important for long-term hemodialysis.

In addition to traditional cardiovascular risk factors, such as aging, diabetes mellitus, dyslipidemia, and high blood pressure, other hemodialysis-related risks, such as phosphate (P) retention, excess calcium (Ca), and prolonged dialysis term contribute to vascular calcification development.<sup>4</sup> Experimental investigations have elucidated several mechanisms of vascular calcification, including endothelial inflammation,<sup>5</sup> high plasma P,<sup>6</sup> lack of Ca chelating fetuin A,<sup>7</sup> lack of vitamin K, and elevated levels of fibroblast growth factor 23, accompanied with high bone turnover. Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor family, binds to receptor activator for nuclear factor κB ligand (RANKL) in osteoblasts.<sup>8</sup> OPG inhibits RANKL-dependent activation of osteoclasts, leading to bone reabsorption. OPG knockout mice showed marked activation of osteoclasts, which resulted in severe osteoporosis and vascular calcification.<sup>9,10</sup>

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This vascular calcification found in OPG (−/−) mice can be averted by systemic supplementation of exogenous OPG but not by transient administration.<sup>11</sup> Accumulation of Ca and P released from bone might induce metastatic calcifications other than bone. Recent reports have described that OPG/RANKL signaling plays a pivotal role in the cardiovascular system and in osteoclast activation because the signal was found to induce inflammation and chondrogenic transformation of vascular endothelial cells.<sup>12</sup> Denosumab, a fully human monoclonal RANKL-directed antibody, prevents RANK receptor binding, thereby decreasing osteoclast-induced bone resorption. Denosumab is used to treat osteoporosis and bone metastasis of tumor cells. To improve osteoporosis, denosumab is administered to elderly patients, by whom it is well-tolerated,<sup>13</sup> and to patients undergoing hemodialysis.<sup>14</sup> Nevertheless, whether denosumab improves vascular calcification in patients undergoing hemodialysis remains unknown.<sup>15</sup> We therefore used an observational study to elucidate denosumab effects on vascular calcification, AoAC.

## METHODS

### Study Design and Population

Using data obtained between January 2013 and January 2019, 58 adults undergoing hemodialysis for >8 years were examined in this single-center, retrospective, open-label study to compare outcomes of patients who received denosumab (28 patients) and a control group (30 patients). We selected patients showing any 1 of 3 phenomena: aortic calcification by chest X-ray (denosumab vs. control group, 20 vs. 22 patients), calcification in carotid arteries (8 vs. 6 patients) by ultrasound echography, or calcification in arteriovenous fistula (6 vs. 6 patients). The selection of patients for the treatment group and the control group took approximately 6 years, and patients and control

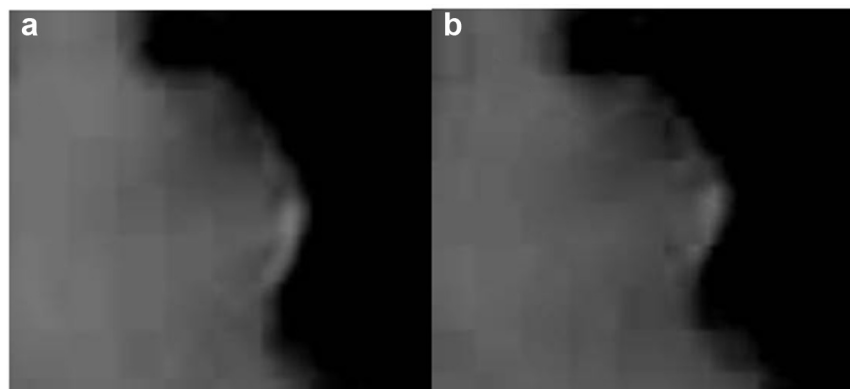
subjects were then followed for 2 years. All patients underwent high-flux hemodiafiltration with 250 to 350 ml/min blood flow with 12 L/hr convective flow. Dialysate contained 2.75 mEq/l Ca. We assessed chronic kidney disease–mineral and bone disorder parameters including laboratory markers, Ca, P, intact parathyroid hormone (iPTH), alkaline phosphatase, tartrate-resistant acid phosphatase 5b, and AoAC. Quantitative calcaneus mineral density was measured using ultrasound echography (AOS-100 SA; Hitachi Ltd., Tokyo, Japan).

The calcified percentile area against AoAC on chest X-ray was measured using computer-based densitometric analysis: the aortic arch was divided into 16 circumferences. Then the number of sectors showing calcification was calculated. The calcified area against the whole aortic area was expressed as a number. The AoAC numbers per 16 areas were highly correlated to the AoAC proportion (%) measured using computed tomography (CT).<sup>1,16</sup>

This study was approved by the ethics committee of Edogawabashi Suzuki Clinic (20200603 no. 001). All patients participating in this study gave written permission for the use of medicines and injection. The patient shown in [Figure 1](#) agreed to disclosure of chest X-ray images.

### Drug Exposure

Patients in the denosumab group were injected with denosumab (60 mg) subcutaneously and were then followed. Denosumab injection was repeated every 6 months until 30 months. Control subjects were not injected. P binders were prescribed individually, injected with maxacalcitol 7.5 to 15 µg/week in both groups. Amounts of these drugs were unchanged throughout the study. The administration of denosumab is known to induce severe hypocalcemia in patients undergoing hemodialysis.<sup>17</sup> Therefore, we



**Figure 1.** Representative aortic arch calcification (AoAC) in the denosumab group. The circular mark drawn on the aorta was divided by 16. The area of calcification was detected. The number of calcified areas was obtained. AoAC of a patient in the denosumab group is shown before (a) and after (b) 30 months of treatment. The AoAC area was changed from 7 of 16 to 3 of 16 areas after treatment.

prescribed a higher dose of 1,25-dihydroxycholecalciferol (0.5–1 µg) orally to prevent hypocalcemia a week before and 2 weeks after denosumab injection. We used no calcimimetic, such as cinacalcet, evocalcet, or etelcalcetide, to prevent hypocalcemia in the denosumab group. Bisphosphonate was not administered to the denosumab group. In the control group, mean serum Ca, P, and iPTH concentrations during the study period were within the target range recommended by Japanese Society for Dialysis Therapy guidelines<sup>18</sup> for the use of calcimimetics. Amounts of calcimimetics were changed to maintain iPTH between 100 and 400 ng/ml. Bisphosphonate was administered monthly to 7 patients in the control group.

### Evaluation of AoAC Using Chest Radiography

We conducted a retrospective review of all patients for AoAC detection. Radiographs were assessed for the presence of AoAC using a specific scale. The scale, which was divided into 16 circumferences, was attached to the aortic arch on chest radiography. The number of sectors with calcification was then divided by 16. The AoAC was calculated after multiplication by 100 to express the result as a percentage. This value was used as an indicator of the AoAC, irrespective of the degree of density.<sup>16</sup>

### Statistical Analysis

Patient demographic information and clinical characteristics were summarized as the mean and standard deviation of continuous variables and as the number and proportion of categorical variables. Categorical and continuous variables were compared respectively using  $\chi^2$  and 2-sample *t* tests. Variables that are assumed to have followed a normal distribution were tested using the *F* test, followed by the Student *t* test; results for which  $P < 0.05$  were considered statistically significant. All analyses were performed using Excel (Microsoft Corp., Redmond, WA) and JMP Pro (SAS Institute Japan Ltd., Tokyo, Japan).

## RESULTS

Baseline data of patients are presented in Table 1. No significant differences were found in age, gender, hemodialysis vintage, underlying diseases, body mass index (20.5 vs. 19.8 kg/m<sup>2</sup>), or earlier fracture (0% vs. 0%; mean values in control vs. denosumab groups) between the control and denosumab groups. Amounts of medicines with chronic kidney disease–mineral and bone disorder were not different: oral alfacalcidol 0.6 versus 0.7 mg/day, Ca carbonate 1.2 versus 1.5 g/day, and lanthanum carbonate hydrate 1.5 versus 1.7 g/day between the control and denosumab groups. No

**Table 1.** Baseline characteristics of the study subjects

	Control group		Denosumab group		<i>P</i> value
	Mean or %	SD	Mean or %	SD	
Patients, n	30		28		
Age, y	68.1	11.6	65.9	13.3	0.50
Male sex, %	76.7		75		
Duration of dialysis, y	8.4	6.6	8.6	6.4	0.94
Primary disease, diabetes, %	43.3		39.3		
Serum calcium, mg/dl	8.9	1.0	8.9	1.1	1.00
Serum phosphate, mg/dl	5.9	1.8	6.6	1.8	0.12
Intact PTH, pg/ml	260	365	230.0	180.1	0.69
Serum albumin, mg/dl	3.7	0.4	3.7	0.26	0.83
Dose of oxarol, µg/w	4.3	2.1	3.6	2.5	0.26
Denosumab duration/observation period, months	32.7	22.0	27.2	14.4	0.26

PTH, parathyroid hormone; SD, standard deviation.

significant differences were found in blood chemical analysis data of the control and denosumab groups: alkaline phosphatase (U/l) 237.5 versus 253.0 (mean values in control vs. denosumab groups), tartrate-resistant acid phosphatase 5b (mU/dl) 282.5 versus 265, and C-reactive protein (mg/dl) 0.87 versus 0.76. No significant difference was found in bone mineral density (−0.85 SD vs. −0.84 SD, mean values in control vs. denosumab groups).

### First Administration and Adverse Effects of Denosumab on Mineral Metabolism

Our preliminary single administration of denosumab to 6 patients before the study showed that hypocalcemia occurred most frequently at 2 to 4 weeks after injection. Therefore, we used a higher dose of active vitamin D<sub>3</sub> 3 weeks before and after denosumab injection to maximize intestinal Ca absorption. An additional preliminary trial conducted before the study showed that injection of evocalcet to reduce serum iPTH after administration of denosumab induced profound and dangerous levels of hypocalcemia (Ca <6.0 mg/dl). We used no calcimimetics for the denosumab group in the present study.

Changes of Ca, P, and Ca × P product are shown in Figure 2a and b. From 1 to 4 weeks after the first denosumab administration in the present denosumab group, serum Ca decreased significantly (from 8.9 ± 1.1 to 8.2 ± 1.4 mg/dl,  $P < 0.05$ ), serum P decreased significantly (from 6.6 ± 1.8 to 5.3 ± 1.8 mg/dL,  $P < 0.01$ ), and iPTH increased significantly (from 230 ± 80 to 604 ± 537 pg/ml,  $P < 0.05$ ), despite preventive prescription of higher amounts of vitamin D<sub>3</sub>. Ca × P product also decreased remarkably during 1 to 4 weeks after the first administration of denosumab. These values were not significantly different between men and women. The changes of Ca, P, and Ca × P were restored from 8 to 24 weeks after the administration of denosumab.

Few adverse events were observed. One patient developed severe asymptomatic hypocalcemia (Ca 5.6 mg/dl) after the first denosumab treatment, which was mitigated gradually by additional supplementation of calcium (Ca lactate 3.0 g/day). Hypocalcemia persisted 3 months after the treatment, without QT prolongation on echocardiography. One patient complained of muscle cramping when Ca fell to <8 mg/dl, which was soon ameliorated by additional supplementation of Ca lactate 4.0 g/day. No other side effect was observed throughout this study. The administration of denosumab was well-tolerated by almost all patients.

### Long-Term Effects of Denosumab on Mineral Metabolism

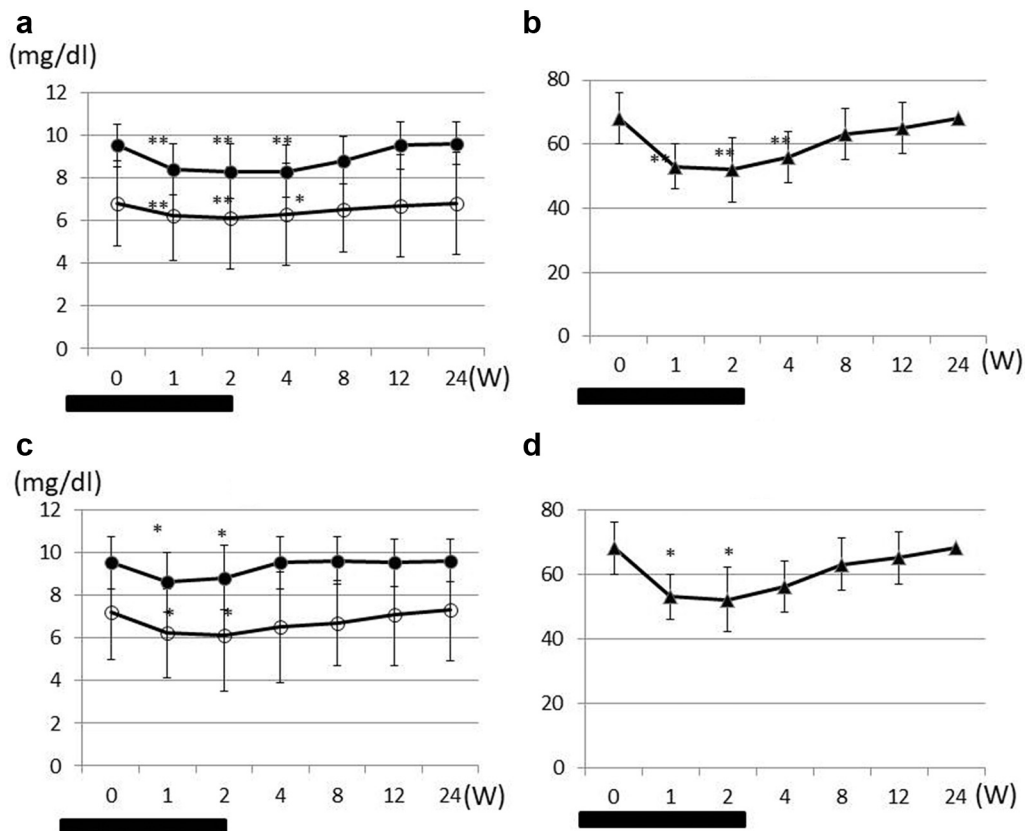
Changes of Ca, P, and Ca × P product after the fourth administration of denosumab in the denosumab group are shown in Figure 2c and d. As seen after the first administration, Ca, P, and Ca × P products were reduced significantly from 1 to 2 weeks after denosumab injection but were restored at 4 weeks and thereafter. Table 2 shows values of mineral metabolism obtained after a 30-month follow-up period in the control group and in the denosumab group. No significant change in Ca, P, iPTH, or albumin was found in

these groups. Moreover, no significant change was found in other blood chemical analyses, alkaline phosphatase, tartrate-resistant acid phosphatase 5b, and C-reactive protein. No significant change was found in bone mineral density (T score) or carotid intima media thickness.

As presented in Figure 2, the Ca and P concentrations fluctuated in the denosumab group. Therefore, we analyzed the Ca and P concentrations as an average of 12 times that were sampled during the previous 6 months. Table 2 shows the results of analyses showing that Ca and P decreased and that iPTH increased significantly in the denosumab group.

### Denosumab Effects on AoAC

Figure 1 shows a representative finding of AoAC for a patient in the denosumab group. The calcified area apparently diminished through 30 months of treatment. Its proportion was counted both before and after the 30-month period in the control and denosumab groups. The progress of AoAC was notable in the control group, where mineral metabolites were maintained as constant; however, the proportion of AoAC was diminished significantly in the denosumab group (Table 3). To estimate the period necessary to diminish



**Figure 2.** Concentrations of Ca (open circle), P (closed circle), and Ca × P product (triangle) after the first (a, b) and fourth administration of denosumab (c, d) are shown. Dark bar represents the term of administration of additional vitamin D<sub>3</sub>. \**P* < 0.05 and \*\**P* < 0.001 compared with levels measured before this study.



**Table 2.** Changes in mineral metabolism, bone mineral density, and carotid intima media thickness at the baseline and after the observation period in the control and denosumab groups

	Baseline		After observation period		P value
	Mean	SD	Mean	SD	
Control group, n = 30					
Serum calcium, mg/dl	9.0	1.0	9.0	0.8	0.12
Serum phosphate, mg/dl	5.9	1.9	5.2	2.0	0.12
Intact PTH, pg/ml	267	377	189	132	0.30
Serum albumin, mg/dl	3.7	0.4	3.8	0.5	0.48
CRP, mg/dl	0.87	0.4	0.78	0.6	0.44
ALP, U/l	237.5	140	242.5	250	0.5
TRACP-5b, mU/dl	282.5	110	315.2	220	0.58
T score, SD	-0.85	0.6	-1.15	0.6	0.42
IMT, mm	0.75	0.4	0.77	0.5	0.32
Denosumab group, n = 28 <sup>a</sup>					
Serum calcium, mg/dl	8.9	1.1	8.9	0.8	0.9
Serum phosphate, mg/dl	6.6	1.8	6.0	1.7	0.05
Intact PTH, pg/ml	230.0	180.1	262	283	0.6
Serum albumin, mg/dl	3.7	0.26	3.7	0.32	0.28
CRP, mg/dl	0.76	0.5	0.92	0.7	0.54
ALP, U/l	253.0	150	252.5	250	0.5
TRACP-5b, mU/dl	265.0	125	242.5	240	0.78
T score, SD	-0.84	0.6	-1.15	0.75	0.52
IMT, mm	0.70	0.4	0.72	0.4	0.32
Denosumab group, n = 28 <sup>b</sup>					
Serum calcium, mg/dl	8.9	1.1	7.9	0.8	0.0004
Serum phosphate, mg/dl	6.6	1.8	5.6	1.7	0.002
Intact PTH, pg/ml	230.0	180.1	490	494	0.0044
Serum albumin, mg/dl	3.7	0.26	3.7	0.3	1.0

ALP, alkaline phosphatase; CRP, C-reactive protein; IMT, intima media thickness; PTH, parathyroid hormone; SD, standard deviation; TRACP-5b, tartrate-resistant acid phosphatase 5b; T score, bone mineral density.

<sup>a</sup>Values immediately before the final administration of denosumab.

<sup>b</sup>Average values of the previous 6 months.

the calcification, we analyzed the AoAC percentile 12 months after the first administration of denosumab. The AoAC proportion was not decreased during this period. It was regarded as necessary for  $\geq 2$  years for decalcification by denosumab. The cardio-ankle vascular index measured before and after the study in the denosumab group was found to have no significance ( $9.0 \pm 1.8$  vs.  $9.2 \pm 1.6$ ;  $n = 24$ ,  $P > 0.05$ ).

We also attempted to evaluate calcification found in the carotid artery, heart, and arteriovenous fistula.

**Table 3.** Changes in aortic arch calcification in the control and denosumab groups

	Baseline		After observational period		P value
	Mean	SD	Mean	SD	
Control group, n = 30					
AoAC areas, %	29.4	20.0	46.3	21.9	<0.0001
Denosumab group, n = 28					
AoAC areas, %	24.8	25.8	12.5	20.0	<0.0001
At 12 months, %			22.3	26.3	>0.05

AoAC, aortic arch calcification; SD, standard deviation.

However, these findings were neither sufficient for quantification nor apparently different between groups.

## DISCUSSION

Generally speaking, vascular calcification is the pathologic deposition of minerals in the vascular system. Vascular calcification exhibits various forms, including intimal calcification and medial calcification, but it can also be found in heart valves. Calcific uremic arteriolopathy, another rare complication of hemodialysis by which arterioles calcify, leads to necrosis of the skin. The AoAC has a complex mechanism with medial layer calcification underlying genetic, inflammatory, and metabolic mechanisms. It can be assessed with CT, but simple in-office techniques using chest X-rays might provide useful information. We compared results obtained using a simple noninvasive technique with those obtained using multidetector CT for AoAC volume (AoACV) in patients with chronic heart disease. The AoAC score (AoACS) estimated from chest X-rays was highly correlated with the AoACV. Measurement of AoAC from chest X-rays presents a simple but useful tool to analyze calcification risk factors,<sup>16</sup> calcification progress,<sup>1</sup> and cardiac mortality<sup>1,19</sup> in patients undergoing hemodialysis. The method used here (chest X-rays) was appropriate for quantitative prediction of AoAC progress.

Histologically, calcified arteries in the OPG (-/-) mice were detectable by 2 weeks and were remarkable by 2 months of age. Aortic calcification in knockout mice was observed primarily in the media, but calcified lesions in the renal arteries were not associated with mineralization.<sup>9</sup> Similarly, the longer the duration of hemodialysis for patients the greater the degree to which severe calcification might be expected to occur in the aorta. We analyzed patients with about 8-year duration of hemodialysis in this study. Our results show that AoAC might progress rapidly in patients with longer durations of hemodialysis than in patients who initiated hemodialysis treatment. It is appropriate to observe the progress of calcification in these patients.

Molecular mechanisms underlying vascular calcification are regarded as initiating chondrogenic transdifferentiation of vascular smooth muscle cells. Various stimuli and various pathways, such as phosphate, fibroblast growth factor 23, and soluble Kloth were presumed to start the transdifferentiation of smooth muscle cells *in vitro*. A recent report described that OPG/RANKL is involved in the mechanisms of this chondrogenic transdifferentiation. Experimentally obtained results indicate that RANKL increased vascular smooth muscle cell calcification directly by binding to

RANK and by increasing bone morphogenetic protein 4 production through the alternative nuclear factor  $\kappa$ B pathway.<sup>20</sup> Reportedly, RANKL promotes smooth muscle cell calcification indirectly by enhancing macrophage paracrine procalcific activity through the release of interleukin-6 and tumor necrosis factor- $\alpha$ .<sup>21</sup> Inflammation induces interleukin-6, which suppresses OPG expression, thereby enhancing RANKL pathway.<sup>22</sup> Deficiency of OPG is manifested by juvenile Paget disease, which includes hearing loss, retinopathy, vascular calcification, and internal carotid artery aneurysm formation, in addition to skeletal deformity.<sup>23</sup> Although denosumab is effective for treating skeletal deformity, the calcification effects remain unclear. These results of studies suggest RANK/RANKL as important in promoting vascular calcification. In addition, OPG is expected to inhibit calcification.

Earlier studies showed higher circulating OPG levels in patients undergoing hemodialysis. The OPG level signals an independent risk of coronary calcification<sup>23</sup> and of all-cause mortality.<sup>24</sup> Genetically, OPG/RANKL polymorphism in hemodialysis and its association with aortic calcification have been proposed.<sup>25</sup> High-level serum RANKL expression stimulated by PTH is also inferred to occur in patients undergoing hemodialysis.<sup>26</sup> These findings suggest OPG/RANKL pathway as a major mechanism of AoAC in hemodialysis. Intervention by denosumab to suppress this pathway is expected to attract further study.

This study found that denosumab, when used for a long time, reverses advanced AoAC in patients undergoing hemodialysis. Restoration or reversal of calcification is surprising because most treatments against calcification merely prevent calcification progress. Interventions such as bisphosphonate,<sup>27</sup> calcimimetics,<sup>28</sup> lanthanum chloride,<sup>29</sup> and SNF472, a novel crystal inhibitor,<sup>30</sup> reportedly prevent the progress of vascular calcification. Sodium thiosulfate fails to stop the progress of calcification.<sup>31</sup> Kidney transplantation offers a means of restoring kidney function and mineral metabolism. Observation of kidney transplant recipients for 2.5 to 4.0 years revealed it as unable to halt progression of coronary artery calcification.<sup>32,33</sup> Similarly, kidney transplantation slowed the progression of AoAC but did not restore it, whereas AoAC is still an independent predictor of poor cardiovascular outcome.<sup>34</sup> It is therefore noteworthy that denosumab reversed advanced calcification in patients undergoing hemodialysis.

Several studies have examined denosumab effects on patients with vascular calcification. One study found that denosumab use for  $\geq 3$  years did not reduce cardiovascular events or AoAC in populations of older people with osteoporosis.<sup>35</sup> That study, however,

examined older patients not undergoing hemodialysis than ours did (74 years of age). Age-related vascular calcification is observed in intimal calcification of endothelial cells that have been damaged by atherosclerosis. As discussed, the OPG/RANKL pathway plays a pivotal role in medial calcification by calcified smooth muscle cells. The mechanism of AoAC in elderly people might be more dependent on age or on the degree of atherosclerosis than on the OPG/RANKL pathway.

Effects on bone density and AoAC by denosumab in cases of hemodialysis with osteoporosis have been reported.<sup>14</sup> Denosumab in that study ameliorated osteoporosis but it did not improve AoAC. However, the observation period was 12 months after the injection, which might have been too short to decalcify AoAC, as seen in our study. Patients with high bone turnover, as seen in osteoporosis, have increased calcium and phosphate release from bone. The excess amount of calcium and phosphate might lead the vascular calcification. By contrast, when bone turnover is low, serum calcium and phosphate levels are maintained frequently at high levels because the reservoir functions of bone decrease. This low bone turnover also induces vascular calcification.<sup>36,37</sup> Denosumab ameliorated high bone turnover. Moreover, it might induce the low bone turnover found in this study. However, that seems unlikely because the last injection of denosumab was associated with a marked decrease in calcium and phosphate (Table 3). This decrease implicates bone as a calcium and phosphate reservoir.

As inferred for OPG ( $-/-$ ) mice, transient supplementation of OPG was insufficient to restore calcification.<sup>11</sup> Decalcification by denosumab might take  $\geq 2$  years to be measurable in human studies.

Chondrogenic transdifferentiation of vascular smooth muscle is induced by high serum phosphate.<sup>23</sup> Denosumab might have indirectly affected AoAC in this study via phosphate metabolism. Administration of denosumab once reduced serum Ca and phosphate; it then recovered. Values had returned to those before injection by  $\geq 6$  months. This pattern was repeated similarly toward the end of the study. Therefore, average Ca and phosphate were maintained as lower for several months during the 30-month observation period (Figure 2 and Table 3). Using the present radiographic analysis, age, Ca  $\times$  P product, PTH, and C-reactive protein levels were shown to be independent risk factors for AoAC.<sup>38</sup> Lower calcium and phosphate products might prevent calcification, at least in part. Nevertheless, no study of reversal of vascular calcification by phosphate restriction has been reported.

Vitamin D also has an important association with a mechanism of vascular calcification. Vitamin D enhances vitamin K-dependent gamma-carboxylation of matrix Gla protein, thereby inhibiting vascular calcification.<sup>39</sup> An earlier study using measurement of AoAC revealed that active vitamin D therapy apparently prevents vascular calcification development.<sup>40</sup> To prevent severe hypocalcemia, we administered active vitamin D for 3 weeks around the injection of denosumab. This additional administration might enhance decalcification of AoAC by the denosumab group, although the amount of vitamin D was slight compared with the total amount of vitamin D and its analogues during the 30-month study period.

One limitation of the present study was its analysis of data obtained from 1 center. Additional studies should be performed at multiple centers. In addition, AoAC and other vascular calcification are closely related to the mortality of patients undergoing hemodialysis. Denosumab effects on cardiovascular events and mortality can be clarified during longer follow-up periods.

In conclusion, the administration of denosumab for a longer period of time, probably via suppression of RANKL-dependent signal, regressed aortic arch calcification in patients undergoing hemodialysis. OPG/RANKL pathway was crucial rather than high phosphate in the calcification at least in the aorta. Denosumab-induced hypocalcemia was protected by vitamin D and was well-tolerated for long periods. Further extended use of denosumab might reduce cardiovascular and all-cause mortality in patients undergoing hemodialysis.

## DISCLOSURE

All authors declared no competing interests.

## ACKNOWLEDGMENTS

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