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Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in non-dialyzed patients with chronic kidney disease stage 3-5

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Abstract

Introduction. Observational studies have shown that high dietary vitamin K2 intake is associated with reduced risk of coronary vascular disease and vascular calcification.

Objectives. This prospective randomized intervention study assesses the impact of vitamin K2 substitution on the progression of atherosclerosis and calcification markers in non-dialyzed 3-5 stage CKD patients.

Patients and methods. The following measurements were taken at baseline and after 270±12 days of supplementation 90 μg vitamin K2 (menaquinone, MK-7) with 10 μg cholecalciferol (K+D group) or 10 μg cholecalciferol (group D) in 42 non-dialyzed CKD patients: Common Carotid Intima Media Thickness (CCA-IMT), Coronary Artery Calcification Score (CACS), serum mineral parameters, lipids, as well as the calcification modulators: matrix Gla protein (MGP), desphosphorylated-uncarboxylated-MGP (dp-ucMGP), osteoprotegerin (OPG), fetuin A, osteocalcin (OC) and fibroblast grown factor 23 (FGF-23).

Results. The increase of CCA-IMT was significantly lower in the K+D group (from 0.95±0.2 mm to 1.01±0.3, *P*=0.003) than in the D group (from 1.02±0.2 mm to 1.16±0.3, *P*=0.003), resulting in a ΔCCA-IMT 0.06±0.08 *vs* 0.136±0.05 mm, *P*=0.005 respectively. The increase in CACS was numerically less in K+D patients (ΔCACS: 58.1±106.5 *vs* 74.4±127.1 Agatson units, *P*=0.7). The level of dp-ucMGP and total OC decreased in K+D patients during the study, OPG increased in both groups.

Conclusions. *A 270-day course of 90 μg vitamin K2 administration may reduced the progression of atherosclerosis*. Vitamin K2 significantly changed the pattern of promoters and calcification inhibitors dp-ucMGP, OC and OPG but failed to affect the progression of coronary artery calcification in CKD patients over the treatment period.

Key words: atherosclerosis, chronic kidney disease, vascular calcification, vitamin K2

Introduction

Atherosclerosis and vascular calcification (VC) are common complications of chronic kidney disease (CKD) and significant risk factors for cardiovascular disease and mortality [1,2].

VC is currently recognized as an actively regulated process dependent on the balance between its inducers and inhibitors [3]. Several studies have shown that the arterial calcification resembles bone formation. In a uremic environment, vascular smooth muscle cells (VSMCs) express osteogenic proteins and deposit a mineralized bone-like matrix [4]. Two of these proteins, matrix Gla protein (MGP) and osteocalcin (OC) are principal regulators of tissue mineralization in the arterial wall and bone [5]. OC and MGP expression is up-regulated by vitamin D and dependent on vitamin K for its calcium-binding capacity [6]. MGP is expressed by VSMCs within the arterial media of the vessel wall. MGP needs to undergo post-translational gammaglutamyl carboxylation to achieve full biologic activity. The carboxylation process is completely dependent on the availability of vitamin K, which is a cofactor of this process [7].

Natural vitamin K consists of phylloquinone (vitamin K1) and the menaquinones MK-4 through MK-10, all vitamin K2. Both vitamin K1 and K2 catalyze the gammaglutamyl carboxylation of all vitamin K-dependent proteins. However, vitamin K1 predominantly accumulates in the liver and is the most important substance for the activation of coagulation factors, while vitamin K2 has a more widespread tissue distribution and is thus more specifically involved in the carboxylation of MGP [8]. Consequently, it exerts a major role in the calcification process. In cases of vitamin K deficiency, MGP is not activated and undercarboxylated MGP predominantly accumulates in areas of VC, and is associated with both intimal and medial calcification [9]. Observational studies have shown that high dietary vitamin K2 intake is associated with reduced risk of coronary vascular disease and VC

[11,12]. The second vitamin exerting multiple functions including a role in the calcification process is vitamin D [13]. Recent data demonstrates a high prevalence of suboptimal levels of vitamin K and D in patients with CKD stage 3 to 5 [14].

The aim of this study was to assess the effect of supplementation of vitamin K2 (menaquinone, MK-7) in combination with a low dose of cholecalciferol compared to cholecalciferol alone on the progression of atherosclerosis and coronary artery calcification (CAC) and on circulating levels of calcification regulators in non-dialysis CKD patients.

Patients and methods.

The prospective, randomized and double-blinded study was conducted between 2009 and 2012. Multislice CT scanning of the thorax to assess the coronary calcification score (CACS) and ultrasonography of the common carotid artery with the measurement of intima media layer thickness (CCA-IMT) was performed on the same day in 75 consecutive non-dialyzed patients in CKD stages 3-5 from a single nephrology outpatient clinic who fulfilled the inclusion and exclusion criteria.

The inclusion criteria were as follows: age 18 to 70 years old, with a history of stable glomerular filtration rate (eGFR<60 ml/min/per1.73m^2) over at least 6 months, not yet requiring dialysis. The exclusion criteria were a history of major cardiovascular complications (myocardial infarction, clinically significant arrhythmia including atrial fibrillation, congestive heart failure, stroke, peripheral vascular disease), history of thrombosis or coagulation disorders, treatment with oral anticoagulants, steroid and other hormonal therapies, and treatment with vitamin D or its analogs. Forty-two screened patients demonstrated CACS values of ≥10 Agatston units (A.u.), and they were randomized to the study. All enrolled patients were non-smoking Caucasians (22 men, mean age 60±3.0 yrs, and 20 women mean age 56±1.5 yrs) and had pharmacologically well-controlled hypertension.

Information concerning patient histories, medications, cardiovascular complications and results of routine laboratory assessments was obtained from chart reviews. *The causes of renal failure were chronic glomerulonephritis in 15 cases, diabetic nephropathy in 8, polycystic kidney disease in 4, hypertensive nephropathy 5, tubulointerstitial nephritis in 3, and unknown in 7 patients.*

The randomization cards were prepared on the assumption that twice the number of patients would be treated with both vitamins: K2 and D, than D alone (active vs control group). Patients were *randomized to each* group by computer. Twenty nine patients in group K+D received an oral dose of 90 μg of vitamin K2 (menaquinone-7, MK-7) plus 10 μg cholecalciferol per day for 270±12 days; thirteen in group D received 10 μg cholecalciferol alone (Figure 1). The tablets containing vitamin K2+D or vitamin D were identical in size and appearance (both kinds of tablets were prepared by NattoPharma, Høvik, Norway). The patients from both groups were treated with statins due to hyperlipidemia. *Four patients from group K+D and two from D received calcium carbonate as a phosphate binder with doses unmodified throughout the study.*

Anthropometric measurements were taken and the fasting blood samples for biochemical, morphological and coagulation test were obtained at the time of randomization and at end of treatment. Routine serum parameters, including creatinine, calcium, phosphate, parathyroid hormone, glucose, albumin, total protein, cholesterol, triglycerides and HDL cholesterol levels were measured with routine laboratory methods, LDL cholesterol was calculated using the Friedewald formula. The eGRF was calculated with a four-variable Modification of Diet in Renal Disease (MDRD) equation. Serum and plasma samples were prepared after standard centrifugation and frozen at -80^{0}C until measurements.

Circulating total MGP was measured using a sandwich enzyme immunoassay by ELISA (USCN Life Science Inc, www.uscnk.com), plasma dp-ucMGP was assessed using the ina*K*tif MGP iSYS kit (Immunodiagnostic Systems; www.idsplc), which is a dual-antibody test based

on the previously described sandwich ELISA (developed by VitaK, Maastricht University, The Netherlands), total serum OC by ELISA (Immunodiagnostic Systems; www.idsplc), serum osteoprotegerin (OPG) by ELISA (Immunodiagnostic Systems; www.idsplc), serum fetuin A by ELISA (Epitope Diagnostics,Inc., www.epitopediagnostics.com), serum 25-hydroxyvitamin D (25OHD) by radioimmunoassay (IBL International; www.IBL-International.com), serum high sensitive CRP by ELISA (IBL International; www.IBL-International.com), plasma FGF-23 was determined using a human FGF-23 ELISA kit (Immutopics, www.immutopicsintl.com).

Within 7 days after the end of active treatment, the CACS level was assessed by multiscan CT and CCA-IMT by ultrasonography.

Written informed consent was obtained from all subjects before entering the study, and the study protocol was approved by the local Ethics Committee.

Imaging procedures

Multislice CT scanning of the thorax was performed using a General Electric Medical Systems Lightspeed 16 scanner to determine CAC. The acquisition parameters were as follows: 120 KVp, 350 mA, slice with 2.5 mm/8i. Data was reconstructed with a standard algorithm using a 512x512 matrix, 50 cm scan field of view and 25 cm display field of view. The system was synchronized with the cardiac cycle to trigger scanning during the diastolic phase. All pixels with an intensity ≥130 Hounsfield units (HU) were counted and the data was analyzed using CardIQ Smart Score software (GE). CAC score (CACS) was determined using the Agatston scoring system, CACS thresholds <10 A.u. were assessed as indicating no calcification.

Ultrasonographic studies were performed with GE “VIVID 7 PRO” apparatus using a 5-14 MHz linear high-resolution probe. Each patient was examined in the supine position in a semi-dark room by the same expert radiologist who was unaware of the purpose of the study, the results of CACS and the allocation to the treatment group. CCA-IMT was defined as a low-level echo grey band that does not project into the arterial lumen and was measured at the diastolic phase as a distance between the leading edge of the first and second echogenic line. CCA-IMT was measured on the longitudinal views of the far wall of the distal segment of the common carotid artery, by means of semiautomatic border-detection program 0.5, 1 and 2 cm below and above the bifurcation in a plaque-free arterial segment and the mean value from all measurements from both carotids was used for statistical analysis [14,15]. Analysis was performed off-line on a workstation equipped with a dedicated software (EchoPac PC, GE Medical System).

Statistical analysis

The results are presented as mean±SD. A χ^2 test was used for sex comparison. The Shapiro-Wilk test was used to confirm the normality of the distribution. For normal distributions, the Student’s T test for unpaired data was used to assess the significance of the differences between the means, and a Bonferroni correction was applied for multiple comparisons. Pearson’s linear regression equations were used to determine the power of association between continuous variables, while Spearman’s rank correlation coefficient was calculated for non-normally distributed parameters. Depending on the data distribution, the comparison of follow-up data *vs* baseline was performed using the *t*-test for dependent variables or Wilcoxon test, respectively. The Wilcoxon test was used to compare the results before and after the treatment in the same group, and the Mann-Whitney U test to compare the results between two treated groups. The ANCOVA was used to adjust the follow up CCA-IMT and CACS for baseline values. A subsequent stepwise linear regression analysis was performed to

identify independent determinants of CCA-IMT and CACS changes according to a gradual modeling approach. The level of statistical significance was set at $P<0.05$.

Results

At baseline, 42 of 75 initially screened patients fulfilled the inclusion criterion of CACS ≥10 Agatston units (A.u). The patients randomized to the vitamin K+D group (14 F, 15 M; mean age 59.4±9.6 years) demonstrated a lower baseline estimated glomerular filtration rate (eGFR) 22.4 ± 10.1 ml/min/1.73m^2 than those in group D: 30.2±12.6 ml/min/1.73m^2, p<0.02 (5 F, 8 M; mean age 55.4±15.2 years), lower serum uric acid level 6.8 ± 1.7 vs 8.5± 1.9 mg/dl (p<0.004), higher serum phosphate 1.4± 0.4 vs 1.1±0.2 mmol/l (p<0.03); higher calcium x phosphate product 3.3±1.06 vs 2.7 ±0.6 mmol^2/l^2 (p<0.03) and lower hemoglobin level 11.7±1.3 vs 13.2 ±1.7 g/l (p<0.004). Other initial routine laboratory parameters were not significantly different between the groups.

Forty patients completed the study. The anthropometric and laboratory parameters of the patients from both groups at the beginning and at the end of the treatment are shown in Table 1. Two patients were withdrawn during the study: one patient from the vitamin K+D group discontinued the treatment period due to bowel discomfort in the fifth week of the study and one patient from the D group died in the second month due to myocardial infarction (this patient had diabetes mellitus and very high CACS - 1902 A.u.). Two patients from the vitamin K+D group started dialysis therapy during the intervention period (the first one 212 days after the start of study and the second one after 234 days, their data were included into the final analysis).

The CACS significantly increased in both groups at the end of the treatment period: in vitamin K+D group from 267.6±414.2 to 325.7±516.9, p<0.001, in vitamin D patients from 398.6±393.2 to 473±507.7, p<0.003 (Figure 2). The change of CACS was numerically less in

the vitamin K+D group treated patients than in the vitamin D group (Table 2). While a decrease of CACS was noticed in 5 patients from vitamin K+D group (5.4±5.2 A.u.), the CACS did not change in 2 patients. The scope of changes ranged from -11.8 to 380 A.u. in patients from vitamin K+D group. In patients treated with vitamin D alone, CACS increased from 4 to 426.5 A.u. When the patients with CACS≥1000 A.u. were excluded from the analysis, the significance of ΔCACS was at the border of statistical significance in patients treated with vitamin K+D versus those receiving vitamin D only, 18.2±29.1 A.u. vs 39.2±49.8, respectively (*P*=0.06).

A significantly lower increase of CCA-IMT during the intervention period was noticed in the vitamin K+D group from 0.95±0.2 to 1.01±0.3, p<0.003 than in D group: from 1.02±0.2 to 1.16±0.3, p<0.003 (Figure 3, Table 2). After nine months of vitamin K2 supplementation a significant decrease of dp-ucMGP was observed. This effect was not observed in D group. The significant increase of serum OPG was found in vitamin K+D group. A significant increase of OC serum concentration was observed in patients treated with vitamin D alone, in contrast to the vitamin K+D group, where the serum concentration of OC decreased. *The borderline increase of FGF-23 level in K+D patients during the treatment was observed in D group it has not changed.* The changes in laboratory parameters between the study groups at the end of the study are presented in Table 1.

A strong linear correlation was noticed between changes over time of ΔCACS and ΔCCA-IMT in patients from vitamin K+D group (r=0.65, *P*=0.0004, *Figure 4*). Significant correlations between serum phosphorus and ΔCACS (r=0.47, P=0.01), similar to the relationship between calcium-phosphate index and ΔCACS (r=0.47, P=0.01) were observed only in the vitamin K+D group. No significant correlation between calcification modulators and CACS and CCA-IMT was found.

In a stepwise multivariate linear regression analysis performed for patients from both groups together, the CACS, allocation to the treatment group and age were found to be independent determinants of the ΔCCA-IMT (Table 3). The ΔCACS was dependent on the CACS, hsCRP, 25OHD, phosphate, triglyceride, FGF-23, LDL-cholesterol and OC levels (Table 4). The allocation to the treatment group had no effect on ΔCACS in this analysis.

Discussion

To the best of our knowledge this study is the first to assess the effect of vitamin K2 administration on atherosclerosis and calcification progression in CKD patients. It shows that the progression of CCA-IMT, is significantly slower in patients treated with both vitamin K2 and cholecalciferol compared with patients substituted with vitamin D alone. Therefore, since all patients received the same dose of vitamin D, but only in one of the arms vitamin K2 was administered, by comparing the treatment results from both groups, we could assume that the differences between the groups were dependent *mainly* on the vitamin K2 effects. This is consistent with a previous observation of Gast et al. that high dietary intake of menaquinones may have a protective effect against the development of coronary heart disease [10]. Shanahan et al. showed that MGP mRNA was present in the normal media, but it expression was greatest in the atheromatous intima [16]. The immunohistochemical data reported by Schurgers et al. demonstrated that in atherosclerotic intima and in media sclerosis undercarboxylated MGP is abundantly present, suggesting local vitamin K deficiency and impaired protection attributable to poor MGP carboxylation [9].

A tendency to slow the progression of CAC in patients who received both vitamin K2 and D was observed in the present study, mostly in patients with less advanced baseline calcification. However, the change between both treated groups was relatively small, probably due to the short observation period, insufficient number of patients and the wide range of

CACS at baseline. Furthermore, in this study, although regression and stabilization of CACS was noticed in a few patients supplemented with MK-7, such effect was not observed in the vitamin D group. Previously, Shea et al. showed that the supplementation of vitamin K1 for 3 years slowed the progression of VC in elderly people with pre-existing calcification [17]. Some studies concluded that these two processes: atherosclerosis and VC are different pathologies [18] other showed that they were closely related and VC represents more advance atherosclerosis process which involves both layers [19,20,21]. Our study showed strong correlation between the changes of CACS and CCA-IMT. Taking into account the common ground of these two pathologies we suspect that supplementing vitamin K2 for a longer time could result in a more distinct inhibition of calcification progress.

Deficiency of carboxylated MGP may contribute substantially to the development and progression of arterial calcification. Areas of calcification in vascular tissue are associated with accumulation of unMGP species, which has also been found to precede the development of clinically overt calcification in children on dialysis [22]. In our study the serum level of dp-ucMGP decreased significantly during vitamin K2 supplementation. The substitution of vitamin K2 could possible cause the increase of MGP carboxylation in vessel's wall and could slow down the progression of atherosclerosis.

Previous studies evaluating the association of OC with VC and cardiovascular disease have been conflicting. One study demonstrates that higher OC levels are associated with lower vascular stiffness and CCA-IMT [23], whereas another shows that the higher OC levels are associated with more advanced VC in animal models [24]. Parker et al. observed no association of OC and aortic calcification in postmenopausal women [25]. In our study, OC level decreased during the substitution of vitamin K2 with vitamin D. This phenomenon seems to be dependent on vitamin K2 concentration as OC concentration was found to increase in patients from the reference group receiving vitamin D alone.

OPG has been proposed as a protective factor against vascular calcification [26]. In our study the serum OPG increased during supplementation with vitamin K2. Our findings confirm the previous experimental [27] and human studies [28] which found that 15 mg/24h of menatetrenone (vitamin K2) prevents the reduction of serum level of OPG in patients treated with glucocorticoids. OPG has previously been reported to play a protective role in preventing calcification during the administration of warfarin and vitamin D [29].

No significant changes in the level of fetiun A and mineral parameters including FGF-23 during vitamin K2 substitution was noted in the present study. Nagasawa et al. showed the total and LDL cholesterol decrease after 6 months supplementation 45 µg vitamin K in peritoneal dialysis patient [30]. We did not observe any significant influence of vitamin K2 substitution on lipids levels, but all our study patients were treated with statins before and during the vitamins substitution.

Our study limitations are small sample size, resulting in the statistical power that does not allow clinically relevant conclusions to be drawn. A follow-up interval of 270 days may have been too short to detect the differences in the progression of atherosclerosis and VC which develop over many years. On the other hand, the development of vascular wall changes in CKD patients is faster than in general population [31]. Our study group was quite homogenous, but the treatment groups were different in baseline eGFR, uric acid, phosphate and calcium x phosphate products *and haemoglobin level*. The baseline values of CACS and CCA-IMT were not significantly different between the K+D and vitamin D groups but were slightly lower in the former, hence the impact of baseline values on our final observation could not be excluded. The power of the test used to compare the effect of vitamin K+D *vs* vitamin D alone on the ΔCACS and ΔCCA-IMT was insufficient due to the small group of patients and a wide range of baseline values. Furthermore only one dose of MK-7 was studied and the vitamin K concentration was not measured before and after the treatment, however,

the patient's compliance may be confirmed by the change of dp-ucMGP and 25OHD levels observed in both groups. In addition, we did not notice any significant differences between the treated groups over time, we only found differences within the groups and between the groups at baseline and at final measurement. The lack of distinguishing between different forms of OC (carboxylated, uncarboxylated) and consequent difficulty in interpretation is another limitation in our study.

In conclusion, *270 day course of 90 μg vitamin K2 administration may reduced the progression of atherosclerosis* in non-dialysis subjects in 3-5 stages of CKD, but does not have a significant effect on CAC progression. Larger studies are needed to confirm whether vitamin K2 needs to be supplemented in CKD patients for the prevention of atherosclerosis and vascular calcification. The mechanisms by which vitamin K2 may exert a protective effect on the progression of vessel damage are still uncertain, but may be connected with the impact of MK-7 on the regulators of calcification, including the impact on the MGP carboxylation process.

Contribution statement: IK designed and performed the study, analyzed the data and wrote the paper, AM-Z, PG and MK performed the study, LS performed the study and contributed to the writing of the paper, CV designed the study and analyzed the data, KM designed the study and analyzed the data, MN designed the study, analyzed the data and wrote the paper. All authors provided intellectual contents of critical importance to the study, edited and approved the final version of the manuscript.

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Table 1. Anthropometric and laboratory parameters at baseline and after 270 days of treatment with vitamin K2+D (K+D group) or vitamin D alone (D group)

Parameter	Vit K+D (n= 28) Mean ± SD Median (P25 - P75)			Vit D (n=*12*) Mean ± SD Median (P25 - P75)			***P****
	Before treatment	**After treatment**	***P***	**Before treatment**	**After treatment**	***P***	
BM (kg/m^2)	30.3 ± 4.6 30.8 (26.9-33.6)	29.8 ± 4.1 30.4(26.8-33.6)	*0.3*	28.7±5 .2 28.3 (23.4-33.6)	28.5± 4.9 28.4 (23.2-33.4)	*0.2*	*0.4*
Serum creatinine (mg/dl)	3.3 ± 1.5 3.1 (2.2-3.5)	4.3 ± 2.7 3.2(2.5-5.5)	*0.01*	2.5 ±0.8 2.1(1.8-3)	2.6 ± 0.9 2.3 (1.7-3.2)	*0.3*	*0.06*
eGFR (ml/min /1.73 m^2)	22.2 ± 9.8 19.5 (14-31)	18.7 ± 11.2 17.0(9.5-25)	*0.08*	30.3±12.7 28.0 (23-39)	30.0 ±13.8 26.0 (21-44)	*0.7*	*0.02*
Uric acid (mg/dl)	6.8 ± 1.4 6.4 (5.8-8.1)	6.5 ± 1.3 6.4(5.5-7.8)	*0. 2*	8.5± 1.9 8.3 (7.6-8.8)	7.9±1.3 8.0 (6.9-8.5)	*0.2*	*0.05*
Total Cholesterol (mg/dl)	208.5 ±66.7 186.5(165-235)	218.9 ±56 194(175-260)	*0.5*	167.5±32.9 160.0 (138-203)	186.8±40.2 187.5 (159-210)	*0.06*	*0.2*
Triglyceride (mg/dl)	215.2±121 175.0 (136-244)	198±113 170 (123.5-212)	*0.4*	140± 48.8 120.0 (106-188)	149.8±52 135.0 (106-190)	*0.5*	*0.3*
LDL (mg/dl)	119.4±49.5 105 (82-143)	125.5±47.3 112.5(92-160)	*0.4*	96.7±21.8 97.0 (78-123)	108±33.1 101.5 (76-123)	*0.06*	*0.4*
HDL (mg/dl)	53.1±15.9 47.5 (38-57)	57.2 ±28.1 47.5(41-63)	*0.9*	45.8±10.0 43.0 (34-55)	51.3±12.2 55.5 (41-61)	*0.02*	*0.4*
Calcium (mmol/l)	2.4±0.1 2.4(2.3-2.5)	2.4 ±0.2 2.4(2.3-2.5)	*0.4*	2.4±0.1 2.4 (2.4-2.5)	2.5 ±0.2 2.4 (2.4-2.5)	*0.2*	*0.1*
Phosphate (mmol/l)	1.4± 0.4 1.3 (1.2-1.4)	1.5 ± 0.6 1.3(1.2-1.8)	*0.08*	1.1±0.2 1.1 (0.9-1.1)	1.2±0.2 1.1 (1.1-1.4)	*0.004*	*0.1*
Ca x P	3.3±1.06	3.7± 1.5	*0.09*	2.7 ±0.6	3.0± 0.6	*0.00*	*0.2*

($mmol^2/l^2$)	3.2 (2.7-3.4)	3.2(2.8-4.2)		2.6 (2.2-2.7)	2.8 (2.6-3.6)	*2*	
PTH (pg/ml)	194±143.1 141 (77-298)	233±245.7 168 (74-246)	*0.3*	134 ± 80.6 131.5 (56-182)	120.8 ±62.4 125.0 (79-147.7)	*0.6*	*0.2*
Hemoglobin (g/l)	11.8±1.4 11.6(10.9-12.7)	11.4 ±1.9 11.3(10.3-12.6)	*0.2*	13.2 ±1.6 14.0 (11.6-14.6)	13.7±1.8 14.0 (12.1-14.8)	*0.02*	*0.001*
Prothrombin time (seconds)	13.2±0.4 13.2 (12.9-13.3)	12.9±0.6 31.5 (21.6-34.4)	*0.8*	13.1±05 12.8 (12.7-13.5)	13.0±0.4 13.0 (12.8-13.5)	*0.7*	*0.9*
25OHD (ng/ml)	20.8±9.8 20.4(12.6-27.5)	32.1±12.1 31.5(21.6-34.4)	*0.004*	24.8±12.9 20.2 (14.7-33.6)	33.4±11.7 28.9 (24.1-38.8)	*0.03*	*0.8*
MGP (pg/ml)	639.6±187 595.1 (533.6-831.2)	742.8±249.1 684 (555.6-888.4)	*0.06*	640.7±195.4 560.3 (516.3-718.2)	615±165.9 594.9 (489.1-680.0)	*0.6*	*0.1*
dp-ucMGP (pmol/l)	1077.1±507.7 1004 (590-1670)	961.5±506.7 812 (510-1580)	*0.02*	793.9±400.3 715(467-1190)	820.7±565.2 710 (490-1119)	*0.7*	*0.5*
FGF-23 (pg/ml)	41.3±120 12.8 (9.0-23.3)	71.5±163 18.05 (9.4-43.7)	*0.06*	16.7±15 12.5 (6.8-18.6)	13.3±8.4 10.1 (7.4-22)	*0.3*	*0.3*
OC (ng/ml)	63.3±41.4 60.2 (43.1-75.7)	56.5±42.0 54.7 (29.9-83.5)	*0.04*	40.8±54 29.3 (19,4 - 64.3)	58±43 50.2 (38.8-78.3)	*0.03*	*0.9*
OPG (pg/ml)	5.7±2.2 4.7(4.6-6.4)	6.3±2.2 5.8(4.7-7.3)	*0.02*	4.7±1.8 4.2 (3.3-6.1)	5.1±1.7 5.7 (3.5-6.5)	*0.08*	*0.1*
Fetuin A (ng/ml)	111.4±43 112.2(90.4-132.2)	113.4±37 108.9(87.1-136.6)	*0.7*	110.6±34.8 103.7 (90.4-129.9)	120.4±30.2 120.0 (84-149)	*0.4*	*0.6*
hsCRP	6.6±4.8	7.9±5.9	*0.19*	4.5±4.9	6.9±5.6	*0.08*	*0.6*

(μg/ml)	4.5 (3.3-9.8)	6.4 (2.6-10.3)		2.0 (1.1-7.7)	5.7 (3.0-10.1)		

* between treatment groups (Vit K+D vs Vit D) after treatment

A.u.: Agatston units; CACS: coronary artery calcification score; CaxP: calcium-phosphorus product; CCA-IMT: common carotid artery intima media thickness; dp-ucMGP: desphosphorylated-uncarboxylated-MGP; FGF-23: fibroblast growth factor 23; hsCRP: high sensitive C-reactive protein; 25OHD: 25-hydroxyvitamin D; MGP: matrix Gla protein; OC: osteocalcin; OPG: osteoprotegerin.

Table 2. The change in coronary artery calcification score and carotid artery intima media thickness from baseline to day 270 of treatment with vitamin K2+D (K+D group) or vitamin D alone (D group)

Parameter	**Units** **Vit K+D (n= 28)** Mean ± SD Median (P25 - P75)	**Vit D (n=*12*)** Mean ± SD Median (P25 - P75)	***P***
ΔCACS A.u	58.1 ± 106.5 11.0 (0 – 55.5)	74.4 ± 127.1 20.5 (8.0 – 119.0)	*0.7* *0.2*
	ANCOVA		*0.3*
	16.7 ± 23.3% 13.9 (0.0 – 26.7)	15.9 ± 12.9% 11.1 (4.8 – 19.7)	*0.9* *0.8*
ΔCCA-IMT mm	0.06 ± 0.08 0.000 (0.000 – 0.1)	0,136 ± 0.05 0.100 (0.1 – 0.2)	*0.005* *0.004*
	ANCOVA		*0.007*
	6.0 ± 7.1% 0.0 (0.0 – 12.5)	13.8 ± 4.9 % 13.3 (10.0 – 18.2)	*0.003* *0.009*

A.u.: Agatston units; ΔCACS: change in coronary artery calcification score; ΔCCA-IMT: change in carotid artery intima media thickness

Table 3. Determinants affecting the change of common carotid artery intima media thickness in a stepwise multivariate linear regression analysis in patients from both treated groups

Variable	β	B±SEM	B	B±SEM	*P*
CACS	0.486473	0.117017	0.000093	0.000022	*0.0003*
Allocation to the treatment group	0.324939	0.123649	0.053876	0.020502	*0.01*
Age	0.281064	0.128746	0.001903	0.000872	*0.04*
OPG	-0.219077	0.119218	-0.008118	0.004418	*0.08*
Total Cholesterol	-0.163047	0.120308	-0.000212	0.000157	*0.2*
FGF-23	0.154598	0.114646	0.000118	0.000088	*0.2*
hsCRP	-0.126798	0.118165	-0.002016	0.001878	*0.3*

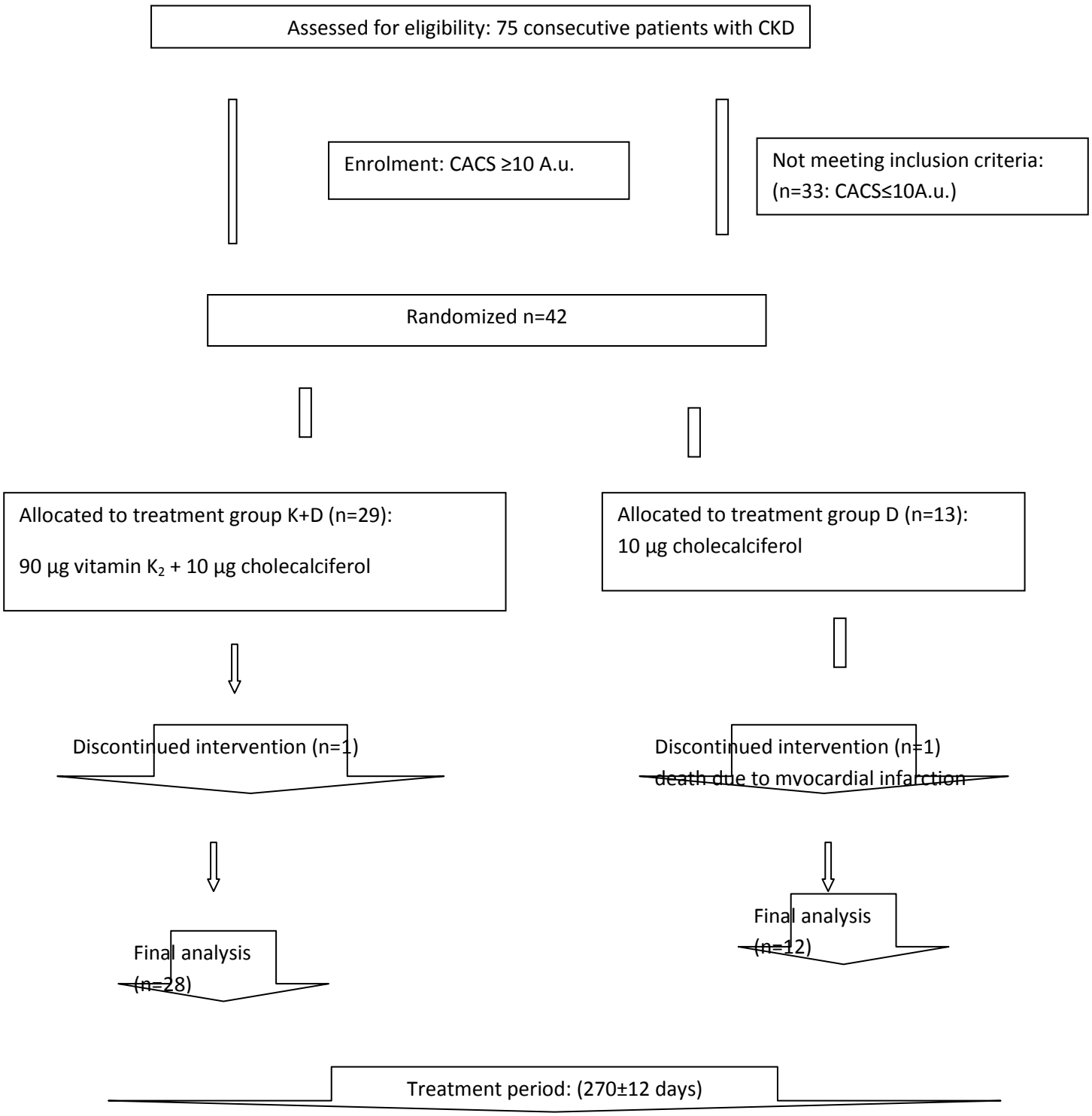
CACS: coronary artery calcification score; FGF-23: fibroblast growth factor 23; hsCRP: high sensitive C-reactive protein; OPG: osteoprotegerin

Table 4. Determinants affecting the change of coronary artery calcification score analyzed in a stepwise multivariate linear regression analysis in patients from both treated groups

Variable	B	B±SEM	B	B±SEM	*P*
CACS	0.893942	0.048580	0.245	0.01332	*0.000*
hsCRP	0.179852	0.046419	4.119	1.06316	*0.001*
25OHD	0.174530	0.052890	1.784	0.54050	*0.003*
Phosphate	0.233145	0.083932	67.625	24.34490	*0.01*
Triglyceride	0.191224	0.070772	0.208	0.07708	*0.01*
FGF-23	-0.188738	0.075961	-0.208	0.08387	*0.02*
LDL	-0.174231	0.072816	-0.453	0.18925	*0.02*
OC	0.141978	0.065692	0.300	0.13881	*0.04*
Age	-0.078647	0.055006	-0.767	0.53647	*0.2*
OPG	-0.065404	0.051691	-3.492	2.75981	*0.2*
MGP	0.042400	0.049333	0.025	0.02906	*0.4*

CACS: coronary artery calcification score; hsCRP: high sensitive C-reactive protein; 25OHD: 25-hydroxyvitamin D; FGF-23: fibroblast growth factor 23; OC: osteocalcin

Figure 1. Schematic illustration of patient selection and randomization and study design



A.u.: Agatston units

CKD: chronic kidney disease

CACS: coronary artery calcification score

Figure 2. Coronary artery calcification score before and after 270±12 days of treatment with

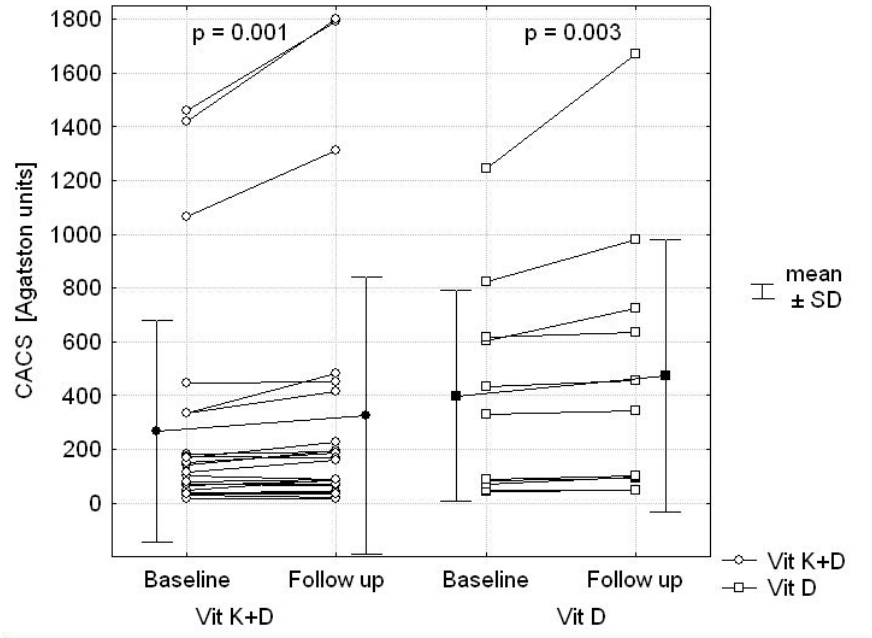


Figure 3. Common carotid intima media thickness before and after 270±12 days of treatment with vitamin K2 and vitamin D (K+D group) or vitamin D alone (D group)

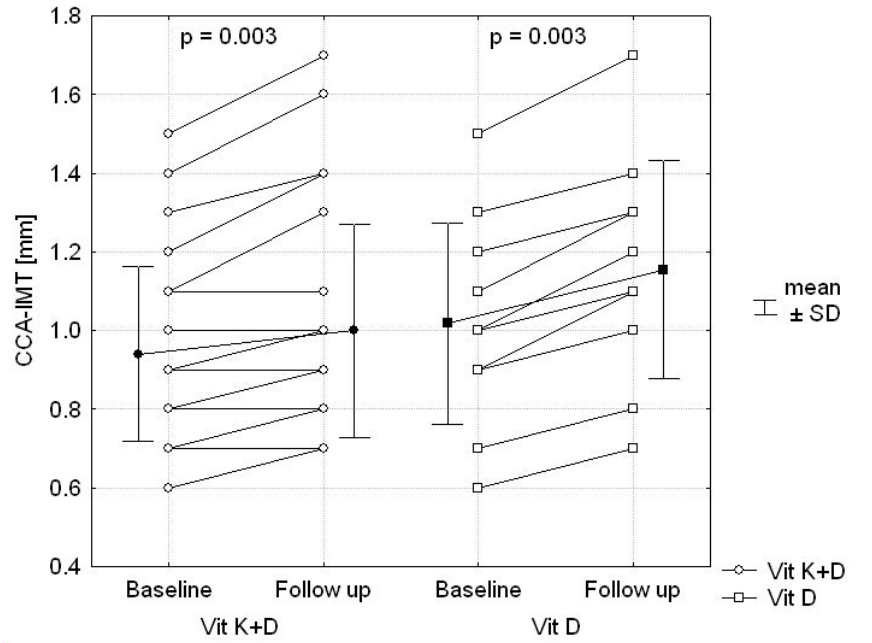


Figure 4. Correlation between the change of coronary artery calcification score (ΔCACS) and the change of common carotid intima media thickness (ΔCCA-IMT) over time of vitamin K2 and D administration (r=0.65, p=0.0004).