

Correlation study of serum vitamin K and Gas6 levels with aortic arch calcification and left ventricular hypertrophy in maintenance hemodialysis patients

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Abstract: Background: Cardiovascular disease is the leading cause of death among MHD patients, with aortic arch calcification and left ventricular hypertrophy as common complications. The role of vitamin K-dependent protein Gas6 in these structural changes remains unclear. **Objectives:** To examine the relationship between serum vitamin K2/Gas6 levels and the presence of aortic arch calcification/left ventricular hypertrophy in MHD patients. **Methods:** This cross-sectional study enrolled 189 patients with MHD. Serum vitamin K2 (MK-7) and Gas6 levels were quantified using enzyme-linked immunosorbent assay (ELISA). LVMI stratified patients into LVH (n=110) and non-LVH (n=79) groups, and by AACS into four calcification severity groups (n=46, 54, 53, 36). Group differences were analyzed using Spearman correlation and multivariate logistic regression. **Results:** LVH patients were older and had higher BNP, creatinine and Gas6 levels, but lower vitamin K2 levels (P<0.05). Across AACS groups, age, glucose, phosphorus, calcium-phosphorus product, vitamin K2 and Gas6 differed significantly. Vitamin K2 correlated positively with calcium (r=0.822) and negatively with Gas6 (r=-0.339). Age >65 years and vitamin K2 <2.39 nmol/L were independent AACS risk factors. **Conclusion:** No correlation was found between serum vitamin K or Gas6 and LVH in MHD patients. AACS negatively correlated with vitamin K and positively with Gas6.

Keywords: Gas 6; Left ventricular hypertrophy; Vessel calcification; Vitamin K

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INTRODUCTION

Patients with chronic kidney disease (CKD) frequently develop mineral and bone metabolism disorders. Cardiovascular disease is the primary cause of mortality in CKD patients, particularly in those with end-stage renal disease (ESRD), an irreversible stage of CKD that is associated with a higher incidence of cardiovascular calcification and an unfavorable prognosis (Marx-Schutt *et al.*, 2025; Li and Lindholm, 2022). Cardiovascular calcification and left ventricular hypertrophy are common complications in CKD patients. Notably, cardiovascular calcification is relatively common in this population and can occur even in younger patients and at early disease stages (Zoccali *et al.*, 2023; Hutcheson and Goettsch, 2023). In maintenance hemodialysis (MHD) patients, the incidence of abdominal aortic calcification and valvular calcification is significantly higher than in the general population. Moreover, CKD patients exhibit more rapid disease progression once cardiovascular calcification develops. The underlying mechanisms of cardiovascular calcification remain incompletely understood (Li *et al.*, 2025; Wang *et al.*, 2024). Left ventricular hypertrophy, a significant risk factor for mortality in MHD patients, is associated with multiple pathogenic factors (Kaur and Singh, 2022; Lafage-Proust, 2023). As CKD progresses, patients often develop disturbances in mineral metabolism,

including hypocalcemia, hyperphosphatemia and elevated parathyroid hormone (PTH) levels, and reduced active vitamin D. These abnormalities contribute to chronic kidney disease–mineral and bone disorder (CKD-MBD), which, in turn, promotes ectopic calcification, including soft-tissue and cardiovascular calcification. These pathological processes contribute to the development of cardiovascular disease in CKD patients, resulting in a poor prognosis. In particular, aortic calcification can induce left ventricular hypertrophy, ventricular remodeling and heart failure (Lee *et al.*, 2024; Izzo *et al.*, 2024).

Vitamin K1 and K2 are essential for the activation of hepatic coagulation factors and the synthesis of vascular proteins. Vitamin K-dependent proteins, such as matrix Gla protein (MGP), serve as potent inhibitors of vascular calcification (Mandatori *et al.*, 2021; Shams *et al.*, 2025). Recent studies suggest that vascular calcification shares similarities with bone development. Gas6, a vitamin K-dependent protein, plays a role in regulating vascular calcification. Previous *in vitro* studies have indicated that Gas6 can inhibit vascular calcification by suppressing the apoptosis of vascular smooth muscle cells (Ke *et al.*, 2021; Lv *et al.*, 2024). However, in the clinical context of CKD, the physiological role of Gas6 may be more complex. Elevated levels of Gas6 may be associated with systemic inflammation, compensatory responses to calcification stress, or variations in detection methods. The exact clinical relationship between Gas6 and vascular

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calcification remains unclear. In patients with CKD, vascular calcification is closely linked to chronic kidney disease–mineral and bone disorder (CKD-MBD) and may be associated with vitamin K deficiency (Lees *et al.*, 2021; Neofytou *et al.*, 2024).

Although basic research identifies vitamin K and Gas6 as potential inhibitors of vascular calcification (Kaur and Singh, 2022) and early detection of calcification is crucial for improving the prognosis of ESRD patients, existing clinical studies present several limitations. First, most investigations focus on the relationship between a single biomarker (e.g., vitamin K) and coronary or peripheral vascular calcification, lacking a systematic assessment of aortic arch calcification—a critical site evaluated by the aortic arch calcification score (AACS). Second, findings on serum Gas6 levels in CKD patients are inconsistent: some reports indicate a negative correlation with calcification, supporting its inhibitory role, while others associate elevated Gas6 with adverse cardiovascular outcomes, suggesting its potential as a disease activity biomarker (Zhou *et al.*, 2021; Ma *et al.*, 2023). These discrepancies may arise from differences in study populations, detection methods, or Gas6 isoforms. Notably, few studies have simultaneously measured both serum vitamin K and Gas6 levels in MHD patients and examined their associations with aortic arch calcification severity and left ventricular hypertrophy. An integrated analysis could clarify their interactions and independent contributions to cardiovascular pathology.

Therefore, to address this gap in the literature, this cross-sectional study aims to simultaneously examine serum levels of vitamin K2 (MK-7) and Gas6 in patients on maintenance hemodialysis (MHD). It further seeks to systematically investigate their individual and combined correlations with aortic arch calcification score (AACS) and left ventricular hypertrophy (LVH), as defined by left ventricular mass index (LVMI). This study is expected to provide novel serological evidence and an empirical basis for assessing cardiovascular risk in MHD patients and elucidating the role of the vitamin K-Gas6 axis in chronic kidney disease–mineral and bone disorder (CKD-MBD).

MATERIALS AND METHODS

Clinical information

This single-center, observational cross-sectional study was conducted at Ningbo Hospital of Integrated Traditional Chinese and Western Medicine. We enrolled patients with end-stage renal disease (ESRD) who were receiving maintenance hemodialysis (MHD). All participants underwent laboratory testing, echocardiography and chest X-ray examinations. The research process is shown in Fig. 1. Patients were recruited between January 2023 and December 2024. Data collection, including laboratory tests, echocardiography and chest X-ray examinations, was completed within two weeks after enrollment.

Inclusion criteria

Inclusion criteria were as follows: (1) diagnosis of ESRD according to the Japanese Society of Nephrology guidelines (2019); (2) receipt of regular hemodialysis for more than 3 months (Thomas *et al.*, 2020); (3) clinically stable condition for at least one month before enrollment; (4) age ≥ 18 years; (5) dialysis frequency of 2–3 times per week; and (6) no use of vitamin K supplements (including vitamin K1 or K2) within the preceding month.

Exclusion criteria

Exclusion criteria included: (1) estimated life expectancy < 6 months; (2) history of cardiac valve disease, cardiomyopathy, atrial fibrillation, malignant tumor, acute kidney injury, or acute myocardial infarction; (3) thyroidectomy; (4) active inflammatory conditions; (5) left ventricular ejection fraction $< 50\%$; (6) prior coronary artery stent implantation; (7) conditions affecting calcium metabolism, such as AIDS or sarcoidosis; (8) limb amputation; and (9) pregnancy or lactation. Among 538 initially screened patients, 349 were excluded due to: cardiovascular diseases ($n=81$), left ventricular ejection fraction $< 50\%$ ($n=52$), active inflammatory conditions ($n=44$), history of malignancy ($n=38$), prior coronary stent implantation ($n=35$), valve disease or atrial fibrillation ($n=29$), thyroidectomy ($n=22$), conditions affecting calcium metabolism ($n=18$), limb amputation ($n=12$), pregnancy or lactation ($n=8$), and estimated life expectancy < 6 months ($n=10$). Some patients met multiple exclusion criteria, resulting in a final cohort of 189 participants.

The mean age of included patients was 63.44 ± 4.23 years, including 112 males (59.3%) and 77 females (40.7%). The mean dialysis duration was 85.26 ± 4.14 months. Based on LVMI, patients were categorized into left ventricular hypertrophy (LVH) group ($n=110$, 58.2%) and non-LVH group ($n=79$, 41.8%). According to AACS, they were stratified into four groups: no calcification ($n=46$, 24.3%), mild calcification ($n=54$, 28.6%), moderate calcification ($n=53$, 28.0%) and severe calcification ($n=36$, 19.1%).

All patients underwent regular hemodialysis with a bicarbonate-based dialysate for 4 hours, three times per week, as recommended for MHD patients (Thomas *et al.*, 2020). The dialysate calcium concentration was set at 1.5 mmol/L and delivered at a flow rate of 500 mL/min via an internal jugular vein catheter or an arteriovenous fistula. Blood flow rates were maintained between 200 and 300 mL/min. Dialysis prescriptions were individualized based on patients' body mass. In addition, 40 healthy volunteers (aged > 18 years) were recruited as the control group, comprising 24 males and 16 females (average age = 63.57 ± 3.78 years). Serum vitamin K and Gas6 levels were measured, and all participants had no chronic renal disease.

Sample size calculation

Based on our unpublished preliminary data from 30 pilot

MHD patients (available upon request), the anticipated difference in serum vitamin K2 levels across AACS groups was used for calculation. With $\alpha=0.05$ (two-tailed) and $\beta=0.20$ (80% statistical power), the sample size was estimated using the ANOVA calculation formula (Rychtar and T Taylor, 2020). The results indicated a requirement of at least 30 patients per group. In this study, patients were stratified into four subgroups based on AACS scores: no calcification, mild calcification, moderate calcification, and severe calcification. To ensure an adequate sample size in each subgroup and account for an anticipated dropout rate, the total planned sample size was set at a minimum of 120 patients. Ultimately, 189 patients were enrolled, meeting the sample size requirement.

Experimental methods

General information collection

We collected demographic and clinical data, including sex, age, body mass index (BMI), dialysis duration, medical history, smoking status, and medication use (e.g., calcium supplements, phosphate binders, and vitamin D analogs).

Phosphate binder usage

Based on routine medication records, all enrolled MHD patients received individualized management for hyperphosphatemia. The use of phosphate binders was as follows: Calcium-containing phosphate binders were prescribed to 78 patients (41.3%). Among them, 65 patients used calcium carbonate alone, 9 used calcium acetate alone and 4 patients alternated between the two. Non-calcium-containing phosphate binders: A total of 92 patients (48.7%) were prescribed these, including sevelamer (n=56), lanthanum carbonate (n=31), and a combination of both (n=5). No phosphate binder use: 19 patients (10.0%) did not use any phosphate binders because their serum phosphorus levels were adequately controlled or they had contraindications to these medications.

Dialysis protocol and related parameters

All patients underwent regular hemodialysis using bicarbonate-based dialysate, with a schedule of three sessions per week, each lasting 4 hours. The dialysate calcium concentration was uniformly set at 1.5 mmol/L. The anticoagulation regimen was selected based on individual patient conditions: the majority of patients received standard heparin (initial dose: 1000 U, maintenance: 500–800 U/h), while those with bleeding risks or heparin contraindications received regional citrate anticoagulation (4% sodium citrate, with a blood flow rate to citrate infusion rate ratio of 1:1.2–1.5). Dialysis adequacy was assessed using the urea clearance index (spKt/V), with a target value of ≥ 1.4 , in accordance with established hemodialysis adequacy guidelines (National Kidney Foundation, 2015). All patients' dialysis vintage (months), individual Kt/V values, dialysate calcium concentration and anticoagulant usage were recorded and included in the analysis.

Clinical assay

Fasting blood samples were collected within one week after chest X-ray examination. Laboratory measurements included serum lipids, glucose, calcium, hemoglobin, phosphorus, albumin, C-reactive protein (CRP) and parathyroid hormone (PTH). The urea clearance index (Kt/V) and calcium–phosphorus product ($\text{Ca} \times \text{P}$) were calculated.

Serum vitamin K and Gas6 assay

Serum levels of vitamin K and Gas6 were measured using enzyme-linked immunosorbent assay (ELISA). All reagent kits were purchased from RandD Systems (USA).

Vitamin K assay: The Human Vitamin K2 (Menaquinone-7, MK-7) ELISA Kit (Catalog #BWJ4396-2016) was used. This kit specifically detects the MK-7 form and can also be used to quantify total vitamin K2 using a standard curve. The detection range was 0.1–10 nmol/L, with a lower limit of detection (LOD) of 0.05 nmol/L. The intra-assay coefficient of variation (CV) was <8% and the inter-assay CV was <10%. Serum samples were stored at -80°C after collection, with a maximum of 2 freeze-thaw cycles.

Gas6 assay: The Human Gas6 ELISA Kit (Catalog #ZK-5352) was used. The detection range was 0.2–50 ng/mL, with an LOD of 0.1 ng/mL. The intra-assay CV was <6% and the inter-assay CV was <9%. All procedures were strictly followed, with standard curves and quality control samples included on each assay plate. Serum samples were centrifuged within 2 hours of collection, aliquoted and stored at -80°C until analysis.

Measurement of matrix Gla protein (MGP) and vascular calcification score

Serum matrix Gla protein (MGP) levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Human MGP ELISA Kit, Catalog #ab216226, Abcam, UK). The detection range was 50–2000 pmol/L, with a lower limit of detection (LOD) of 20 pmol/L. The intra-assay coefficient of variation (CV) was <8% and the inter-assay CV was <10%. Serum samples were stored at -80°C and analyzed according to the manufacturer's instructions.

The vascular calcification score was assessed using the Adragao method on lateral lumbar spine radiographs. Briefly, the anterior and posterior walls of the abdominal aorta at the L1–L4 vertebral levels were examined. Calcification at each of the four vertebral segments was scored as 0 (no calcification), 1 (calcification involving <50% of the wall length), or 2 (calcification involving $\geq 50\%$ of the wall length). The total score ranged from 0 to 16. All radiographs were evaluated independently by two senior radiologists blinded to clinical data. The intraclass correlation coefficient (ICC) for the vascular calcification score was 0.89 (95% CI: 0.84–0.93), indicating good inter-rater reliability. In case of disagreement, a third radiologist arbitrated, and the majority score was adopted.

Comparability of assessment methods across groups: All laboratory measurements (including vitamin K2, Gas6, MGP, and routine biochemical parameters) were performed using the same equipment, reagent lots, and protocols for all participants. Echocardiography and chest X-ray examinations were conducted by the same group of trained technicians and interpreted by the same two senior radiologists throughout the study period. The control group (healthy volunteers) underwent the same blood collection and ELISA protocols as the MHD patients.

Aorta arch calcification evaluation

All patients underwent postero-anterior standing chest X-ray examinations. The Aortic Arch Calcification Score (AACS) was assessed by two senior radiologists using the Symeonidis method (Muscogiuri *et al.*, 2025). Briefly, scores were assigned as follows: 0 points for no calcification; 1 point for a single lamellar or dot-like calcification; 2 points for single or multiple areas of calcification; and 3 points for circular calcification. To evaluate consistency among raters, the intraclass correlation coefficient (ICC) was used. The results showed that the ICC for the AACS score was 0.92 (95% CI: 0.88-0.95), indicating excellent inter-rater consistency. In the event of any discrepancy in the scoring, a third senior radiologist shall arbitrate, and the majority opinion shall be taken as the final score.

Left ventricular mass index (LVMI)

Patients underwent an echocardiogram (Model Sonos 5500, Philips) to measure left ventricular diastolic diameter (LVDD), intraventricular septal thickness (IVST), and left ventricular posterior wall thickness (LVPWT). Left ventricular mass (LVM, in g) = $0.8 \times 1.04 \times [(LVDD + IVST + LVPWT)^3 - LVDD^3] + 0.6$, LVMI = LVM/BSA. Judgment of left ventricular hypertrophy was identified (Muscogiuri *et al.*, 2025) as higher than 100g/m² LVMI in females and higher than 131g/m² in males. The effects of vitamin K and Gas6 levels on calcium-phosphorus metabolism and cardiovascular complications in patients with CKD were analyzed.

Dialysis parameters

Carbohydrate buffer (1.5mmol/L) and model FX60 dialysis apparatus (1.4m² polysulfone membrane, 46ml/(mmHg.h) ultra-filtering parameter, Fresenius, Germany) were used and were disposable. Blood flow velocity was controlled at 220~280ml/min and dialysis buffer flow velocity was maintained around 500ml/min.

Statistical analysis

Statistical analyses were performed using SPSS 19.0. Categorical variables were compared using the chi-square test or Fisher's exact test. Normality was assessed with the Kolmogorov-Smirnov test. Normally distributed data are expressed as mean \pm standard deviation (SD); skewed data are presented as median (interquartile range) [M (Q1-Q3)] and non-parametric tests or transformations were applied

as appropriate. Group comparisons were conducted using analysis of variance (ANOVA), followed by LSD post hoc tests. Spearman correlation analysis was used to examine associations between serum vitamin K2/Gas6 levels and other variables. Multivariate logistic regression was performed to identify risk factors for left ventricular hypertrophy and aortic arch calcification, with adjustments for age, sex, diabetes history, serum phosphorus, calcium, PTH and use of phosphate binders and vitamin D analogs. A two-tailed p-value <0.05 was considered statistically significant. There were no missing data for the primary exposure (vitamin K2, Gas6) or outcome variables (AACS, LVMI) in the final analytic cohort of 189 patients. For secondary laboratory parameters, complete data were available for all included participants. Sensitivity analysis was performed by excluding patients with extreme calcium levels (n=3) to assess the robustness of the correlation between vitamin K2 and serum calcium.

RESULTS

Baseline characteristics and serum biomarker levels of study participants

A total of 189 MHD patients and 40 healthy controls were included in the analysis. There were no significant differences between the two groups in terms of age or gender distribution ($P > 0.05$). As shown in Table 1, compared to the control group, MHD patients exhibited significantly higher values for left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), left ventricular posterior wall thickness (LVPWT) and serum Gas6 levels ($P < 0.05$). In contrast, the E/A ratio and serum vitamin K2 (MK-7) levels were significantly lower ($P < 0.05$). Serum vitamin K2 levels displayed a skewed distribution, with a median (interquartile range) of 2.28 (1.76-2.66) nmol/L. Gas6 levels followed a normal distribution, with a mean value of 20.11 ± 3.45 ng/mL. Spearman correlation analysis revealed no significant correlations between serum levels of either vitamin K2 or Gas6 and any of the echocardiographic parameters (LVEF, LVMI, LVPWT, E/A ratio) ($P > 0.05$; see Table 2).

Comparison of indicators between left ventricular hypertrophy (LVH) and non-hypertrophy groups

Based on LVMI, patients were categorized into an LVH group (n=110) and a non-LVH group (n=79). As shown in Table 3, patients in the LVH group had significantly higher levels of age, serum B-type natriuretic peptide (BNP), serum creatinine, and Gas6 than the non-LVH group ($P < 0.05$), while their serum vitamin K2 levels were significantly lower ($P < 0.05$). No statistically significant differences were observed between the two groups in terms of serum phosphorus, calcium-phosphorus product, parathyroid hormone (PTH), blood lipids, C-reactive protein (CRP), or other related indicators ($P > 0.05$). Similarly, there were no significant differences in the proportion of patients using phosphate binders, vitamin D analogs, or calcium supplements between the two groups.

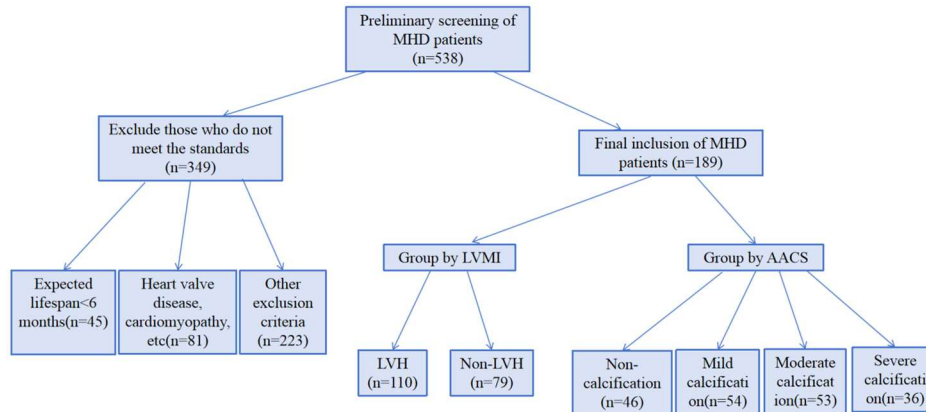


Fig. 1: Study flow diagram. A total of 538 patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis (MHD) were initially screened. After applying inclusion and exclusion criteria (including 81 patients with cardiovascular diseases), 349 patients were excluded. The final analytic cohort consisted of 189 MHD patients, who were stratified by left ventricular mass index (LVMI) into non-left ventricular hypertrophy (non-LVH, n=79) and LVH (n=110) groups, and by aortic arch calcification score (AACCS) into no (n=46), mild (n=54), moderate (n=53) and severe calcification (n=36) groups.

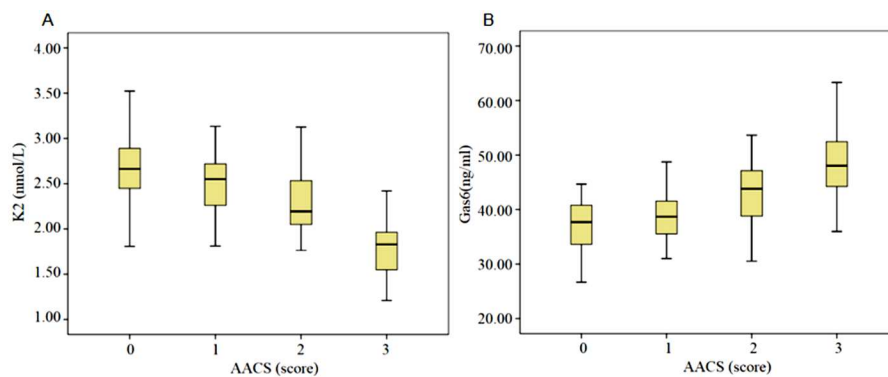


Fig. 2: Serum vitamin K2 (MK-7) and Gas6 levels across different aortic arch calcification score (AACCS) groups in maintenance hemodialysis (MHD) patients. (A) Serum vitamin K2 levels (nmol/L) showed a significant stepwise decrease with increasing AACCS severity (no calcification, mild, moderate, severe); (B) Serum Gas6 levels (ng/mL) showed a significant stepwise increase with increasing AACCS severity. Data are presented as mean \pm SD. * $P < 0.05$ compared to the no-calcification group; # $P < 0.05$ compared to the mild calcification group; $\Delta P < 0.05$ compared to the moderate calcification group. Abbreviations: AACCS, aortic arch calcification score; K2, vitamin K2 (menaquinone-7); Gas6, growth arrest-specific protein 6; SD, standard deviation; n.s., not significant (detailed P -values are provided in Table 4).

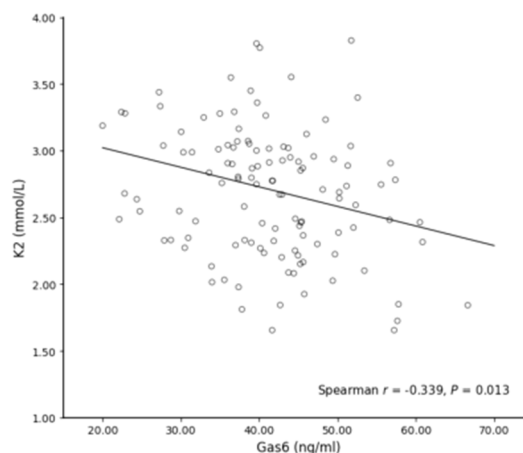


Fig. 3: Correlation between serum K2 and Gas6 levels.

Table 1: Serum vitamin K and gas6 levels.

Index	Control (n=40)	MHD (n=189)	P value
LVEF (%)	52.35±7.38	61.27±7.19	<0.05
LVMi (g/m ²)	95.28±10.16	121.44±14.38	<0.05
LVPWT (mm)	8.11±0.72	10.94±1.23	<0.05
E/A value	1.24±0.42	0.86±0.29	<0.05
K2 (nmol/L)	4.21±0.55	2.34±0.56	<0.05
Gas6 (ng/ml)	43.21±6.77	20.11±3.45	<0.05
MGP (pmol/L)	209.34±24.55	530.18±17.69	<0.05
Vascular calcification score	0.45±0.11	5.02±0.22	<0.05

Table 2: Correlation between serum vitamin, Gas6 and ultrasound cardiogram indexes.

Index	Gas6 (ng/ml)		K2 (nmol/L)	
	R value	P value	R value	P value
LVEF (%)	0.22	>0.05	0.19	>0.05
LVMi (g/m ²)	0.17	>0.05	0.18	>0.05
LVPWT (mm)	0.21	>0.05	0.22	>0.05
E/A value	0.23	>0.05	0.28	>0.05

Table 3: Comparison of indices between left ventricular hypertrophy and non-hypertrophy MHD patients.

Index	Non-LVH (n=79)	LVH (n=110)	P value
Age (years)	60.88±4.23	65.28±4.57	<0.05
1,25(OH)2D3 (ng/ml)	31.23±5.11	32.06±4.09	>0.05
Corrected Ca (mmol/L)	2.31±0.34	2.32±0.28	>0.05
P (mmol/L)	1.71±0.21	1.72±0.27	>0.05
Ca×P (mg ² /dl ²)	50.24±5.24	51.22±6.17	>0.05
Log PTH (ng/L)	2.40±0.34	2.44±0.31	>0.05
ALB (g/L)	40.18±4.16	41.22±4.09	>0.05
Hb (g/L)	104.55±11.21	106.24±12.04	>0.05
TC (mmol/L)	4.24±0.65	4.31±0.77	>0.05
TG (mmol/L)	2.30±0.76	2.34±0.75	>0.05
LDL-c (mmol/L)	2.11±0.54	2.26±0.65	>0.05
CRP (mg/L)	3.26±0.87	3.31±0.76	>0.05
Log BNP (pg/ml)	2.21±0.35	2.64±0.38	<0.05
Gas6 (ng/ml)	19.55±1.76	20.77±3.63	<0.05
K2 (nmol/L)	2.78±0.49	2.03±0.43	<0.05
Use of Phosphate Binders, n (%)	65 (82.28)	94 (85.45)	>0.05
Use of Vitamin D Analogs, n (%)	45 (56.96)	70 (63.64)	>0.05
Use of Calcium Supplements, n (%)	30 (37.97)	50 (45.45)	>0.05

Comparison of indicators across different severity groups of aortic arch calcification (AACS)

Patients were stratified into four groups based on AACS score: no calcification (n=46), mild (n=54), moderate (n=53) and severe calcification (n=36). Intergroup comparisons were performed using analysis of variance (ANOVA), followed by LSD post hoc tests. As shown in Table 4, with increasing severity of AACS, there was a significant stepwise increase in patient age, serum phosphorus, calcium-phosphorus product, glucose (GLU) and serum Gas6 levels (pairwise comparisons, $P < 0.05$), while serum vitamin K2 levels exhibited a significant stepwise decrease (pairwise comparisons, $P < 0.05$). No

significant differences were observed among the groups for serum calcium, PTH, albumin, hemoglobin, blood lipids, or CRP ($P > 0.05$). This trend is visually presented in Fig. 2.

Correlation analysis of serum vitamin K2 and gas6 with other indicators

Spearman correlation analysis (Table 5, Fig. 3) revealed that among MHD patients, serum vitamin K2 levels showed a significant negative correlation with the AACS score ($r = -0.603$, $P < 0.001$) and a significant positive correlation with serum calcium levels ($r=0.412$ (after excluding 3 patients with extreme calcium levels;

Table 4: Indexes among AACS groups of MHD patients.

Index	Non-calcification (n=46)	Mild calcification (n=54)	Moderate calcification (n=53)	Severe calcification (n=36)	P value
Age (Years)	49.37±4.55	64.68±5.19*	68.26±5.14*#	73.11±6.42*#Δ	0.00
Sex	27/19	32/22	30/23	21/15	>0.05
1,25(OH)2D3 (ng/ml)	33.88±4.21	33.34±4.12	32.34±5.01	31.38±4.15	>0.05
P (mmol/L)	1.23±0.29	1.50±0.36*	1.78±0.44*#	2.05±0.53*#Δ	0.00
Corrected Ca (mmol/L)	2.26±0.32	2.31±0.22	2.32±0.33	2.33±0.26	>0.05
Ca×P (mg2/dl2)	33.89±5.22	42.57±6.25*	49.82±6.29*#	55.99±6.24*#Δ	0.00
Log PTH (ng/L)	2.40±0.41	2.35±0.36	2.32±0.41	2.39±0.43	>0.05
ALB (g/L)	40.76±3.27	38.96±4.07	38.29±3.25	39.05±4.01	>0.05
Hb (g/L)	106.92±10.27	104.92±11.02	103.45±10.03	101.77±8.99	>0.05
GLU (mmol/L)	6.08±1.03	7.31±1.15	7.46±1.07	7.93±1.35	0.00
TC (mmol/L)	4.20±0.78	4.25±0.83	4.33±0.92	4.45±1.03	>0.05
TG (mmol/L)	2.09±0.75	2.26±0.73	2.38±0.83	2.49±0.84	>0.05
LDL-c (mmol/L)	2.05±0.54	2.10±0.72	2.25±0.66	2.33±0.69	>0.05
CRP (mg/L)	3.11±0.71	3.19±0.76	3.29±0.74	3.39±0.83	>0.05
Gas6 (ng/ml)	36.48±4.02	40.11±5.02	43.11±6.18	47.83±8.11	0.00
K2 (nmol/L)	2.66±0.341	2.51±0.42*	2.28±0.40*#	1.76±0.32*#Δ	0.00
Use of Phosphate Binders, n (%)	36 (78.26)	45 (83.33)	45 (84.91)	36 (78.26)	>0.05
Use of Vitamin D Analogs, n (%)	25 (54.35)	33 (61.11)	33 (62.26)	24 (66.67)	>0.05
Use of Calcium Supplements, n (%)	16 (34.78)	22 (40.74)	24 (45.28)	18 (50.00)	>0.05

*, p<0.05 comparing to non-calcification group; #, p<0.05 comparing to mild calcification group; Δ, p<0.05 comparing to moderate calcification group.

Table 5: Spearman correlation analysis of serum vitamin K2, Gas6 and clinical parameters in MHD patients.

Parameter	vitamin K2		Gas6	
	r value	P value	r value	P value
AACS Score	-0.603	<0.001	0.564	<0.001
Serum Calcium	0.822*	<0.001	-	-
Gas6	-0.339	<0.05	-	-
CRP	-0.069	0.123	0.188	0.014

*Correlation attenuated to r=0.412 after sensitivity analysis excluding outliers.

Table 6: Analysis for AACS risk factors in MHD patients.

Correlation factor	χ^2	Wald χ^2	P value	OR value	95%CI
Elder age (> 65 years)	0.683	4.782	0.000	1.980	1.125~3.483
Serum K2 (< 2.39 nmol/L)	-0.317	5.473	0.000	0.728	0.572~0.917

sensitivity analysis), P<0.001). Serum Gas6 levels demonstrated a significant positive correlation with the AACS score (r = 0.564, P < 0.001). Furthermore, a significant negative correlation was observed between serum vitamin K2 and Gas6 levels (r = -0.339, P < 0.05). Further analysis indicated that serum CRP levels were weakly positively correlated with Gas6 (r = 0.188, P = 0.014) but showed no significant correlation with vitamin K2 (r = -0.069, P = 0.123).

Multivariate logistic regression analysis of aortic arch calcification

Using the AACS score as the dependent variable

(dichotomized at the median or based on clinical significance), variables with P < 0.25 in the univariate analysis were included in the multivariate logistic regression model. After adjusting for potential confounders such as age, gender, serum phosphorus, PTH, use of phosphate binders and use of vitamin D analogs, the results (Table 6) showed that age >65 years (OR =1.980, 95% CI:1.125-3.483, P<0.05) was an independent risk factor for higher AACS, while serum vitamin K2 level <2.39 nmol/L was associated with a lower risk of severe AACS (OR = 0.728, 95% CI: 0.572-0.917, P<0.05). The model demonstrated good fit according to the Hosmer-Lemeshow test ($\chi^2 = 6.132, P = 0.631$). The area under the ROC curve

(AUC) was 0.829 (95% CI: 0.765–0.881), indicating good discriminative ability of the model.

DISCUSSION

CKD is associated with a relatively high incidence of cardiovascular events, with age being a well-established risk factor for cardiovascular calcification. Arterial calcification itself is a significant contributor to cardiovascular morbidity and mortality. Among MHD patients, the extent of vascular calcification varies and hyperphosphatemia is known to promote its progression. Previous studies have demonstrated a correlation between coronary artery calcification and serum phosphorus levels and the calcium–phosphorus product in MHD patients. Various imaging modalities, including chest X-ray and multislice computed tomography (CT), are used to assess vascular calcification. In particular, the aortic arch calcification score (AACS) derived from chest X-ray has been shown to reliably reflect calcification burden in dialysis patients (Wang *et al.*, 2025, Fanadka *et al.*, 2023). In advanced CKD, hyperphosphatemia is often associated with severe renal dysfunction, Klotho protein deficiency and elevated fibroblast growth factor 23 (FGF23) levels, which collectively impair phosphorus homeostasis. Consequently, hyperphosphatemia is a key risk factor for vascular calcification in patients with CKD–mineral and bone disorder (CKD-MBD) (Li *et al.*, 2023, Ye *et al.*, 2021). Vascular calcification reduces arterial wall compliance, promotes cardiac hypertrophy or dilation and diminishes coronary artery perfusion.

Thus, the severity of arterial calcification serves as an important prognostic indicator in CKD. Vitamin K acts as a cofactor for the post-translational carboxylation of proteins such as matrix Gla protein (MGP), thereby inhibiting vascular calcification—a mechanism demonstrated in warfarin-induced rat models. Gas6, another vitamin K-dependent protein, exerts diverse biological effects through binding to tyrosine kinase receptors (Hariri *et al.*, 2021, Shi *et al.*, 2025). However, few studies have comprehensively examined the relationships among vitamin K, Gas6, vascular calcification and left ventricular hypertrophy in clinical populations. The present study aimed to address this gap.

Our findings indicate that CKD patients with left ventricular hypertrophy were older and had higher levels of B-type natriuretic peptide (BNP), serum creatinine and Gas6, along with lower vitamin K2 levels, compared to those without hypertrophy. No significant difference in serum phosphorus was observed between the two groups. Neither vitamin K2 nor Gas6 showed a significant correlation with echocardiographic parameters. However, serum vitamin K2 was inversely correlated with Gas6. Given that Gas6 is a vitamin K-dependent protein implicated in the inhibition of calcification, vitamin K

deficiency may impair its function, thereby contributing to vascular calcification. In the management of hyperphosphatemia, calcium-based phosphate binders may also bind vitamin K, potentially exacerbating vitamin K deficiency. Vitamin K supplementation could therefore influence the status of vascular calcification. Many MHD patients are vitamin K-deficient and supplementation has been shown to increase carboxylated MGP, an active inhibitor of calcification. Prior research suggests that reduced activity of calcification inhibitors, such as MGP, contributes to vascular calcification in CKD. Vitamin K is essential for activating MGP, and its deficiency may promote calcification by reducing MGP activity, although clinical evidence remains limited (Stepien *et al.*, 2022; Roumeliotis *et al.*, 2022). Consistent with the above, we found no significant association between vitamin K2 and echocardiographic measures. Vitamin K2 was positively correlated with serum calcium and inversely correlated with Gas6. Moreover, higher AACS scores were associated with progressively lower vitamin K2 levels. Multivariate analysis identified older age and low vitamin K2 as independent risk factors for aortic arch calcification, underscoring the potential role of vitamin K deficiency in promoting vascular calcification in MHD patients. This suggests that vitamin K supplementation might be a feasible intervention in this population. Notably, the absence of a significant correlation between vitamin K2 or Gas6 and echocardiographic parameters (LVEF, LVMI, LVPWT, E/A ratio) may reflect the multifactorial pathogenesis of LVH in MHD patients. Factors such as pressure overload, volume overload, anemia, and neurohormonal activation likely dominate over vitamin K-dependent pathways in driving LVH. Future studies with larger sample sizes and longitudinal follow-up are needed to detect potential weak associations.

This study found a positive correlation between Gas6 and AACS, which appears to contradict the traditional understanding of Gas6 as a calcification inhibitor. One possible explanation is that in MHD patients, the elevated Gas6 may represent a compensatory response to progressive vascular calcification. Alternatively, Gas6 might serve as a marker of inflammation or tissue damage, with its increase reflecting the severity of vascular pathology. Furthermore, the Gas6 measured by ELISA may include inactive forms, necessitating further functional studies to verify its biological activity.

This study is limited by its small sample size and cross-sectional design, and lacking long-term follow-up. Therefore, larger-scale studies are needed to explore the relationships between left ventricular hypertrophy and vitamin K or Gas6 in CKD patients, as well as their underlying mechanisms. Dietary phosphorus intake and detailed medical history were not collected, so their potential influence on the results remains unclear. During data analysis, we observed that the strong correlation between vitamin K2 and serum calcium ($r = 0.822$) was largely driven by a few patients w

with extremely high calcium levels. After excluding these three outliers, the correlation coefficient dropped to 0.412. Since sensitivity analyses were not pre-specified, the robustness of this finding may be compromised. In addition, our ELISA method measures total Gas6 levels without distinguishing its biologically active forms. It is possible that some of the detected Gas6 represents inactive or post-translationally modified protein, which might explain why we observed a positive correlation between Gas6 and calcification, contrary to the traditionally proposed protective role. Finally, we only assessed aortic arch calcification on chest X-ray; coronary or peripheral vascular calcification was not evaluated. Whether our conclusions can be generalized to other vascular beds requires further investigation.

CONCLUSION

In MHD patients, serum vitamin K or Gas6 levels do not correlate significantly with left ventricular hypertrophy. Aortic arch calcification was negatively correlated with serum vitamin K levels and positively correlated with serum Gas6 levels. Vitamin K deficiency may play an important role in aortic arch calcification in maintenance hemodialysis patients.

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None.

Authors' contributions

Mingxiao Gan: Conceptualization, methodology, investigation, formal analysis, writing – original draft preparation, data curation and project administration; Jingna Wang: Conceptualization, methodology, validation, resources, writing–review and editing, supervision and corresponding author responsibility.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval

This study was approved by the ethics committee of Ningbo Hospital of Integrated Traditional Chinese and Western Medicine (Approval No.: 2025-029). This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest

The authors declare no conflict of interest.

Supplementary data

<https://www.pjps.pk/uploads/2026/06/SUP1781344052.pdf>

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