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Association of serum phosphate concentration with the incidence of intervention for peripheral artery disease in patients undergoing hemodialysis: 10-year outcomes of the Q-Cohort Study

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HIGHLIGHTS

- The present study is a longitudinal cohort study of 3505 hemodialysis patients.
- Hyperphosphatemia was associated with the risk of major adverse limb events (MALE).
- The competing risk model did not change the effect of hyperphosphatemia on MALE.
- The presence of diabetic nephropathy mitigated the impact of hyperphosphatemia on MALE.

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ABSTRACT

Background and aims: Peripheral artery disease (PAD) is mainly caused by atherosclerosis and is a critical cardiovascular complication in patients undergoing hemodialysis. Although hyperphosphatemia is a risk factor for cardiovascular events, whether serum phosphate concentration is associated with PAD remains unclear. This study was performed to clarify the relationship between serum phosphate concentration and the risk of intervention for PAD in patients undergoing hemodialysis.

Methods: In total, 3505 patients undergoing hemodialysis registered in the Q-Cohort Study were followed up for 10 years. The primary outcome was the incidence of major adverse limb events (MALE) as a surrogate endpoint of intervention for PAD. The patients were divided into quartiles according to the baseline serum phosphate concentration: Q1 (n = 886), < 4.2 mg/dL; Q2 (n = 837), 4.2–4.8 mg/dL; Q3 (n = 909), 4.9–5.6 mg/dL; and Q4 (n = 873), ≥5.7 mg/dL. A multivariable-adjusted Cox proportional hazards risk model was employed to examine the association between the serum phosphate concentration and the risk of MALE.

Results: During a median follow-up period of 8.2 years, 257 patients required intervention with MALE. The Cox proportional hazards risk model showed that the risk of MALE in Q4 was significantly higher than that in Q1 (hazard ratio, 1.81; 95% confidence interval, 1.25–2.63). Every 1-mg/dL increase in serum phosphate concentration was also significantly associated with the increased incidence of MALE (hazard ratio, 1.24; 95% confidence interval, 1.10–1.39).

Conclusions: An elevated serum phosphate concentration was associated with an increased risk of MALE in patients undergoing hemodialysis.

1. Introduction

Peripheral artery disease (PAD) is caused by impaired blood flow in the aorta and branched blood vessels other than the coronary and cerebral arteries. Several disorders may cause PAD, including vasculitis, trauma, anatomical abnormality, dysplasia, and thrombosis. However,

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the most common cause is atherosclerosis. The prevalence of PAD is increasing, and more than 200 million people are estimated to have PAD worldwide [1]. Age, sex, smoking, diabetes mellitus, hypertension, and dyslipidemia are major risk factors for PAD [2–4]. Chronic kidney disease (CKD) also contributes to the pathogenesis of PAD [5].

The risks of cardiovascular morbidity and mortality are higher in patients with CKD and those undergoing hemodialysis. Although the mechanisms of cardiovascular complications associated with CKD are complex, there are at least two types of vascular diseases: atherosclerosis and arteriosclerosis. Atherosclerosis is characterized by intima–media thickening and subsequent plaque formation, while arteriosclerosis is characterized by age-related loss of elastic fibers, increased stiffness, and reduced cushioning function [6]. The pathogenesis of PAD, a critical cardiovascular complication in patients undergoing hemodialysis, is thought to be related to both types. Importantly, PAD often leads to critical limb ischemia, amputation, decreased activities of daily living, and even death. According to one study, the prevalence of PAD in patients undergoing hemodialysis was as high as 25.3% [7]. This highlights the urgent need to identify modifiable risk factors for PAD and decrease the risk of PAD-associated death.

Accumulating evidence has shown that an increased serum phosphate concentration is closely associated with cardiovascular events in patients with CKD and in the general population [8–10]. An elevated serum phosphate concentration is also associated with coronary atherosclerosis and calcification [11]. Elevated phosphate promotes vascular calcification by osteochondrogenic differentiation and apoptosis of vascular smooth muscle cells (VSMCs) [12]. Hyperphosphatemia also causes endothelial dysfunction through increased production of reactive oxygen species [13]. However, whether hyperphosphatemia and phosphate loading play a role in the development and progression of PAD in the CKD population remains unclear.

This study was performed to clarify the relationship between the serum phosphate concentration and the risk of PAD in patients undergoing hemodialysis. For this purpose, we analyzed the data of the Q-Cohort Study, which was a multicenter, longitudinal, observational cohort of patients undergoing maintenance hemodialysis in Japan. In the present study, the incidence of major adverse limb events (MALE) was used as a surrogate endpoint of intervention for PAD because revascularization and amputation in patients with lower extremity PAD have been recently referred to as MALE, and the incidence of MALE has been increasingly used as an outcome in clinical studies [14,15].

2. Materials and methods

2.1. Study population

The Q-Cohort Study was a multicenter, longitudinal, observational study designed to identify risk factors for morbidity and mortality in patients with end-stage kidney disease undergoing hemodialysis in Japan [16–18]. The study population comprised 3598 outpatients aged ≥18 years undergoing hemodialysis in 39 dialysis facilities from December 31, 2006 to December 31, 2007. The patients were followed up until December 31, 2016. Of the 3598 patients, 93 were excluded from our study because of missing data on either baseline characteristics or outcomes. We enrolled the remaining 3505 patients in this study. The present study was performed according to the Ethics of Clinical Research established in the Declaration of Helsinki. The study protocol was approved by the Clinical Research Ethics Committee of the Institutional Review Board at Kyushu University (Approval Number 20–31) and was registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (UMIN ID: 000000556). Written informed consent was obtained from all patients at the start of the study. The ethics committee of all participating institutions granted approval to waive the requirement for written informed consent for the additional follow-up survey from 2011 to 2016 because of the retrospective nature of the study.

2.2. Outcomes and covariates

The primary outcome was the incidence of MALE, including percutaneous or surgical revascularization and amputation.

The main exposure was the serum phosphate concentration at baseline. Potential confounders were age, sex, body mass index (BMI), systolic blood pressure, dialysis vintage (i.e., length of time on dialysis), use of anti-hypertensives, use of phosphate binders, use of vitamin D receptor activators (VDRAs), presence of diabetic nephropathy, history of cardiovascular events, history of stroke, history of bone fracture, and concentrations of blood hemoglobin, serum albumin, albumin-corrected calcium, creatinine, total cholesterol, alkaline phosphatase (ALP), C-reactive protein (CRP), and parathyroid hormone (PTH). Blood samples were collected before starting hemodialysis on the first dialysis day of the week (i.e., Monday or Tuesday). Biochemical parameters were measured at enrollment using the blood that had been collected before starting hemodialysis. Routine biochemical parameters, including the serum concentrations of albumin, creatinine, calcium, phosphate, total cholesterol, ALP, and CRP, were measured at different laboratories using an automated analyzer with standard procedures. The serum concentration of PTH was measured using whole or intact PTH assays. The values measured by the two different assays were converted using the following formula: intact PTH (pg/mL) = 1.7 × whole PTH (pg/mL) [19]. The corrected serum calcium concentration was calculated using the serum albumin concentration based on Payne's formula: corrected serum calcium = serum calcium + (4 − serum albumin) [20]. The target ranges of the serum concentration of corrected calcium, phosphate, and PTH were based on the guideline set by the Japanese Society for Dialysis Therapy (JSDT): calcium, 8.5–10.0 mg/dL; phosphate, 3.5–6.0 mg/dL; and intact PTH, 60–180 pg/mL [19].

2.3. Statistical analysis

Normally distributed continuous variables, non-normally distributed continuous variables, and categorical data are described as mean ± standard deviation, median (interquartile range), and number (percentage), respectively. The patients were divided into quartiles according to their baseline serum phosphate concentration: Q1 (n = 886), < 4.2 mg/dL; Q2 (n = 837), 4.2–4.8 mg/dL; Q3 (n = 909), 4.9–5.6 mg/dL; and Q4 (n = 873), ≥5.7 mg/dL. The distribution of baseline characteristics stratified by serum phosphate quartiles was compared using the following trend analyses: the Cochran–Armitage test was used for categorical variables, and the Jonckheere–Terpstra test was used for continuous variables. The event-free survival rate for MALE according to the serum phosphate quartiles was depicted by the Kaplan–Meier method. Unadjusted and multivariable-adjusted Cox proportional hazards risk models were employed to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the outcome. The assumption of the proportional hazards was tested using analysis of the Schoenfeld residuals. The multivariable-adjusted association of the serum phosphate concentration with the HR and 95% CI for MALE was plotted using spline curves. A Fine–Gray proportional subdistribution hazards model with all-cause death as a competing risk was used to take into account the impact of competing death risk on the association between the serum phosphate concentration and MALE. Furthermore, the patients were divided into three groups using known criteria for the serum phosphate concentration based on the guidelines set by the JSDT or the Kidney Disease Outcomes Quality Initiative (KDOQI) (JSDT, 3.5–6.0 mg/dL; KDOQI, 3.5–5.5 mg/dL) [19,21], and the association between the serum phosphate concentration and the risk of MALE was examined. The multivariable-adjusted models included the following covariates: age, sex, BMI, systolic blood pressure, dialysis vintage, use of anti-hypertensives, use of phosphate binders, use of VDRAs, presence

of diabetic nephropathy, history of cardiovascular events, history of stroke, history of bone fracture, blood hemoglobin concentration, and serum concentrations of albumin, corrected calcium, creatinine, total cholesterol, ALP, CRP, and PTH. A two-tailed p value of < 0.05 was considered statistically significant in all analyses. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R version 3.4.1 (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander (version 2.4-0) that was designed to add statistical functions frequently used in biostatistics [22].

3. Results

3.1. Baseline characteristics according to distribution of serum phosphate concentration

The mean age of the 3505 patients was 63.5 years, and 59.3% were male. The serum phosphate concentration showed a normal distribution (Supplementary Fig. 1). The mean serum phosphate concentration was 4.9 mg/dL. The baseline characteristics of the Q-Cohort Study population stratified by serum phosphate quartiles are shown in Table 1. Patients with a higher serum phosphate concentration were significantly younger and had a higher mean BMI, longer median dialysis vintage, lower prevalence of diabetic nephropathy, lower prevalence of history of both stroke and bone fracture, and higher blood hemoglobin concentration. The serum ALP and CRP concentrations were significantly lower in patients with a higher than lower serum phosphate concentration, whereas the serum albumin, creatinine, total cholesterol, corrected calcium, and PTH concentrations were significantly higher in patients with a higher than lower serum phosphate concentration. Patients with a higher serum phosphate concentration used phosphate binders and VDRAs significantly more frequently.

3.2. Association of serum phosphate concentration with the incidence of MALE

During a median observational period of 8.2 years (interquartile range, 1360–3654 days), 257 (7.3%) patients required intervention with MALE. Among these patients, 63.0% (n = 162) were male and the mean age was 65.5 years. The incidence of MALE in each group according to the serum phosphate quartiles was as follows: 59 in Q1, 61 in Q2, 63 in Q3, and 74 in Q4.

The event-free survival rates for MALE according to the serum phosphate quartiles are shown in Supplementary Fig. 2. A higher serum phosphate concentration was associated with a lower event-free survival rate for MALE in the multivariable-adjusted model.

In the multivariable-adjusted Cox proportional hazards models, patients in the highest serum phosphate quartile (Q4) showed a higher HR for MALE than patients in the lowest serum phosphate quartile (Q1) (Table 2). Furthermore, the HR (95% CI) for a 1-mg/dL increase in the serum phosphate concentration was 1.24 (1.10–1.39). In the multivariable-adjusted spline curve, the HR for MALE increased as the serum phosphate concentration increased (Fig. 1).

During the observation period, the all-cause mortality rate was 49.5% (n = 1735). When the competing risk model with all-cause death as a competing risk was applied to the examined population, a higher serum phosphate concentration was still significantly associated with a higher HR for MALE (Table 3). When the patients were divided into three groups (low, target range, and high) according to the known target ranges for the serum phosphate concentration recommended by the JSDT or KDOQI, hyperphosphatemia was similarly associated with an increased risk of MALE (Supplementary Table 1 and Supplementary Fig. 3).

Table 1
Baseline characteristics stratified by serum phosphate concentration quartiles.

	Serum phosphate concentration quartiles				
	Q1 (n = 886)	Q2 (n = 837)	Q3 (n = 909)	Q4 (n = 873)	p for trend
Serum phosphate concentration, mg/dL	0.9–4.1	4.2–4.8	4.9–5.6	5.7–10.6	
Age, years	66.7 ± 12.6	65.3 ± 12.6	62.4 ± 12.3	59.8 ± 12.8	< 0.001
Male	520 (58.7)	494 (59.0)	545 (60.0)	519 (59.5)	0.66
Presence of diabetic nephropathy	273 (30.8)	273 (32.6)	255 (28.1)	223 (25.5)	0.003
History of cardiovascular events	220 (24.8)	174 (20.8)	212 (23.3)	180 (20.6)	0.11
History of stroke	157 (17.7)	136 (16.2)	140 (15.4)	112 (12.8)	0.005
History of bone fracture	99 (11.2)	93 (11.1)	80 (8.8)	74 (8.5)	0.021
Dialysis vintage, years	4.6 (1.7–10.3)	5.2 (2.1–11.0)	5.8 (2.4–12.2)	6.0 (2.2–11.3)	< 0.001
Body mass index, kg/m^2	20.6 ± 2.9	21.2 ± 3.2	21.2 ± 2.9	21.5 ± 3.3	< 0.001
Systolic blood pressure, mmHg	152 ± 25	154 ± 22	153 ± 23	153 ± 23	0.85
Blood hemoglobin, g/dL	10.3 ± 1.1	10.5 ± 1.1	10.6 ± 1.2	10.7 ± 1.2	< 0.001
Serum albumin, g/dL	3.7 ± 0.5	3.8 ± 0.4	3.9 ± 0.4	3.9 ± 0.4	< 0.001
Serum creatinine, mg/dL	9.0 ± 2.5	10.0 ± 2.5	10.5 ± 2.5	11.3 ± 2.7	< 0.001
Serum total cholesterol, mg/dL	151 (127–175)	153 (131–177)	153 (133–178)	153 (131–181)	0.024
Serum C-reactive protein, mg/dL	0.15 (0.07–0.44)	0.13 (0.06–0.30)	0.13 (0.05–0.30)	0.13 (0.06–0.30)	< 0.001
Albumin-corrected serum Ca, mg/dL	9.3 ± 0.7	9.4 ± 0.7	9.4 ± 0.8	9.5 ± 0.8	< 0.001
Serum alkaline phosphatase, U/L	247 (192–322)	234 (184–311)	227 (176–308)	225 (173–296)	< 0.001
Serum PTH (intact assay), pg/mL	79 (37–148)	106 (54–211)	114 (53–232)	134 (57–277)	< 0.001
Use of anti-hypertensives	530 (59.8)	545 (65.1)	570 (62.7)	550 (63.0)	0.32
Use of phosphate binders	647 (73.0)	688 (82.2)	756 (83.2)	754 (86.4)	< 0.001
Use of Ca-containing phosphate binders	586 (66.1)	586 (70.0)	637 (70.1)	619 (70.9)	0.038
Use of non-Ca-containing phosphate binders	159 (17.9)	210 (25.1)	289 (31.8)	383 (43.9)	< 0.001
Use of VDRAs	578 (65.2)	606 (72.4)	652 (71.7)	619 (70.9)	0.016

Baseline data are expressed as mean ± standard deviation, median (interquartile range), or number (percentage). The Cochran–Armitage test was used to determine p for trend of categorical variables. The Jonckheere–Terpstra trend test was used to determine p for trend of continuous variables. A two-tailed p-value of < 0.05 was considered statistically significant.

Conversion factors for units: phosphate in mg/dL to mmol/L, ×0.323; hemoglobin in g/dL to g/L, ×10; albumin in g/dL to g/L, ×10; creatinine in mg/dL to μmol/L, ×88.4; total cholesterol in mg/dL to mmol/L, ×0.0259; C-reactive protein in mg/dL to nmol/L, ×9.524; albumin-corrected Ca in mg/dL to mmol/L, ×0.25; PTH in pg/mL to ng/L, ×1.

Abbreviations: Ca, calcium; PTH, parathyroid hormone; Q, quartile of serum phosphate concentration; VDRA, vitamin D receptor activator.

Table 2
Hazard ratios for major adverse limb events by the Cox proportional hazards model according to serum phosphate concentration quartiles.

Serum phosphate concentration quartiles	Unadjusted			Age- and sex-adjusted			Multivariable-adjusted		
	HR (95% CI)	*p*-value	*p* for trend	HR (95% CI)	*p*-value	*p* for trend	HR (95% CI)	*p*-value	*p* for trend
Q1	1.00 (reference)			1.00 (reference)			1.00 (reference)		
Q2	1.00 (0.70–1.42)	0.98	0.76	1.04 (0.72–1.48)	0.85	0.096	1.09 (0.75–1.57)	0.65	0.003
Q3	0.90 (0.63–1.28)	0.56		1.03 (0.72–1.48)	0.86		1.15 (0.79–1.66)	0.46	
Q4	1.09 (0.77–1.53)	0.64		1.36 (0.96–1.93)	0.08		1.81 (1.25–2.63)	0.002	
Every 1-mg/dL increase in serum phosphate concentration	1.04 (0.94–1.15)	0.48		1.13 (1.01–1.25)	0.032		1.24 (1.10–1.39)	< 0.001	

Unadjusted and multivariable-adjusted HRs were analyzed by the Cox proportional hazards model. Covariates included age; sex; body mass index; systolic blood pressure; dialysis vintage; use of anti-hypertensives; use of phosphate binders; use of vitamin D receptor activators; presence of diabetic nephropathy; history of cardiovascular events; history of stroke; history of bone fracture; blood hemoglobin concentration; and serum concentrations of albumin, albumin-corrected calcium, creatinine, total cholesterol, alkaline phosphatase, C-reactive protein, and parathyroid hormone. A two-tailed *p*-value of < 0.05 was considered statistically significant.
HR, hazard ratio; CI, confidence interval; Q, quartile of serum phosphate concentration.

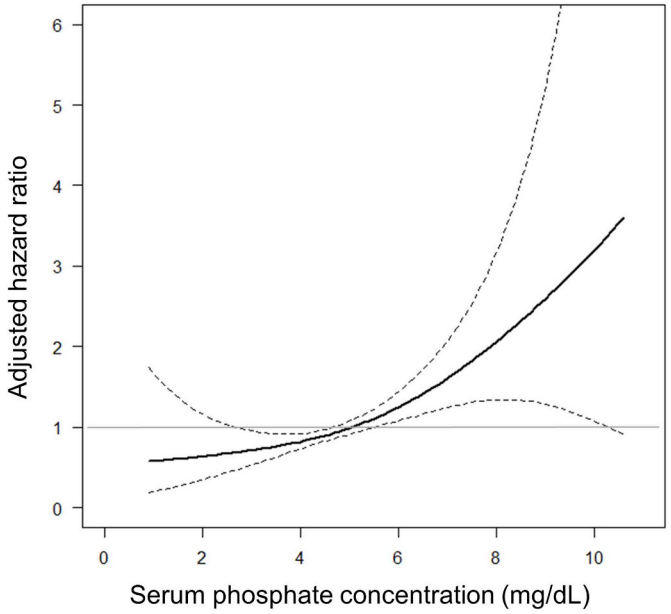

Fig. 1. Multivariable-adjusted spline plots of the HR for MALE according to the serum phosphate concentration.
The solid line represents the adjusted HR, and the dotted line represents the 95% confidence interval. The horizontal gray line corresponds to the normal reference HR of 1.0. The multivariable-adjusted model was adjusted for age, sex, body mass index, systolic blood pressure, dialysis vintage, use of anti-hypertensives, use of phosphate binders, use of vitamin D receptor activators, presence of diabetic nephropathy, history of cardiovascular events, history of stroke, history of bone fracture, blood hemoglobin concentration, and serum concentrations of albumin, albumin-corrected calcium, creatinine, total cholesterol, alkaline phosphatase, C-reactive protein, and parathyroid hormone.
HR, hazard ratio; MALE, major adverse limb events.

3.3. Subgroup analysis stratified by baseline characteristics

Subgroup analyses were performed to assess the consistency of the association between the serum phosphate concentration and the incidence of MALE across a variety of baseline clinical parameters (Fig. 2). A significant interaction was observed between the incidence of MALE and the following baseline clinical parameters. A clearer association between hyperphosphatemia and a higher rate of MALE was observed in patients without than with diabetic nephropathy. Effect modification was observed between the serum phosphate concentration and the following baseline parameters regarding MALE: age, history of cardiovascular events, and serum CRP, ALP, and PTH concentrations. Specifically, the effect of hyperphosphatemia on the risk of MALE was significantly enhanced in patients with an older mean age, higher prevalence of a history of cardiovascular events, and higher serum CRP, ALP, and PTH concentrations. There was no evidence of a significant interaction between the serum phosphate concentration and the other baseline parameters.

4. Discussion

In this longitudinal cohort study of patients undergoing maintenance hemodialysis, we demonstrated that hyperphosphatemia was associated with an increased risk of MALE in the multivariable-adjusted Cox proportional hazards risk model and Fine–Gray proportional subdistribution hazards model with all-cause death set as a competing risk. In the spline curve analysis, the HR abruptly increased when the serum phosphate concentration exceeded the upper limit of 6 mg/dL. Our results suggest that control of the serum phosphate concentration below the upper limit of the target range may be beneficial for the prevention of PAD progression in patients undergoing maintenance hemodialysis.

In a nested case-control study, which included 11 patients with PAD and 22 healthy controls, the multivariable-adjusted logistic regression analysis showed that the serum phosphate concentration was a significant predictor of PAD (odds ratio, 2.4; 95% CI, 1.01–5.74) [23]. Another prospective observational study showed that the risk of hospitalization for new amputation in the group with the highest serum phosphate concentration was significantly higher than that in the group with the serum phosphate concentration in the target range (HR, 1.48; 95% CI, 1.24–1.77) [24]. These findings are consistent with our current observation and support our hypothesis that hyperphosphatemia increases the risk of PAD and MALE in patients undergoing hemodialysis. Considering that our main outcome covered all events from the relatively early events of PAD (such as percutaneous or surgical revascularization) to amputation, the most advanced form of PAD, our data confirmed that phosphate plays critical roles in the multiple stages of PAD progression.

Calciprotein particles (CPPs) are nanoparticles containing calcium phosphate and fetuin-A [25], which systemically increase in response to phosphate loading. Increasing evidence has shown that CPPs are now regarded as inducers of inflammation as well as the true culprit of vascular calcification [26–28]. Actually, CPPs induce the expression and secretion of tumor necrosis factor (TNF)-α and interleukin-1β in macrophages and activate the TNF-α/TNF receptor-1 system in VSMCs [29,30]. Because macrophages recruited to the atherosclerotic plaque play key roles in the progression of atherosclerosis via inflammation and increased oxidative stress [31,32], elevated CPPs induced by

Table 3
Hazard ratios for major adverse limb events by the Fine–Gray proportional subdistribution hazard model according to serum phosphate concentration quartiles.

Serum phosphate concentration quartiles	Unadjusted			Age- and sex-adjusted			Multivariable-adjusted		
	HR (95% CI)	*p*-value	*p* for trend	HR (95% CI)	*p*-value	*p* for trend	HR (95% CI)	*p*-value	*p* for trend
Q1	1.00 (reference)			1.00 (reference)			1.00 (reference)		
Q2	1.09 (0.76–1.56)	0.63	0.20	1.11 (0.78–1.60)	0.56	0.065	1.07 (0.74–1.55)	0.73	0.024
Q3	1.04 (0.73–1.49)	0.83		1.11 (0.78–1.59)	0.56		1.13 (0.78–1.64)	0.52	
Q4	1.28 (0.91–1.81)	0.15		1.43 (1.00–2.04)	0.049		1.57 (1.08–2.23)	0.019	
Every 1-mg/dL increase in serum phosphate concentration	1.09 (0.99–1.21)	0.09		1.13 (1.02–1.26)	0.023		1.17 (1.04–1.31)	0.008	

Unadjusted and multivariable-adjusted HRs were analyzed by the Fine–Gray proportional subdistribution hazards model with all-cause death as a competing risk. Covariates included age; sex; body mass index; systolic blood pressure; dialysis vintage; use of anti-hypertensives; use of phosphate binders; use of vitamin D receptor activators; presence of diabetic nephropathy; history of cardiovascular events; history of stroke; history of bone fracture; blood hemoglobin concentration; and serum concentrations of albumin, albumin-corrected calcium, creatinine, total cholesterol, alkaline phosphatase, C-reactive protein, and parathyroid hormone. A two-tailed *p*-value of < 0.05 was considered statistically significant.
HR, hazard ratio; CI, confidence interval; Q, quartile of serum phosphate concentration.

phosphate loading may cause inflammation in the atherosclerotic plaque, thereby accelerating the progression of PAD in hyperphosphatemic patients undergoing hemodialysis.

Several studies have shown that phosphate is also involved in endothelial dysfunction [13,33]. Phosphate has direct toxic effects on endothelial cells, including induction of apoptosis, increased reactive oxygen species production, and reduced nitric oxide production [34]. In an experiment using uremic apolipoprotein E-deficient mice, reducing phosphate with phosphate binders suppressed the progression of aortic plaque lesions that had developed following endothelial dysfunction [35]. In the first *in vivo* study of a direct effect of phosphate on endothelial dysfunction, high dietary phosphate loading significantly reduced flow-mediated dilation [13]. Previous clinical studies also revealed that endothelial dysfunction was associated with future cardiovascular events in patients with coronary artery disease [36,37]. Considering that endothelial dysfunction greatly contributes to the pathobiology of atherosclerotic cardiovascular disease by dysregulated thrombosis and induction of the inflammatory response in the arterial wall [38], our data suggest that hyperphosphatemia-induced endothelial dysfunction may accelerate the progression of PAD in patients undergoing hemodialysis.

Control of the serum phosphate concentration within an optimal range has been a cornerstone treatment to reduce the risk of cardiovascular morbidity and mortality in patients undergoing hemodialysis. In patients undergoing hemodialysis, phosphate overload is often managed by aggressive phosphate removal through sufficient hemodialysis, reduction in the total amount of intestinal phosphate absorption through dietary therapy and use of phosphate binders, and control of phosphate efflux from bone through management of secondary hyperparathyroidism. Despite the use of combinations of these treatment modalities, management of serum phosphate remains unacceptable, and many patients undergoing hemodialysis are at increased risk of phosphate-induced cardiovascular toxicity. Hence, novel approaches to reduce the phosphate burden, including new pharmaceutical therapies, are mandatory to further reduce the risk of phosphate-induced PAD progression in patients undergoing hemodialysis.

Our subgroup analysis revealed significant interactions between the serum phosphate concentration and several of the baseline parameters. Diabetes mellitus is known as a strong predictor of PAD. A diabetic foot lesion is caused by a combination of microangiopathy, atherosclerosis, neuropathy, and immune depression. These abnormalities may cause the foot lesion to progress rapidly, and the lesion is thus likely to be treated before the long-term effects of hyperphosphatemia on PAD become overt. Therefore, the effect of hyperphosphatemia on the progression of PAD may be blunted in patients with diabetes. Inflammation is another risk factor for PAD [2]. Because the vascular toxicity of phosphate is partially exerted by inflammation [34], inflammation and hyperphosphatemia may work synergistically to cause PAD progression in patients with higher serum CRP concentrations. ALP and PTH are associated with bone metabolism, and elevation of ALP and PTH indicates enhanced bone turnover. When bone turnover is enhanced, increased release of calcium and phosphate from bone may enhance the formation of circulating CPPs and accelerate the progression of PAD in patients with higher ALP and PTH concentrations. Patients of advanced age and patients with a history of cardiovascular events may accumulate risk factors for atherosclerosis and arteriosclerosis, thereby intensifying the impact of hyperphosphatemia on PAD progression. To confirm our hypotheses, further studies are necessary to elucidate the mechanisms underlying the interaction between an increasing serum phosphate concentration and the clinical factors identified in the current study.

Our study has two main strengths. First, this is the first longitudinal cohort study to demonstrate the association of serum phosphate with an increased risk of MALE, including percutaneous or surgical revascularization as well as amputation, in patients undergoing maintenance hemodialysis. Second, our results showed that the serum phosphate concentration was associated with the incidence of MALE independently of traditional risk factors by applying conventional and advanced statistical methods.

Our study also had several limitations. First, the blood test data, including the serum phosphate concentration, were obtained at only one time point (baseline), and serial changes in the serum phosphate concentration were not considered. In this regard, when the time-average model or time-varying model was applied, some of the patients might have been misclassified and the associations revealed in this study might have been weakened. Second, although an elevated serum low-density lipoprotein cholesterol level and hypertriglyceridemia are strong risk factors for atherosclerotic diseases [39], we had data only on total cholesterol, not low-density lipoprotein cholesterol or triglycerides. Hence, we were unable to assess the impact of dyslipidemia on the association between the serum phosphate concentration and MALE. In addition, we were unable to consider the impact of treatments for dyslipidemia, such as statins. Third, we did not measure the baseline ankle-brachial pressure index, which is useful for the diagnosis and detection of PAD. Accordingly, our outcome did not include asymptomatic PAD that would be detected by the ankle-brachial pressure index. Fourth, our data did not contain information on the presence or absence of PAD at baseline; therefore, whether PAD was present or absent and whether an intervention for PAD was required at the time of enrollment remain unclear. Fifth, our data did not include the number of patients transferred for kidney transplantation, which could be a potentially competing risk. Therefore, the impact of cases that were censored

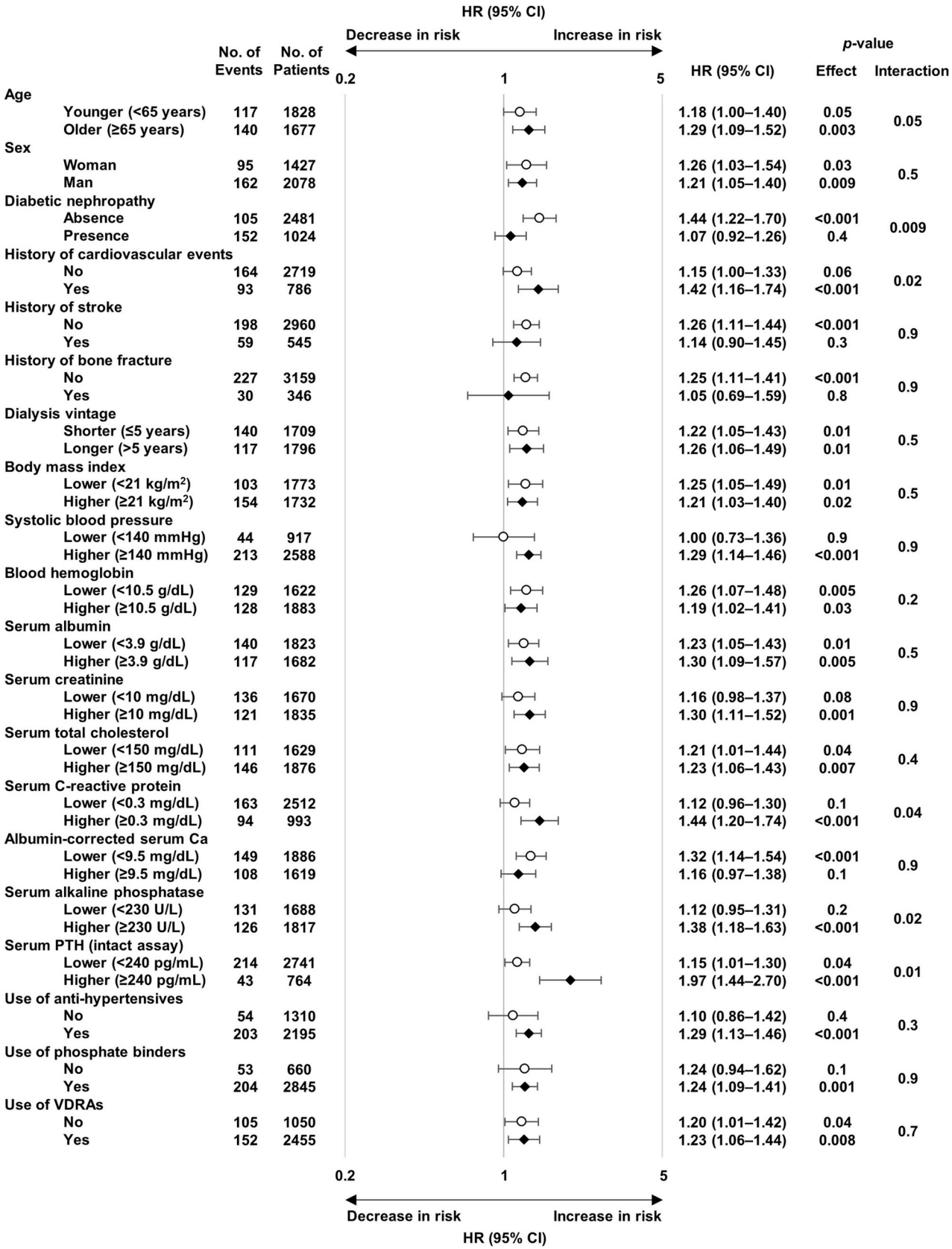

Fig. 2. Multivariable-adjusted HRs and 95% CIs for MALE by each 1-mg/dL increase in the serum phosphate concentration in subgroups of baseline characteristics. Open circles and black rhombi indicate the point estimate of the HRs, and the error bars represent the 95% CI. The multivariable-adjusted model was adjusted for age, sex, body mass index, systolic blood pressure, dialysis vintage, use of anti-hypertensives, use of phosphate binders, use of VDRAs, presence of diabetic nephropathy, history of cardiovascular events, history of stroke, history of bone fracture, blood hemoglobin concentration, and serum concentrations of albumin, albumin-corrected Ca, creatinine, total cholesterol, alkaline phosphatase, C-reactive protein, and PTH. Variables relevant to the subgroups were excluded from each model. A two-tailed p value of < 0.05 was considered statistically significant. HR, hazard ratio; CI, confidence interval; MALE, major adverse limb events; Ca, calcium; PTH, parathyroid hormone; VDRA, vitamin D receptor activator.

because of transfer for kidney transplantation could not be considered using the competing risk model. Sixth, because of the nature of observational studies, we could not prove causality from our current observations. Finally, known and unknown residual confounders that were not included in our study could lead our present observation to a null hypothesis.

In conclusion, we have demonstrated that a high serum phosphate concentration is closely associated with a higher risk of MALE in patients undergoing maintenance hemodialysis. Our results suggest that maintaining the serum phosphate concentration in the optimal range by combination of sufficient dialysis, dietary therapy, use of phosphate binders, and control of secondary hyperparathyroidism may reduce the risk of the development of PAD in the hemodialysis population. Further clinical studies are necessary to determine whether lowering the serum phosphate concentration can retard the progression of PAD in patients undergoing hemodialysis.

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CRediT authorship contribution statement

Sho Shimamoto: Conceptualization, Methodology, Visualization, Formal analysis, Writing - original draft. **Shunsuke Yamada:** Conceptualization, Formal analysis, Validation, Writing - review & editing. **Hiroto Hiyamuta:** Investigation, Data curation. **Hokuto Arase:** Writing - review & editing. **Masatomo Taniguchi:** Resources, Writing - review & editing. **Masanori Tokumoto:** Writing - review & editing. **Toshiaki Nakano:** Project administration, Writing - review & editing. **Kazuhiko Tsuruya:** Project administration. **Takanari Kitazono:** Supervision.

Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

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