

Serum phosphate optimal timing and range associated with patients survival in haemodialysis: the COSMOS study

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ABSTRACT

Background. Serum phosphate is a key parameter in the management of chronic kidney disease-mineral and bone disorder (CKD-MBD). The timing of phosphate measurement is not standardized in the current guidelines. Since the optimal range of these biomarkers may vary depending on the duration of the interdialytic interval, in this analysis of the Current management of secondary hyperparathyroidism: a multicentre observational study (COSMOS), we assessed the influence of a 2- (mid-week) or 3-day (post-weekend) dialysis interval for blood withdrawal on serum levels of CKD-MBD biomarkers and their association with mortality risk.

Methods. The COSMOS cohort (6797 patients, CKD Stage 5D) was divided into two groups depending upon midweek or post-weekend blood collection. Univariate and multivariate Cox's models adjusted hazard ratios (HRs) by demographics and comorbidities, treatments and biochemical parameters from a patient/centre database collected at baseline and every 6 months for 3 years.

Results. There were no differences in serum calcium or parathyroid hormone levels between midweek and post-weekend patients. However, in post-weekend patients, the mean serum phosphate levels were higher compared with midweek patients (5.5 ± 1.4 versus 5.2 ± 1.4 mg/dL, $P < 0.001$). Also, the range of serum phosphate with the lowest mortality risk [$HR \leq 1.1$; midweek: 3.5–4.9 mg/dL (95% confidence interval, CI: 2.9–5.2 mg/dL); post-weekend: 3.8–5.7 mg/dL (95% CI: 3.0–6.4 mg/dL)] showed significant differences in the upper limit ($P = 0.021$).

Conclusion. Midweek and post-weekend serum phosphate levels and their target ranges associated with the lowest mortality risk differ. Thus, clinical guidelines should consider the timing of blood withdrawal when recommending optimal target ranges for serum phosphate and therapeutic strategies for phosphate control.

Keywords: calcaemia, chronic haemodialysis, epidemiology, hyperparathyroidism, phosphataemia

ADDITIONAL CONTENT

An author video to accompany this article is available at: https://academic.oup.com/ndt/pages/author_videos.

INTRODUCTION

Serum levels of calcium, phosphate and parathyroid hormone (PTH) are the main parameters used for the diagnosis and treatment of abnormalities of bone and mineral metabolism associated with the progression of chronic kidney disease-mineral and bone disorder (CKD-MBD).

Kidney Disease Outcomes Quality Initiative (KDOQI) [1] and Kidney Disease: Improving Global Outcomes (KDIGO) [2–4] guidelines recommend monitoring serum calcium and phosphate levels every 1–3 months and serum PTH levels at least every 3–6 months in CKD Stage 5D patients and to maintain serum levels within recommended ranges without considering the timing of blood sampling. Recent international efforts have been directed to improve current recommendations regarding reference levels for serum calcium, phosphate and PTH in haemodialysis. In fact, two large observational studies, Dialysis Outcomes and Practice Patterns Study (DOPPS), analysing a 10-year follow-up database in 22 000 patients from 12 countries, and Current management of secondary hyperparathyroidism: a multicentre observational study (COSMOS), examining the dialysis practices in 6797 patients from 227 centres from 20 European countries with a 3-year follow-up, have provided important new information regarding serum levels of calcium, phosphate and PTH, which are associated with the lowest risk of mortality [5, 6].

Most patients undergo haemodialysis three times per week, and according to the practice of each dialysis centre, blood withdrawal takes place after either 2 (midweek) or 3 (post-weekend) days after the last dialysis. A study in a cohort of 100 haemodialysis patients showed significantly higher serum phosphate levels in post-weekend compared with midweek measurements in the same patient [7]. Since serum phosphate may vary depending on the duration of the interdialytic interval, the identification of optimal ranges for midweek and post-weekend sampling remains an important open question for clinical practice.

To this end, the present study reanalyses the COSMOS database to assess whether serum calcium, PTH and phosphate levels are affected by the duration of the interdialytic interval prior to blood collection and, more importantly, whether midweek and post-weekend measurements may influence the optimal ranges associated with the lowest risk of all-cause mortality.

MATERIALS AND METHODS

COSMOS is a 3-year, multicentre, open cohort, prospective, observational study carried out in 227 dialysis facilities randomly selected from 20 European countries including data from chronic adult haemodialysis patients. Each facility was required to recruit 20 patients randomly, totalling to 4500 patients. In addition, 2297 new patients on haemodialysis for <1 year were recruited to replace those patients leaving the study for any reason, making a total of 6797 patients. Recruitment began in February 2005, and the study ended in July 2010. Additional detailed information on the study and data collection has already

been published [6, 8, 9]. The institutional review boards of the participating centres approved the study, and patients provided informed consent for study participation. The research was conducted according to principles of the Declaration of Helsinki.

At baseline and every 6 months, a patient-specific form collected 27 itemized variables (185 items), including demographics, comorbidities, treatments, monthly laboratory values for serum PTH, phosphate, calcium, albumin and haemoglobin, and also the average value of the previous 6 months for each of these parameters was calculated. Every centre also completed a centre-specific form (15 variables and 119 items) including site characteristics (type, funding and size), clinical practice patterns for the management of CKD-MBD, midweek or post-weekend blood withdrawal and the assay used for intact or bio-intact PTH measurements.

In this analysis, patients were divided into two groups according to midweek (47%) or post-weekend (53%) blood collection. These percentages remained similar across the 3-year follow-up (midweek patients ranged from 46.3% to 48.1% and post-weekend patients from 51.9% to 53.7%).

Serum PTH values were multiplied by a correcting factor of 1.95 for centres using the Bio-intact PTH assay (3.5%) [9–11] or converted to the Allegro-intact PTH (Nichols Institute, San Clemente, CA, USA) according to the correcting factors reported by Souberbielle *et al.* [12] for centres using intact PTH measurements (79.3%) or remained uncorrected in the remaining 20.7% of centres not reporting the PTH assay used [9].

Statistical analysis

Results were expressed as mean and SD, median and interquartile range or percentage of patients. Comparisons between midweek and post-weekend groups were assessed using Student's *t*-test for continuous variables and Chi-squared test for categorical variables. Nonparametric test (Mann–Whitney *U* test) was used for serum PTH with a non-normal distribution.

Kernel density estimation was used to estimate the probability density function of serum phosphate and PTH in midweek and post-weekend patients. Forward stepwise regression was used to adjust for potential confounders including the 22 variables used in the Cox analyses described below. The subgroup effect was assessed by analysing both the differences in serum phosphate in the patients' subgroups and also the interaction between each variable and the interdialytic interval.

Cox proportional hazard regression analysis with time-dependent variables was used to assess the association between all-cause mortality and serum phosphate in midweek and post-weekend patients. Serum phosphate was introduced in the Cox models as a continuous time-dependent variable and fitted using penalized spline smoothing [6]. Univariate and three progressive multivariate models were used. Model 1 adjusted for demographic characteristics and comorbidities (10 variables): age, sex, body mass index (BMI), smoking habit, vintage, vascular or valvular calcification, aetiology of CKD, diabetes, cardiovascular disease history and parathyroidectomy. Model 2 included the variables of Model 1 plus treatments (eight variables): dialysis type, calcium concentration in the dialysate, hours of haemodialysis per week, treatment with erythropoietin-stimulating agents, prescription of active vitamin D

Table 1. Baseline patients' characteristics

	All patients	Midweek	Post-weekend	P-value ^a	SMD
Sex (male), <i>n</i> (%)	6679 (60.5)	3139 (60.3)	3540 (60.7)	0.8	0.008
Age (years), <i>n</i> (mean $\pm$ SD)	6679 (64.1 $\pm$ 14.4)	3139 (63.6 $\pm$ 14.6)	3540 (64.5 $\pm$ 14.2)	0.016	0.059
BMI (kg/m ²), <i>n</i> (mean $\pm$ SD)	6673 (25.4 $\pm$ 5.1)	3138 (25.3 $\pm$ 5.2)	3535 (25.4 $\pm$ 4.9)	0.3	0.026
Current smokers (%)	6674 (13.8)	3139 (12.9)	3535 (14.5)	0.07	0.045
Diabetes mellitus, <i>n</i> (%)	6678 (31.1)	3139 (29.3)	3539 (32.6)	0.004	0.071
History of cardiovascular disease, <i>n</i> (%)	6675 (71.7)	3139 (71.8)	3536 (71.7)	1.0	0.002
Parathyroidectomy, <i>n</i> (%)	6679 (4.8)	3139 (4.1)	3540 (5.5)	0.011	0.064
Vintage (months), <i>n</i> (mean $\pm$ SD)	6679 (38.2 $\pm$ 49.0)	3139 (39.0 $\pm$ 50.4)	3540 (37.5 $\pm$ 47.8)	0.2	0.030
Vintage ($\leq$ 1 year), <i>n</i> (%)	2736 (41.0)	1288 (41.0)	1448 (40.9)	0.9	0.003
Hours of dialysis per week, <i>n</i> (mean $\pm$ SD)	6679 (12.0 $\pm$ 2.1)	3139 (11.9 $\pm$ 2.1)	3540 (12.1 $\pm$ 2.2)	<0.001	0.093
Type of haemodialysis, <i>n</i>	6678	3138	3540	<0.001	0.149
Conventional low flux (%)	53.5		49.8		56.8
Conventional high flux (%)	37.4		41.0		34.1
Hemodiafiltration and other (%)	9.1		9.2		9.1
Calcium conc. in the dialysate (mEq/L), <i>n</i>	6004	2733	3271	<0.001	0.209
2.5 (%)	29.6		28.4		30.6
3.0 (%)	51.4		48.1		54.1
3.5 (%)	19.1		23.5		15.3
Prescription of phosphate binders, <i>n</i> (%)	6678 (84.9)	3139 (84.8)	3539 (85.0)	0.9	0.004
Prescription of active vitamin D, <i>n</i> (%)	6676 (47.7)	3137 (45.0)	3539 (50.1)	<0.001	0.101
Prescription of calcimimetics, <i>n</i> (%)	6629 (6.2)	3091 (6.8)	3538 (5.8)	0.1	0.042
Prescription of ESAs, <i>n</i> (%)	6511 (90.8)	2989 (90.7)	3512 (90.8)	0.9	0.003

Values in parentheses indicate mean $\pm$ SD or percentage of patients.

^aStudent's *t*-test for continuous variables and Chi-square test for categorical variables were used.

n, number of patients; SMD, standardized mean difference; ESAs, erythropoiesis-stimulating agents.

metabolites/analogues (calcitriol, alfacalcidol or paricalcitol), native vitamin D or calcidol, phosphate-binding agents (PBAs) (calcium-containing PBAs, sevelamer, aluminium-containing PBAs, lanthanum carbonate or other PBAs) and calcimimetics. Model 3 (full model) included all previous variables plus four biochemical parameters: haemoglobin, albumin, calcium and PTH. Variables included in Models 2 and 3 as well as BMI and parathyroidectomy in Model 1 were used as time-varying covariates. All multivariate models were stratified by centre.

The serum phosphate with the minimum log (hazard ratio, HR) within either midweek or post-weekend populations was used as reference (HR = 1.0). In addition, the lowest mortality range was calculated as the range of serum phosphate with an HR $\leq$ 1.1 ($\leq$ 10% increase in the relative risk of mortality). Also, complementary sensitivity analyses were done using different cut-off values from 5% to 25%. Furthermore, a naive bootstrapping technique was used to check the equality between the parameters mentioned earlier (P-value was approximated from 5000 iterations). Additional analyses categorized serum phosphate levels as concentrations below, within or above the range of serum phosphate with the lowest mortality. Cox regression analyses were conducted for serum phosphate below and above the lower and upper limits of the reference range for midweek or post-weekend patient populations.

All statistical analyses were done using R Statistical Software version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

After exclusion of patients with missing values of CKD-MBD biochemical parameters, the final analysis was carried out in

6679 patients, which represents 98.3% of the COSMOS cohort. Blood collection to monitor biochemical parameters were performed midweek in 3139 (47%) patients and post-weekend in 3540 (53%) patients. The number and percentage of centres using midweek and post-weekend policy are shown in [Supplementary data](#), Table S1.

Table 1 shows baseline patients characteristics. In the post-weekend group, patients were older, with a higher proportion of diabetics, history of parathyroidectomy and prescription of active vitamin D. Groups also differed significantly in the duration and modality of dialysis and in dialysate calcium. Standardized differences were small (<0.2) for all the variables described in **Table 1** except for the calcium concentration in the dialysate, with a medium standardized difference (<0.5) [13].

Post-weekend patients showed mean serum phosphate levels 0.3 mg/dL higher than midweek patients and also probability density curve shifted to the right (**Table 2**; **Figure 1** left panel). Serum calcium did not differ between midweek and post-weekend patients. In post-weekend patients, the median serum PTH levels were significantly lower (4 pg/mL) compared with midweek patients, with similar probability density curves of log-transformed serum PTH (**Figure 1** right panel). Both the groups showed similar serum haemoglobin, and although serum albumin differed significantly, the observed average difference was only 0.1 g/dL.

Table 3 shows that the higher serum phosphate in post-weekend patients persisted among 43 subgroups of patients when categorized by 18 variables (demography, comorbidities, treatments and biochemical parameters). Only the prescription of calcimimetics showed a strong interaction with the interdialytic interval when used as predictors for serum phosphate levels.

Table 2. Biochemical parameters in midweek and post-weekend patients

	All patients	Midweek patients	Post-weekend patients	P-value ^a
Serum phosphate (mg/dL), <i>n</i> (mean ± SD)	6679 (5.4 ± 1.4)	3139 (5.2 ± 1.4)	3540 (5.5 ± 1.4)	<0.001
Serum PTH (pg/mL), <i>n</i> [median (IQR)]	6374 [200.1 (102.5–369.2)]	2952 [203.0 (103.5–384.6)]	3422 [199.0 (101.7–351.5)]	0.047 ^b
Serum Ca (mg/dL), <i>n</i> (mean ± SD)	6432 (9.1 ± 0.7)	3002 (9.1 ± 0.8)	3340 (9.1 ± 0.7)	0.5
Haemoglobin (g/dL), <i>n</i> (mean ± SD)	6597 (11.4 ± 1.4)	3084 (11.4 ± 1.4)	3513 (11.5 ± 1.3)	0.2
Serum albumin (g/dL), <i>n</i> (mean ± SD)	6009 (3.8 ± 0.5)	2896 (3.7 ± 0.5)	3113 (3.8 ± 0.4)	<0.001

Values are expressed as mean ± SD or median (lower and upper limits of IQR) according to normal and non-normal distribution, respectively. Conversion factors for units: serum phosphate in mg/dL to mmol/L, 0.323; serum PTH in pg/mL to pmol/L, 0.106; serum calcium in mg/dL to mmol/L, 0.2495; serum albumin from g/dL to g/L, 10 and haemoglobin in g/dL to g/L, 10.

^aStudent's *t*-test.

^bMann–Whitney *U* test.

n, number of patients; IQR, interquartile range.

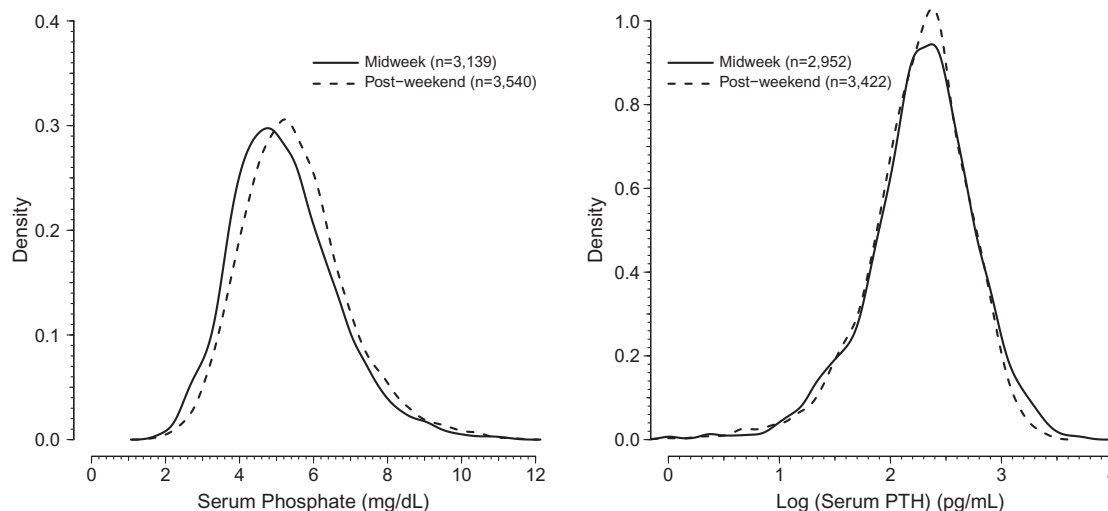


FIGURE 1: Density plot for serum phosphate and PTH. Kernel density estimation for serum phosphate (left, *n* = 6679) and log-transformed serum PTH (right, *n* = 6374) in midweek and post-weekend patients. The number of patients is shown in parenthesis represented as '*n*'. Conversion factors for units: serum phosphate in mg/dL to mmol/L, 0.323 and serum PTH in pg/mL to pmol/L, 0.106.

A multivariate analysis confirmed that the duration of the interdialytic period prior to blood collection remained associated with serum phosphate after adjustment for all other potential confounders ($P < 0.001$).

The post-weekend group showed a significantly higher percentage of patients with serum phosphate above the upper limit of KDOQI and COSMOS targets (Figure 2) and above any other serum phosphate level that may be used as upper limit of normal ranges (KDIGO) (Supplementary data, Figure S1).

After exclusion of patients with no follow-up data ($n = 388$), the analysis of the association between serum phosphate and mortality was carried out in 6291 patients (2913 midweek and 3378 post-weekend). Crude all-cause mortality was 13.3 deaths per 100 patient-years (13.4 midweek and 13.2 post-weekend). The curves for the relative risk of mortality versus serum phosphate for midweek and post-weekend patients were different (Figure 3). The serum phosphate values with the minimum log (HR) (lowest mortality rate, HR = 1.0) were 4.2 and 4.7 mg/dL for midweek and post-weekend patients, respectively (Figure 3 middle panel). The ranges for serum phosphate associated with the lowest mortality (optimal ranges, $\leq 10\%$ increase in the relative risk of mortality, HR ≤ 1.1) were 3.5–4.9 mg/dL [95% confidence interval (95% CI): 2.9–5.2 mg/dL] for midweek and 3.8–5.7 mg/dL (95%

CI: 3.0–6.4 mg/dL) for post-weekend blood collection. Cut-off levels for 5–25% increases in HR are shown in Supplementary data, Figure S2. The upper limit of the serum phosphate range associated with the lowest mortality risk for midweek blood collection (4.9 mg/dL) was lower than the post-weekend one (5.7 mg/dL) in 4897 of the 5000 (97.9%) bootstrap iterations ($P = 0.021$). There were no differences for the lower limit.

Additional analyses comparing the relative risk of mortality for midweek and post-weekend values for serum phosphate within the fourth quintile (5.5 to 6.3 mg/dL, depicted in grey in the upper central panel of Figure 3) showed that midweek measurements were associated with a higher risk of mortality compared with post-weekend measurements [HR = 1.35 (95% CI: 1.01–1.82)].

Accordingly, Table 4 shows that serum phosphate levels below the lower or above the upper limits of their respective lowest mortality ranges in both groups of patients were associated with a higher relative risk of mortality. However, while the percentage of patients with serum phosphate below the lower limit of the optimal range was similar between the two groups (8.4% and 10.0%), the percentage of patients with serum phosphate above the optimal upper limits were different. Furthermore, the midweek group showed a higher percentage of patients above

Table 3. Serum phosphate in different subgroups of midweek and post-weekend patients

	Midweek		Post-weekend		P-value ^a	P-value for interaction ^b
	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD		
Sex						0.045
Males	1894	5.2 $\pm$ 1.4	2150	5.5 $\pm$ 1.4	<0.001	
Females	1245	5.3 $\pm$ 1.4	1390	5.5 $\pm$ 1.4	<0.001	
Age (years)						0.9
<65	1446	5.6 $\pm$ 1.5	1488	5.9 $\pm$ 1.5	<0.001	
$\geq$ 65	1639	4.9 $\pm$ 1.2	2052	5.2 $\pm$ 1.3	<0.001	
BMI (kg/m ²)						0.1
<25	1699	5.1 $\pm$ 1.4	1845	5.3 $\pm$ 1.4	<0.001	
$\geq$ 25	1439	5.3 $\pm$ 1.4	1690	5.7 $\pm$ 1.4	<0.001	
Diabetes						0.5
No	2218	5.2 $\pm$ 1.4	2385	5.5 $\pm$ 1.5	<0.001	
Yes	921	5.1 $\pm$ 1.3	1154	5.5 $\pm$ 1.3	<0.001	
History of cardiovascular disease						0.9
No	886	5.3 $\pm$ 1.4	1001	5.6 $\pm$ 1.4	<0.001	
Yes	2253	5.2 $\pm$ 1.4	2535	5.4 $\pm$ 1.4	<0.001	
Parathyroidectomy						0.2
No	3010	5.2 $\pm$ 1.4	3346	5.5 $\pm$ 1.4	<0.001	
Yes	129	5.4 $\pm$ 1.5	194	5.5 $\pm$ 1.6	0.7	
Vintage (months)						0.2
<19	1542	5.3 $\pm$ 1.4	1729	5.5 $\pm$ 1.4	<0.001	
$\geq$ 19	1597	5.1 $\pm$ 1.4	1811	5.5 $\pm$ 1.4	<0.001	
Hours of HD per week						0.2
$\leq$ 12	2499	5.2 $\pm$ 1.4	2502	5.4 $\pm$ 1.4	<0.001	
>12	640	5.4 $\pm$ 1.5	1038	5.7 $\pm$ 1.4	<0.001	
Type of dialysis						
Conventional low flux	1562	5.3 $\pm$ 1.4	2010	5.5 $\pm$ 1.4	<0.001	
Conventional high flux	1288	5.2 $\pm$ 1.4	1207	5.6 $\pm$ 1.4	<0.001	0.011
Haemodiafiltration and Other	288	5.1 $\pm$ 1.3	323	5.3 $\pm$ 1.4	0.09	0.8
Calcium in the dialysate (mEq/L)						
2.5	775	5.4 $\pm$ 1.4	1001	5.8 $\pm$ 1.4	<0.001	
3.0	1315	5.1 $\pm$ 1.3	1769	5.4 $\pm$ 1.4	<0.001	0.4
3.5	643	5.2 $\pm$ 1.5	501	5.3 $\pm$ 1.4	0.3	0.022
Prescription of PBAs						0.8
No	476	4.5 $\pm$ 1.3	532	4.8 $\pm$ 1.3	0.002	
Yes	2663	5.3 $\pm$ 1.4	3007	5.6 $\pm$ 1.4	<0.001	
Prescription of VDRA						0.8
No	1725	5.2 $\pm$ 1.4	1767	5.5 $\pm$ 1.4	<0.001	
Yes	1412	5.3 $\pm$ 1.4	1772	5.5 $\pm$ 1.4	<0.001	
Prescription of calcimimetics						0.007
No	2881	5.2 $\pm$ 1.4	3334	5.5 $\pm$ 1.4	<0.001	
Yes	210	5.2 $\pm$ 1.4	204	5.9 $\pm$ 1.5	<0.001	
Prescription of ESAs						0.7
No	277	5.1 $\pm$ 1.4	323	5.3 $\pm$ 1.4	0.051	
Yes	2712	5.2 $\pm$ 1.4	3199	5.5 $\pm$ 1.4	<0.001	
Serum PTH (pg/mL)						0.3
<200	1456	5.0 $\pm$ 1.3	1717	5.3 $\pm$ 1.3	<0.001	
$\geq$ 200	1496	5.4 $\pm$ 1.4	1705	5.7 $\pm$ 1.4	<0.001	
Serum calcium (mg/dL)						0.4
<9.1	1537	5.2 $\pm$ 1.4	1720	5.5 $\pm$ 1.5	<0.001	
$\geq$ 9.1	1465	5.2 $\pm$ 1.4	1710	5.5 $\pm$ 1.4	<0.001	
Haemoglobin (g/dL)						0.042
<11.5	1526	5.3 $\pm$ 1.5	1713	5.5 $\pm$ 1.4	<0.001	
$\geq$ 11.5	1558	5.1 $\pm$ 1.3	1800	5.5 $\pm$ 1.4	<0.001	
Serum albumin (g/dL)						0.2
<3.8	1437	5.1 $\pm$ 1.4	1386	5.3 $\pm$ 1.3	<0.001	
$\geq$ 3.8	1459	5.3 $\pm$ 1.4	1727	5.6 $\pm$ 1.4	<0.001	

Values are expressed as mean $\pm$ SD. Conversion factors for units: serum phosphate in mg/dL to mmol/L, 0.323; serum PTH in pg/mL to pmol/L, 0.106; serum calcium in mg/dL to mmol/L, 0.2495; serum albumin from g/dL to g/L, 10 and haemoglobin in g/dL to g/L, 10.

^aStudent's *t*-test.

^bInteraction between each variable and interdialytic interval as predictors of serum phosphate.

VDRA, vitamin D receptor activators; ESAs, erythropoiesis-stimulating agents.

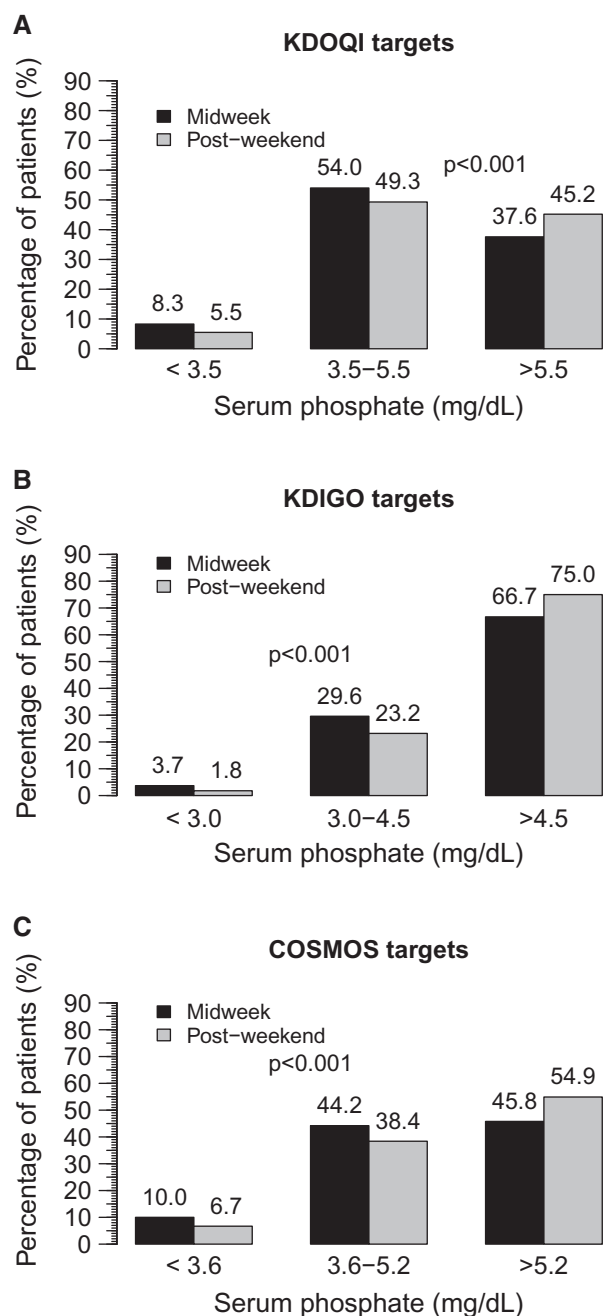


FIGURE 2: Percentage of patients within different KDOQI, KDIGO and COSMOS targets for serum phosphate according to the time of blood collection. Serum phosphate was categorized as below, within and above the recommended targets from KDOQI and KDIGO guidelines and COSMOS targets. Bars depict the percentage of patients within each guideline category for midweek and post-weekend blood collections. To convert serum phosphate in mg/dL to mmol/L, multiply by 0.323.

the optimal upper target and also a higher HR compared with the post-weekend group (55.1% versus 39.7% and HR of 1.42 versus 1.24, respectively).

DISCUSSION

This analysis of the COSMOS study has shown that midweek and post-weekend patients differ significantly not only in their

mean serum phosphate levels but also in their respective ranges for serum phosphate associated with the lowest risk for all-cause mortality (Figure 3), indicating that the time of blood withdrawal should be taken into account when recommending the optimal and safest serum phosphate targets in haemodialysis patients.

Among all CKD-MBD serum biomarkers examined in midweek and post-weekend patients, only mean serum phosphate levels were distinctly higher after the longer interdialytic interval. In fact, there were no significant differences between the groups in serum calcium or haemoglobin levels and the minimal significant differences observed in serum levels of PTH (4 pg/mL) and albumin (0.1 g/dL) are clinically irrelevant.

Phosphate is a recognized uraemic toxin [14, 15] with a limited removal by dialysis [16–18], because only a small percentage of the phosphate body burden is distributed in the extracellular space [19]. Therefore, the higher mean serum phosphate in post-weekend blood collections, in which there is an extra day for phosphate accumulation, was not surprising.

The difference between post-weekend and midweek serum phosphate levels of observed herein (0.3 mg/dL) is similar to that reported by Sigrist *et al.* in a study specifically designed to evaluate the impact of the interdialytic period on serum phosphate, as midweek and post-weekend measurements were conducted in the same patient [7]. In addition, a similar study by Yokoyama *et al.*, measuring serum phosphate midweek and post-weekend in the same hyperphosphataemic patient (>5.6 mg/dL), corroborated a significantly higher post-weekend serum phosphate [20]. In contrast, Tentori *et al.* [5] failed to find significant differences in serum phosphate between samples collected on Monday/Tuesday (post-weekend) versus Wednesday/Thursday (midweek) in a study that was not designed to address this key issue. The COSMOS difference of 0.3 mg/dL between midweek and post-weekend serum phosphate is clinically relevant, as differences of a similar magnitude have been used to evaluate the efficacy of interventions with phosphate binders [21].

Importantly, a higher serum phosphate in post-weekend patients persisted after patient stratification and multivariate adjustment, reflecting the expected time-dependent phosphate accumulation in body fluids due to a longer interdialytic period.

A previous COSMOS study reported serum phosphate concentrations between 3.6 and 5.2 mg/dL as the optimal and safest range [6]. This subanalysis of the COSMOS database, discriminating measurements from midweek and post-weekend blood collection strategies, clearly shows that the percentage of patients having a serum phosphate above the desired KDOQI, KDIGO or COSMOS targets was always higher for post-weekend blood collections (Figure 2 and Supplementary data, Figure S1).

It is well established that high levels of serum phosphate associate with a higher risk of mortality in worldwide studies [5, 22], in multinational regional studies from North America [23, 24], Latin America [25], Europe [6, 26] and also in individual country studies from The Netherlands [27], Ireland [28], France [29], Sweden [30] and Japan [31]. Accordingly, a critical clinical contribution of this COSMOS subanalysis has been the identification, for the first time, of distinct optimal ranges for serum phosphate associated with the minimum relative risk of mortality for midweek or post-weekend blood collection. Indeed, the serum phosphate associated with the lowest

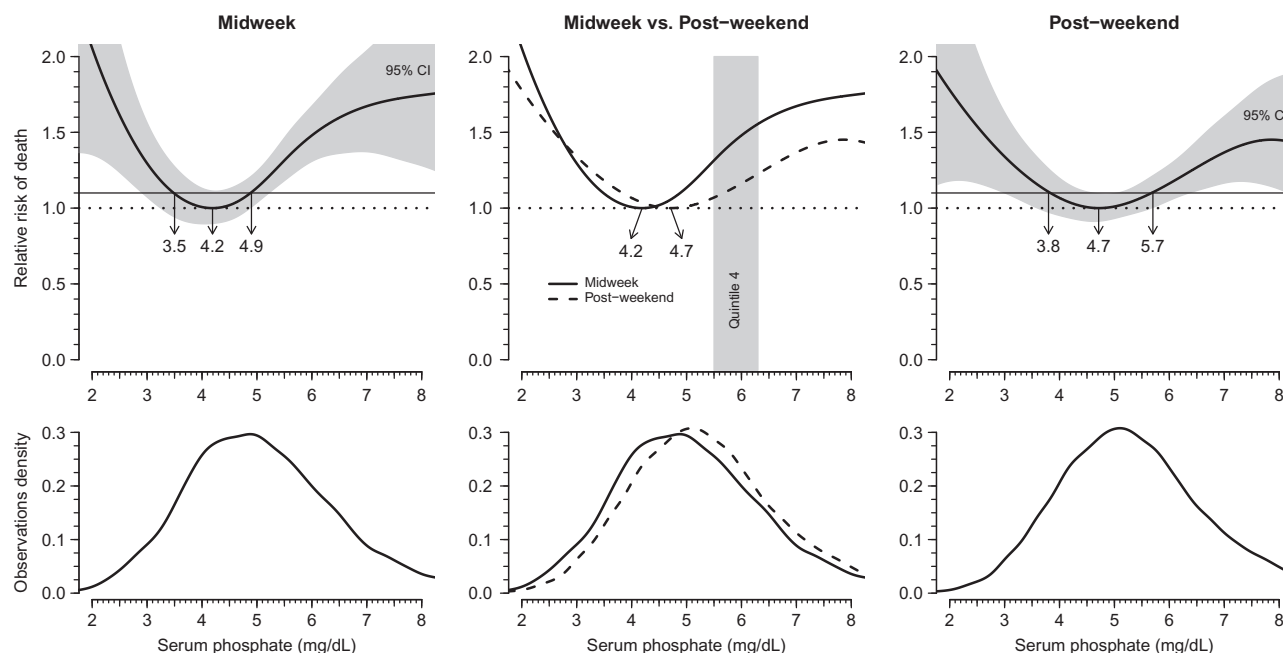


FIGURE 3: Association between all-cause mortality and serum phosphate in midweek and post-weekend patients ($n = 6291$, 2913 midweek and 3378 post-weekend). The top middle panel shows the comparison between midweek (left panel top) and post-weekend patients (right panel top) when using the full adjusted model. The bottom graphs show the Kernel density estimation of observations density. Arrows show the serum phosphate with the minimum log(HR) ($HR = 1.0$) and the lowest mortality ranges ($<10\%$ increase in the relative risk of mortality, $HR = 1.1$). HR was adjusted by age, sex, BMI, smoking habit, time on haemodialysis, aetiology of CKD, diabetes, cardiovascular disease history, parathyroidectomy, dialysis type*, calcium concentration in the dialysate*, hours of haemodialysis per week*, treatment with erythropoietin-stimulating agents (ESAs)*, prescription of vitamin D metabolites/analogues (calcitriol, alfacalcidol or paricalcitol)*, native vitamin D or calcidol*, phosphate-binding agents (calcium-containing PBAs, sevelamer, aluminium-containing PBAs, lanthanum carbonate or other PBAs)*, calcimimetics*, haemoglobin*, albumin*, calcium* and PTH*. Asterisks represents time-varying covariates. Multivariate model was stratified by centre. The grey box in the middle panel shows the fourth quintile of serum phosphate. To convert serum phosphate to mmol/L, multiply by 0.323.

Table 4. Relative risk of mortality according to serum phosphate

Midweek			
Serum phosphate categories (mg/dL)	<3.5	3.5–4.9	>4.9
No. of patients (%)	244 (8.4)	1063 (36.5)	1606 (55.1)
Univariate	1.95 (1.59–2.38)	1.0	1.06 (0.90–1.24)
Model 1	1.88 (1.51–2.34)	1.0	1.27 (1.07–1.51)
Model 2	1.62 (1.28–2.04)	1.0	1.31 (1.09–1.57)
Model 3	1.30 (1.01–1.69)	1.0	1.42 (1.17–1.74)
Post-weekend			
Serum phosphate categories (mg/dL)	<3.8	3.8–5.7	>5.7
No. of patients (%)	338 (10.0)	1700 (50.3)	1340 (39.7)
Univariate	2.00 (1.69–2.38)	1.0	0.90 (0.77–1.05)
Model 1	1.82 (1.51–2.19)	1.0	1.15 (0.97–1.36)
Model 2	1.65 (1.35–2.00)	1.0	1.25 (1.05–1.48)
Model 3	1.34 (1.09–1.66)	1.0	1.24 (1.03–1.49)

The different categories according to the lowest mortality ranges were obtained from Figure 3 considering an $HR \leq 1.1$ ($\leq 10\%$ increase in the relative risk of mortality). In the univariate and multivariate models, values indicate the HRs and 95% CI. Multivariate analyses were stratified by centre. To convert serum phosphate in mg/dL to mmol/L, multiply by 0.323.

mortality risk was 4.2 mg/dL for midweek and 4.7 mg/dL for post-weekend patients (Figure 3 upper panel). The safest and optimal serum phosphorus target ranges resulting after applying to these values a $\leq 10\%$ increase in the relative risk of mortality ($HR \leq 1.1$) were 3.5–4.9 and 3.8–5.7 mg/dL, respectively, with different but also clinically meaningful differences in the amplitude of the target ranges (Figure 3 upper panel and Figure 4). In midweek patients, the amplitude of the range was 1.4 mg/dL

whereas in post-weekend patients it was 1.9 mg/dL. Moreover, the upper limit of the serum phosphate range associated with the minimum risk of mortality was higher by 0.8 mg/dL in post-weekend blood samples (4.9 mg/dL midweek and 5.7 mg/dL post-weekend, $P = 0.021$), exceeding the maximum serum phosphate target values proposed in the current guidelines and in COSMOS [1–4, 6]. Instead, the 4.9 mg/dL optimal upper limit for midweek measurements resulted lower than prior KDOQI

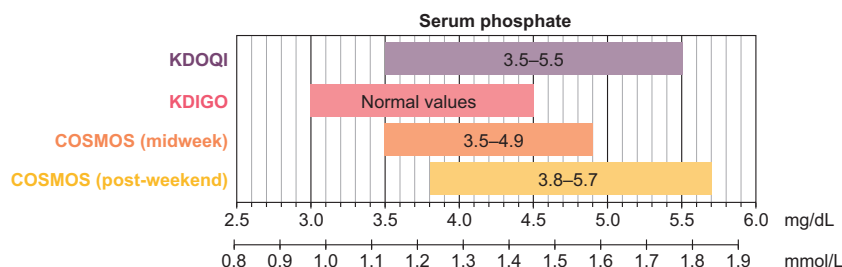


FIGURE 4: Comparison of KDOQI [1] and KDIGO [2] recommended targets and COSMOS lowest mortality ranges (midweek and post-weekend).

and COSMOS targets, thereby leaving 55% of midweek patients above the desired phosphate target compared with only 39% of post-weekend patients not achieving optimal target levels (Table 4). This finding may have relevant clinical implications, as midweek patients also presented a higher HR of 42% compared with the HR of 24% in post-weekend patients. Further support for harder outcomes in midweek patients came from their significantly higher risk of mortality compared with post-week patients for serum phosphate levels within the fourth quintile (5.5–6.3 mg/dL), depicted in Figure 3 (grey bar, central upper panel).

Clearly, the recommendation of a single range of serum phosphate regardless of the timing for blood sampling after the last dialysis may be insufficient as a target reference value and not accurate enough for the clinical practice.

These findings also pose an important consideration regarding the pathophysiological significance of a higher optimal upper limit for post-weekend patients compared with prior recommendations, but a significantly lower one in the case of midweek blood withdrawal associated with an adverse impact survival outcome. A potential explanation is that the higher post-weekend serum phosphate reflects the levels achieved in the worst scenario: the longest interval of the three weekly inter-dialytic periods, responsible for an exposure to a significant 0.3 mg/dL higher serum phosphorus. However, it is important to stress that this high exposure happens only 1 day per week. On the contrary, midweek serum phosphate reflects the exposure to serum phosphate occurring 6 days per week. Thus, mid-week serum phosphate may be a better marker of the abnormalities in the mechanisms regulating mean serum phosphorus levels and the resulting exposure during 6 out of the 7 days of the week in dialysis patients.

The main limitations of the present analysis are that COSMOS is an observational study and that the comparisons between midweek and post-weekend measurements were not performed in the same patients. Additional limitations are that serum biochemical parameters were obtained from medical records, not measured in a central laboratory. The use of midweek or post-weekend policy was heterogeneous across participating countries. Despite multivariate adjustments, centres may be geographically clustered resulting in residual confounding and bias. In addition, serum phosphate may also be influenced by diurnal rhythm or fasting conditions at withdrawal, which unfortunately were not collected.

The strength of COSMOS is the random selection of centres and patients proportional to the haemodialysis population of each of the 20 participating countries, which allowed us to study

a large European representative sample (6797 patients) during a long follow-up period of 3 years. In addition, the facility-level analysis carried out minimizes the effect of unknown or un-measured confounders due to the fact that midweek or post-weekend blood withdrawal was a characteristic of the centre and not of the patient.

In summary, the timing of blood withdrawal influences not only serum phosphate levels but also the association between serum phosphate levels and the optimal ranges with the lowest mortality risk. Among them, the upper limits of these ranges are of paramount clinical importance, as they are currently used to guide the therapeutic management of hyperphosphataemia. Although both optimal limits associate with the lowest risk of mortality, midweek serum phosphate could better reflect the prolonged weekly exposure to an abnormally high serum in haemodialysis patients.

According to these results, it is advisable that in the future clinical guidelines consider the timing of blood withdrawal when recommending optimal target ranges for serum phosphate. Undoubtedly, the use of different serum phosphorus target ranges for midweek and post-weekend blood withdrawal strategies should have a positive practical impact in the current clinical practice.

For COSMOS participating centres, see [Supplementary Appendix 1](#).

SUPPLEMENTARY DATA

[Supplementary data](#) are available at [ndt online](#).

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CONFLICT OF INTEREST STATEMENT

None declared.

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