SUPPLEMENTAL MATERIAL

High oxalate concentrations increase risk for sudden cardiac death in dialysis patients

Table of Contents.

Supplemental Figure S1. Oxalate concentration and its correlation with residual kidney function in 104 US patients with kidney failure requiring chronic dialysis (US cohort).

Supplemental Figure S2. Risk of (**A**) all-cause mortality, (**B**) death due to heart failure, (**C**) myocardial infarction, and (**D**) stroke as a function of continuous oxalate exposure in the 4D Study (restricted splines).

Supplemental Figure S3. Kaplan-Meier curves for overall survival in 104 US patients with kidney failure requiring chronic dialysis (US cohort) comparing (**A**) the highest oxalate quartile vs. quartiles 1-3 and (**B**) the highest oxalate quintile vs. quintiles 1-4.

Supplemental cohort (US cohort)

Study design and participants

Preliminary results underlying the 4D Study were obtained from a cross-sectional study in the US with plasma oxalate concentration as primary outcome variable. For this study, 104 patients, aged ≥18 years, were recruited from four outpatient dialysis centers in Connecticut. Participants received either thrice weekly hemodialysis (HD) for 3-5 hours per treatment session or daily home peritoneal dialysis (PD). Patients eligible for this study had to be (1) on HD or PD treatment and (2) medically stable with no infections or hospitalizations for a minimum of 3 months. HD patients had to have (3) an access blood flow of ≥250 mL/min and (4) a dialysate flow of ≥500 mL/min. Patients with an established diagnosis of primary or secondary hyperoxaluria at the time of recruitment were excluded from the study. Between April and September 2016, one-time blood samples were collected from all patients to measure plasma oxalate concentrations. Local authorities (Western Institutional Review

Board Study No. 1162867) approved the study and all patients gave their written informed consent before inclusion.

Data collection

Clinical and supplementary laboratory data were collected from electronic health records and complemented by reports from the treating physician. Residual kidney function was assessed in both HD and PD patients via self-report, and defined as urine output > 300 mL/min. Single pool Kt/V (spKt/V) was calculated using the second generation Daugirdas formula.[1] Mean spKt/V was calculated from three previous HD treatments. In PD patients, Kt/V (peritoneal and urinary) were obtained as single measurements every three months.
For explorative analysis, mortality data were collected in November 2018.

Sample handling and oxalate measurement

Oxalate in HD and PD patients was measured in EDTA plasma samples obtained prior to dialysis therapy at the first appointment after a long interval and at the monthly clinic appointment, respectively**.** Samples were immediately put on ice, centrifuged, deproteinized, acidified, aliquoted, and stored at -80°C within 2 hours, as previously reported.[2] The frozen and acidified filtrate was thawed, and oxalate was measured enzymatically using oxalate oxidase (Trinity Biotech; Bray, Co. Wicklow, Ireland).[2, 3] To evaluate the validity of one-time sampling, we collected repeat blood samples from 11 HD patients (weekly over 2-4 consecutive weeks) and 13 PD patients (monthly over 2-4 consecutive months). The intraindividual variability (median CV) was 9.4% in the HD and 16.7% in the PD group.

Statistical analyses

We calculated means (SDs) or medians (interquartile ranges [IQRs]) for continuous and frequency tables for categorical variables. Clinical determinants of oxalate were determined by Spearman correlation and regression modeling. After a follow-up of 2.5 years, we analyzed survival stratified by oxalate concentration below or above the highest quartile or quintile, respectively, using the Kaplan–Meier method and Cox regression. P-values are two-sided. Statistical analyses were conducted using IBM SPSS Statistics, Version 25 (IBM Corp; Armonk, NY, USA).

References

1. Daugirdas JT: Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *Journal of the American Society of Nephrology : JASN,* 4**:** 1205-1213, 1993

2. Ermer T, Kopp C, Asplin JR, Granja I, Perazella MA, Reichel M, et al.: Impact of Regular or Extended Hemodialysis and Hemodialfiltration on Plasma Oxalate Concentrations in Patients With End-Stage Renal Disease. *Kidney international reports,* 2**:** 1050-1058, 2017 10.1016/j.ekir.2017.06.002

3. Ladwig PM, Liedtke RR, Larson TS, Lieske JC: Sensitive spectrophotometric assay for plasma oxalate. *Clinical chemistry,* 51**:** 2377-2380, 2005 10.1373/clinchem.2005.054353

Supplemental Table S1. Characteristics of 104 US patients with kidney failure requiring chronic dialysis (US cohort).

Characteristics	All patients (n=104)	Patients with oxalate levels in the highest quartile (n=26)	Patients with oxalate levels in the highest quintile (n=21)
Oxalate [µM]*	23.8 (10.3) range: <2-59.6	34.5 (9) range: 28.4-59.6	35.1 (7.4) range: 31.8-59.6
Deceased within observation period, No.	15	6	6
Age [years]	65.9 (14.6)	63.8 (16.8)	66.7 (15.9)
Male, No. (%)	53 (51)	17 (65.4%)	14 (66.7)
Race/ethnicity , No. (%)			
White	47 (45.2)	15 (57.7)	12 (57.1)
Black	51 (49)	10 (38.5)	9 (42.9)
Hispanic	4 (3.8)	1 (3.8)	0
Other	2 (2)	0	0
History of kidney stones, No. (%)	3 (2.8)	2 (7.7)	1 (4.8)
Dialysis mode, No. (%)			
HD	81 (77.9)	21 (80.8)	18 (85.7)
PD	23 (22.1)	5 (19.2)	3 (14.3)
Weight [kg]	89.1 (25.2)	94.6 (28.2)	95.1 (28.6)
BMI [kg/m^2]	31.4 (8.3)	33.1 (9.2)	33.8 (9.6)
Kt/V HD patients	1.7 (0.33)	1.5 (0.32)	1.5 (0.34
Kt/V PD patients	1.6 (0.53)	2.1 (0.32)	2.1 (0.32)
Time on dialysis [months]*	41.5 (52)	42 (56)	42 (57)

Urine output, No. (%)			
Diuresis > 300 mL/day	28 (26.9)	2 (7.7)	1 (4.8)
Diuresis < 300 mL/day	63 (60.6)	23 (88.5)	19 (90.5)
Not defined	13 (12.5)	1 (3.8)	1 (4.8)
Dialysis access, No. (%)			
Tunneled catheter	8 (7.7)	2 (7.7)	2 (9.5)
AV fistula	61 (58.7)	14 (53.9)	11 (52.4)
AV graft	10 (9.6)	4 (15.4)	4 (19)
Tenckhoff catheter	23 (22.1)	5 (19.2)	3 (14.3)
Not documented	2 (1.9)	1 (3.8)	1 (4.8)

HD: hemodialysis; PD: peritoneal dialysis; BMI: body mass index, calculated as weight in kilograms divided by height in meters squared; AV: arteriovenous. In HD patients, Kt/V was calculated from three previous HD treatments using the second generation Daugirdas formula.[1] In PD patients, Kt/V was obtained as single measurement every three months. For analysis, just peritoneal (not urinary) Kt/V was used. Continuous variables are expressed as mean (SD) or median (IQR)* where appropriate, categorical variables as No. (%).

Supplemental Table S2. Correlation between oxalate concentration and clinical parameters in 104 US patients with kidney failure requiring chronic dialysis (US cohort).[a]

	Oxalate concentration	
Parameter	r	P
Age	0.02	0.87
BMI	0.05	0.61
Kt/V	0.04	0.70
Time on dialysis	0.30	0.002

[a] Spearman´s rank correlation. R: correlation coefficient. BMI: body mass index, calculated as weight in kilograms divided by height in meters squared. In HD patients, Kt/V was calculated from three previous HD treatments using the second generation Daugirdas formula.[1] In PD patients, Kt/V was obtained as single measurement every three months. For analysis, only peritoneal (not urinary) Kt/V was used.

Supplemental Table S3. Multivariable regression of oxalate concentration, age, BMI, Kt/V, time on dialysis, and residual kidney function in 104 US patients with kidney failure requiring chronic dialysis (US cohort).[a]

	Oxalate concentration	
Covariates	r	P
Age	0.06	0.63
BMI	0.12	0.44
Kt/V	-0.20	0.15
Ln(time on dialysis)	0.29	0.01
Residual kidney function	-0.36	0.002

[a] Dependent variable: oxalate concentration; covariates: age, BMI, Kt/V, time on dialysis, and residual kidney function; time on dialysis was transformed to its natural logarithm to obtain normally distributed values; categorical variable: residual kidney function, defined as urine output >300 mL/day. BMI: body mass index, calculated as weight in kilograms divided by height in meters squared. In HD patients, Kt/V was calculated from three previous HD treatments using the second generation Daugirdas formula.[1] In PD patients, Kt/V was obtained as single measurement every three months. For analysis, only peritoneal (not urinary) Kt/V was used.

Supplemental Table S4. Adjustment models used in the 4D Study.

Model	Variables
Model 1	Age, sex, use of atorvastatin, time on HD, and use of diuretics
Model 2 (core model)	Model 1 + C-reactive protein (CRP), BMI, hemoglobin, albumin, and previous coronary artery disease
Model 3 (mediating model)	Model 2 + NT-proBNP

Supplemental Table S5. Risk of all-cause mortality, combined cardiovascular events, sudden cardiac death, death due to heart failure, myocardial infarction, and stroke, using cause-specific hazards models, stratified by quartiles of oxalate concentration at baseline (4D Study, n=1108).

	HRs stratified by oxalate quartiles at baseline				
Outcome	**Quartile 1 ≤29.6 µM n=274**	**Quartile 2 29.7-42.3 µM n=279**	**Quartile 3 42.4-59.6 µM n=274**	**Quartile 4 ≥59.7 µM n=281**	**Global P-value**
All-cause mortality					
Crude HR (95% CI)	1	1.01 (0.78-1.30)	1.02 (0.81-1.29)	1.06 (0.83-1.35)	0.63
Adjusted[a] HR (95% CI)	1	1.06 (0.81-1.39)	1.11 (0.88-1.42)	1.17 (0.92-1.50)	0.19
Adjusted[b] HR (95% CI)	1	1.05 (0.80-1.38)	1.16 (0.91-1.47)	1.23 (0.97-1.56)	0.08
Cardiovascular events[c]					
Crude HR (95% CI)	1	0.90 (0.67-1.20)	0.92 (0.70-1.20)	1.25 (0.98-1.59)	0.07
Adjusted[a] HR (95% CI)	1	0.92 (0.69-1.24)	0.97 (0.74-1.28)	1.33 (1.04-1.72)	0.03
Adjusted[b] HR (95% CI)	1	0.94 (0.70-1.27)	1.01 (0.76-1.33)	1.40 (1.08-1.81)	0.01
Sudden cardiac death					
Crude HR (95% CI)	1	1.18 (0.74-1.88)	1.34 (0.83-2.18)	1.39 (0.91-2.12)	0.13
Adjusted[a] HR (95% CI)	1	1.23 (0.77-1.97)	1.45 (0.88-2.39)	1.50 (0.96-2.34)	0.07
Adjusted [b] HR (95% CI)	1	1.26 (0.76-2.07)	1.50 (0.89-2.55)	1.62 (1.03-2.56)	0.04
Death due to heart failure					
Crude HR (95% CI)	1	1.01 (0.37-2.78)	1.21 (0.46-3.23)	1.77 (0.69-4.55)	0.24
Adjusted[a] HR (95% CI)	1	1.12 (0.40-3.12)	1.48 (0.57-3.87)	2.31 (0.91-5.83)	0.08
Adjusted[b] HR (95% CI)	1	1.07 (0.37-3.07)	1.47 (0.57-3.83)	2.40 (0.99-5.80)	0.05
Myocardial infarction					
Crude HR (95% CI)	1	0.98 (0.64-1.51)	0.88 (0.58-1.35)	1.28 (0.88-1.86)	0.20

Adjusted[a] HR (95% CI)	1	1.00 (0.65-1.54)	0.91 (0.60-1.38)	1.29 (0.88-1.89)	0.19
Adjusted[b] HR (95% CI)	1	1.03 (0.67-1.57)	0.94 (0.62-1.42)	1.34 (0.91-1.98)	0.14
Stroke					
Crude HR (95% CI)	1	0.55 (0.30-1.01)	0.63 (0.36-1.10)	1.08 (0.67-1.73)	0.75
Adjusted[a] HR (95% CI)	1	0.55 (0.30-1.00)	0.64 (0.35-1.15)	1.12 (0.69-1.82)	0.66
Adjusted[b] HR (95% CI)	1	0.54 (0.29-0.99)	0.68 (0.38-1.22)	1.19 (0.71-2.00)	0.50

HR: hazard ratio; 95% CI: 95% confidence interval.

[a] Adjusted HR: adjustments were made for age, sex, use of atorvastatin, time on hemodialysis, and use of diuretics.

[b] Adjusted HR: adjustments were made for age, sex, use of atorvastatin, time on hemodialysis, use of diuretics, C-reactive protein, body mass index, hemoglobin, albumin, and previous coronary artery disease.

[c] Combined cardiovascular events were defined as a composite of death from cardiac causes, fatal or nonfatal stroke, and nonfatal myocardial infarction, whichever occurred first. Death from cardiac causes comprised death due to congestive heart failure, sudden cardiac death, fatal myocardial infarction, death due to coronary artery disease during or within 28 days after an intervention, and all other deaths attributable to coronary artery disease.

Supplemental Table S6. Risk of combined cardiovascular events, sudden cardiac death, death due to heart failure, and the composite outcome of both, myocardial infarction, and stroke, using subdistribution hazards models, stratified by quartiles of oxalate concentration at baseline (4D Study, n=1108) (Fine-Gray competing risk regression).

	HRs stratified by oxalate quartiles at baseline				
Outcome	**Quartile 1 ≤29.6 µM n=274**	**Quartile 2 29.7-42.3 µM n=279**	**Quartile 3 42.4-59.6 µM n=274**	**Quartile 4 ≥59.7 µM n=281**	**Global P-value**
Cardiovascular events[a]					
Crude HR (95% CI)	1	1.03 (0.77-1.39)	1.00 (0.76-1.31)	1.35 (1.04-1.75)	0.02
Adjusted[b] HR (95% CI)	1	1.67 (0.79-1.44)	1.05 (0.79-1.40)	1.46 (1.11-1.92)	0.007
Adjusted[c] HR (95% CI)	1	1.08 (0.79-1.47)	1.06 (0.80-1.42)	1.49 (1.13-1.97)	0.005
Sudden cardiac death					
Crude HR (95% CI)	1	1.53 (0.87-2.68)	1.55 (0.86-2.79)	1.44 (0.86-2.42)	0.17
Adjusted[b] HR (95% CI)	1	1.59 (0.90-2.84)	1.66 (0.91-3.03)	1.52 (0.89-2.59)	0.13
Adjusted[c] HR (95% CI)	1	1.63 (0.89-2.98)	1.64 (0.88-3.05)	1.57 (0.93-2.67)	0.09
Death due to heart failure					
Crude HR (95% CI)	1	0.97 (0.34-2.77)	0.71 (0.23-2.23)	1.51 (0.58-3.96)	0.40
Adjusted[b] HR (95% CI)	1	1.12 (0.39-3.24)	0.92 (0.30-2.87)	2.18 (0.84-5.69)	0.11
Adjusted[c] HR (95% CI)	1	1.02 (0.34-3.03)	0.83 (0.25-2.68)	2.01 (0.81-5.03)	0.13
Sudden cardiac death and death due to heart failure combined[d]					
Crude HR (95% CI)	1	1.38 (0.87-2.21)	1.30 (0.83-2.03)	1.46 (0.93-2.29)	0.10
Adjusted[a] HR (95% CI)	1	1.47 (0.91-2.37)	1.45 (0.92-2.30)	1.68 (1.06-2.67)	0.03
Adjusted[b] HR (95% CI)	1	1.48 (0.89-2.46)	1.40 (0.86-2.26)	1.71 (1.09-2.71)	0.02

Myocardial infarction					
Crude HR (95% CI)	1	1.10 (0.71-1.72)	1.01 (0.66-1.56)	1.41 (0.94-2.12)	0.10
Adjusted[a] HR (95% CI)	1	1.10 (0.70-1.72)	1.00 (0.65-1.53)	1.36 (0.90-2.05)	0.15
Adjusted[b] HR (95% CI)	1	1.12 (0.71-1.75)	1.02 (0.66-1.57)	1.40 (0.92-2.15)	0.12
Stroke					
Crude HR (95% CI)	1	0.67 (0.28-1.59)	0.77 (0.35-1.72)	1.09 (0.59-2.03)	0.78
Adjusted[a] HR (95% CI)	1	0.68 (0.30-1.57)	0.80 (0.36-1.80)	1.16 (0.64-2.08)	0.62
Adjusted[b] HR (95% CI)	1	0.70 (0.31-1.61)	0.83 (0.37-1.86)	1.21 (0.67-2.17)	0.53

HR: hazard ratio; 95% CI: 95% confidence interval.

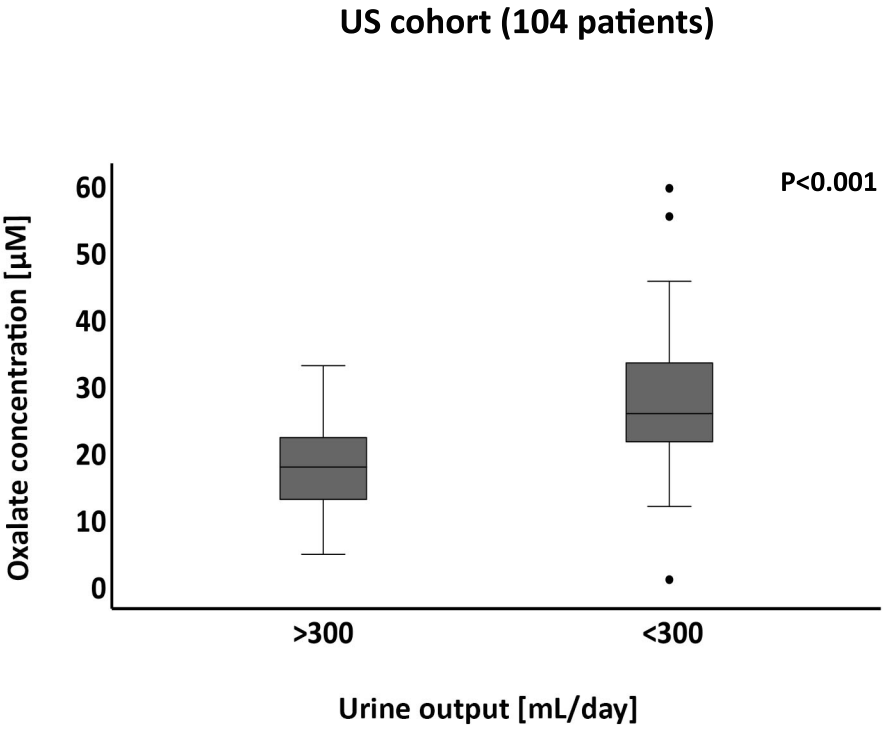
[a] Combined cardiovascular events were defined as a composite of death from cardiac causes, fatal or nonfatal stroke, and nonfatal myocardial infarction, whichever occurred first. Death from cardiac causes comprised death due to congestive heart failure, sudden cardiac death, fatal myocardial infarction, death due to coronary artery disease during or within 28 days after an intervention, and all other deaths attributable to coronary artery disease.

[b] Adjusted HR: adjustments were made for age, sex, use of atorvastatin, time on hemodialysis, and use of diuretics.

[c] Adjusted HR: adjustments were made for age, sex, use of atorvastatin, time on hemodialysis, use of diuretics, C-reactive protein, body mass index, hemoglobin, albumin, and previous coronary artery disease.

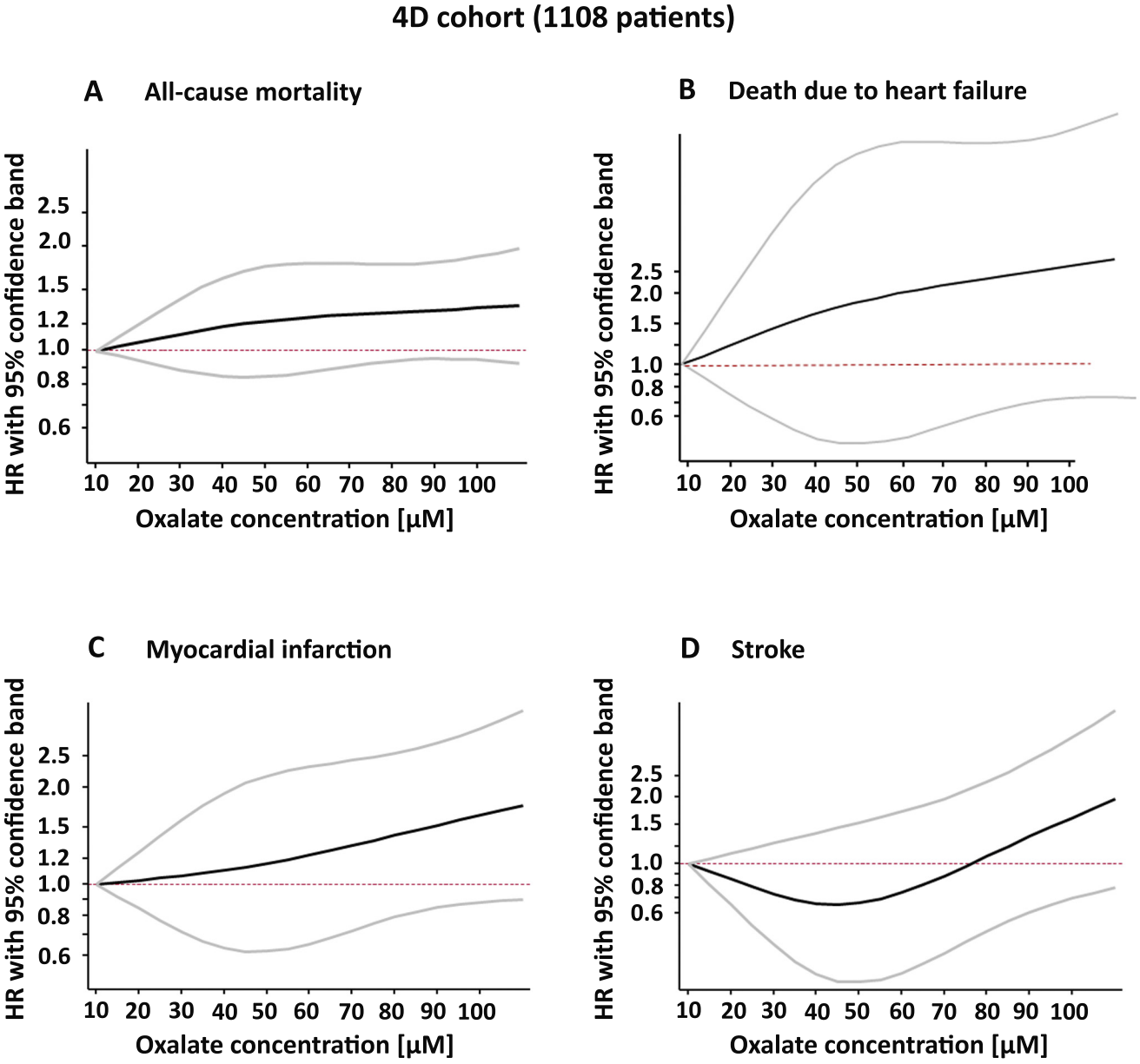
[d] For competing risk regression, sudden cardiac death and death due to heart failure were combined as composite endpoint.

Figure S1. Oxalate concentration and its correlation with residual kidney function in 104 US patients with kidney failure requiring chronic dialysis (US cohort).[a]



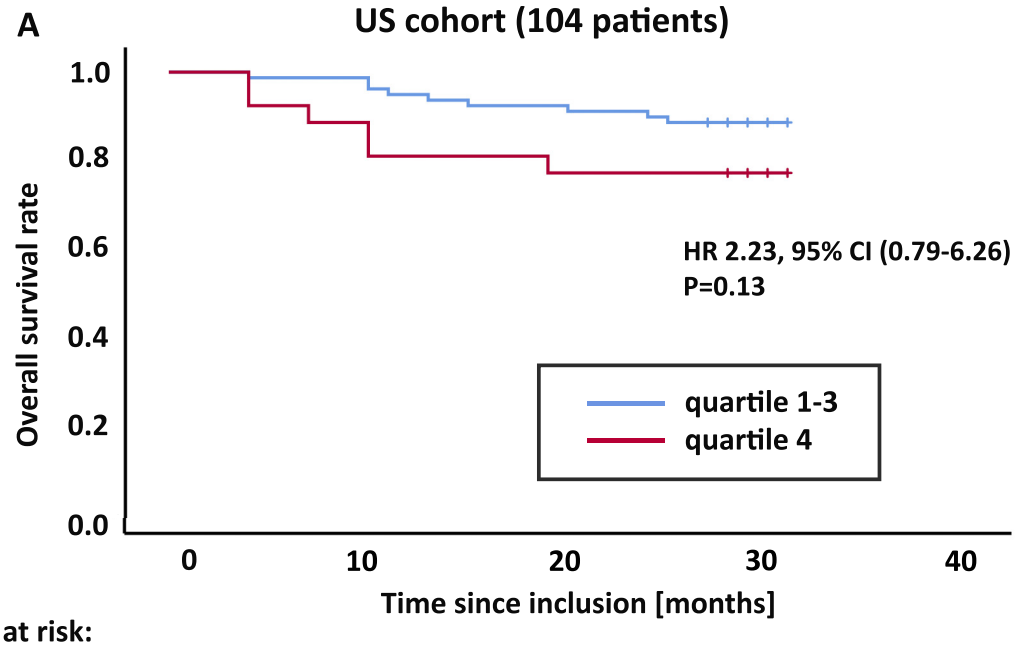
[a] Means were compared via unpaired t-test.
Residual kidney function was defined as urine output > 300 mL/day, and was assessed in both HD and PD patients.

Figure S2. Risk of (A) all cause mortality, (B) death due to heart failure, (C) myocardial infarction, and (D) stroke as a function of continuous oxalate exposure in the 4D Study (restricted splines).[a]

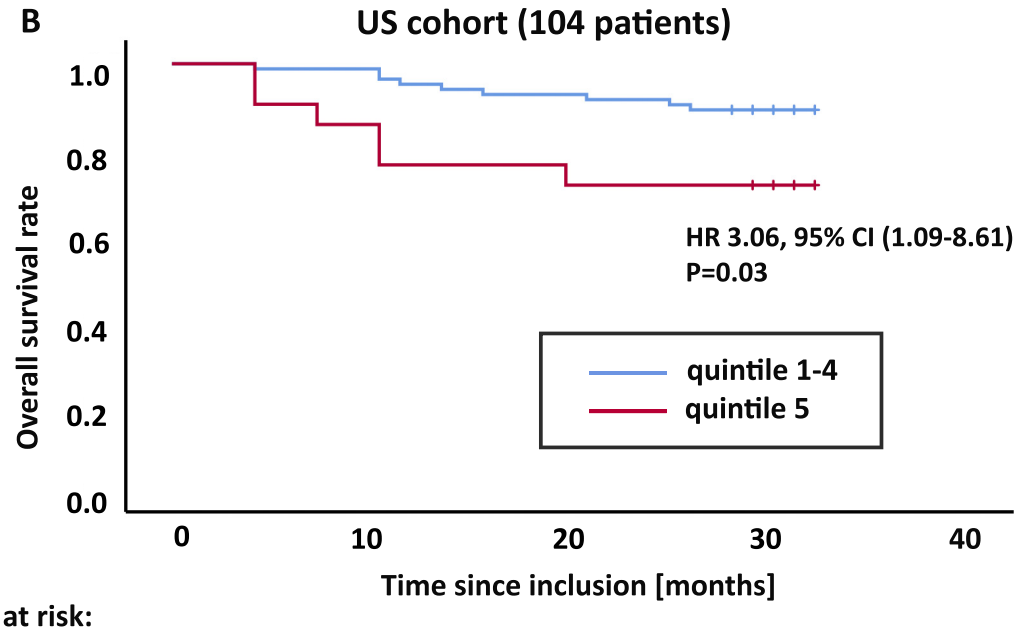


[a] Risk was assessed using restricted cubic spline modeling, and adjusted for age, sex, use of atorvastatin, time on dialysis, use of diuretics, C-reactive protein, body mass index, hemoglobin, albumin, and previous coronary artery disease (n=1108).
The black line represents the hazard ratios (HR), the grey lines indicate the upper and lower 95% CIs. The pink dashed line marks a HR of 1.0. CI: confidence interval.

Figure S3. Kaplan-Meier curves for overall survival in 104 US patients with kidney failure requiring chronic dialysis (US cohort) comparing (A) the highest oxalate quartile vs. quartiles 1-3, and (B) the highest oxalate quintile vs. quintiles 1-4.



No. at risk:				
quartile 1-3:	78	75	71	69
quartile 4:	26	21	20	20



No. at risk:				
quintile 1-4:	83	80	76	74
quintile 5:	21	16	15	15