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Circulating Calcitriol Concentrations and Total Mortality

Armin Zittermann,[1*] Stefanie S. Schleithoff,[1] Sabine Frisch,[1] Christian Götting,[2] Joachim Kuhn,[2] Heinrich Koertke,[1] Knut Kleesiek,[2] Gero Tenderich,[1] and Reiner Koerfer[1]

BACKGROUND: Evidence is accumulating that vitamin D supplementation of patients with low 25-hydroxyvitamin D concentrations is associated with lower cardiovascular morbidity and total mortality during long-term follow-up. Little is known, however, about the effect of low concentrations of the vitamin D hormone calcitriol on total mortality. We therefore evaluated the predictive value of circulating calcitriol for midterm mortality in patients of a specialized heart center.

METHODS: This prospective cohort study included 510 patients, 67.7% with heart failure (two-thirds in end stage), 64.3% hypertension, 33.7% coronary heart disease, 20.2% diabetes, and 17.3% renal failure. We followed the patients for up to 1 year after blood collection. For data analysis, the study cohort was stratified into quintiles of circulating calcitriol concentrations.

RESULTS: Patients in the lowest calcitriol quintile were more likely to have coronary heart disease, heart failure, hypertension, diabetes, and renal failure compared to other patients. They also had low 25-hydroxyvitamin D concentrations and high concentrations of creatinine, C-reactive protein, and tumor necrosis factor α. Eighty-two patients (16.0%) died during follow-up. Probability of 1-year survival was 66.7% in the lowest calcitriol quintile, 82.2% in the second quintile, 86.7% in the intermediate quintile, 88.8% in the fourth quintile, and 96.1% in the highest quintile ($P < 0.001$). Discrimination between survivors and nonsurvivors was best when a cutoff value of 25 ng/L was applied (area under the ROC curve 0.72; 95% CI 0.66–0.78).

CONCLUSIONS: Decreased calcitriol levels are linked to excess midterm mortality in patients of a specialized heart center.

Based on a metaanalysis of randomized controlled trials in studies with osteoporotic patients, it has recently been demonstrated that vitamin D supplementation is linked to lower total mortality in middle-aged and elderly subjects with low 25-hydroxyvitamin D [25(OH)D][3] concentrations compared with unsupplemented subjects *(1)*. Risk reduction was 7% during a mean follow-up of 5.7 years. These data are in line with the fact that animal and human studies demonstrate a broad range of vitamin D actions *(2–4)* and that vitamin D deficiency is associated with various chronic diseases such as specific types of cancer, musculoskeletal diseases, cardiovascular disease, autoimmune diseases, and diabetes *(5)*.

The assessment of serum 25(OH)D concentrations is generally considered to be the best approach for determining human vitamin D status *(5, 6)*. This assumption is supported by the fact that renal and extrarenal synthesis of the vitamin D hormone, calcitriol (1,25-dihydroxyvitamin D), are substrate dependent, i.e., dependent on circulating 25(OH)D levels *(6, 7)*. The stages of vitamin D status according to serum 25(OH)D concentrations have been defined and include deficiency (<10 μg/L), insufficiency (10–20 μg/L), hypovitaminosis (>20 to 32–40 μg/L), adequacy (>40–100 μg/L), and toxicity (>100 μg/L) *(2)*. Recently published data demonstrate that low serum 25(OH)D concentration is an independent predictor of cardiovascular morbidity and mortality during long-term follow-up *(8–10)*.

The assessment of circulating concentrations of calcitriol may be another important and hitherto neglected predictor of clinical outcome. At present, no generally accepted cutoff concentrations for calcitriol exist. It is known, however, that low circulating calcitriol concentrations are associated with chronic kidney disease and markedly increased concentrations of proinflammatory cytokines *(11, 12)*. Patients with end-stage renal disease or HIV infection, for example, have very low circulating calcitriol concentrations and

[1] Department of Cardio-Thoracic Surgery and [2] Institute for Laboratory and Transfusion Medicine, Heart Center North Rhine-Westfalia, Ruhr University Bochum, Bad Oeynhausen, Germany.
* Address correspondence to this author at: Department of Cardio-Thoracic Surgery, Heart Center North Rhine-Westfalia, Ruhr University Bochum, Georgstraβe 11, D-32545 Bad Oeynhausen, Germany. Fax +49-5731-97-2020; e-mail azittermann@hdz-nrw.de.
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[3] Nonstandard abbreviations: 25(OH)D, 25-hydroxyvitamin D; LOD, limit of detection; hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor α; iPTH, intact parathyroid hormone; HR, hazard ratio.

high mortality rates *(11, 13)*, whereas administration of calcitriol or vitamin D analogs is associated with reduced mortality in chronic kidney disease *(14)*. Moreover, it has been demonstrated that calcitriol concentrations are independently associated with all-cause and cardiovascular mortality in patients scheduled for angiography at a tertiary center *(10)*. Although studies with isotopically labeled vitamin D metabolites suggest a relatively short half-life of only 12–36 h for calcitriol in the human circulation *(15)* but a longer half-life (3 weeks) for 25(OH)D *(16)*, it is reasonable to assume that circulating calcitriol is present in a relatively steady state. We therefore hypothesize that the importance of low calcitriol concentrations as a risk marker for short- or midterm survival might be underestimated. This led us to investigate whether circulating calcitriol concentrations predict 1-year total mortality risk in patients of a specialized heart center. We also tried to evaluate whether there is a threshold of optimal circulating calcitriol for survival.

Materials and Methods

PATIENTS

To study the predictive value of circulating calcitriol on 1-year mortality, we enrolled 510 patients in our data analysis. Detailed information of the study participants has been published *(17–19)*. Briefly, we included 228 patients with end-stage heart failure who were on a waiting list for cardiac transplantation and 282 other patients who participated in 2 randomized vitamin D supplementation trials. Of these 282 patients, 104 were noncardiac transplant candidates who attended our outpatient clinic for heart failure patients, and the remaining 178 patients were overweight or obese non–heart failure patients who attended a weight reduction program. Patients of the 2 randomized controlled trials were included in our investigation after they had finished the randomized trial. These trials included a placebo-controlled daily vitamin D dose of 50 μg in heart failure patients or 83 μg in overweight and obese patients. At enrollment into the present study and during the entire follow-up period, none of the patients was taking vitamin D supplements. Exclusion criteria were age <18 years or cardiac transplantation during follow-up. All participants were white and were from the 2 federal German states North Rhine-Westfalia and Lower Saxonia (geographic latitude 50.5–54° N). Characteristics for patients with end-stage heart failure and other patients at study enrollment are given in Table 1. These data demonstrate that patients with end-stage heart failure were slightly older and leaner than other patients. In addition, they suffered more often from various chronic diseases and were taking various medications more frequently than other patients. All study procedures were approved by the local ethics committee, and informed written consent was obtained from all participants.

Table 1. Characteristics of the study cohort at enrollment.[a]

Characteristics	End-stage heart failure	Other	*P*
n	228	282	
Male	83.6	54.5	<0.001
Age, years	56.5 (10.6)	51.2 (10.1)	<0.001
Body mass index, kg/m^2	26.1 (3.6)	29.7 (5.2)	<0.001
Current smoker	7.7	29.0	<0.001
History of chronic disease			
Coronary heart disease	50.0	21.4	<0.001
Heart failure	100	56.6	<0.001
Hypertension	74.6	56.6	<0.001
Diabetes	32.7	10.7	<0.001
Renal insufficiency	29.5	9.3	<0.001
Biochemical parameters			
25(OH)D, μg/L	14.5 (23.7)	23.2 (19.5)	<0.001
iPTH, ng/L	81.7 (66.2)	42.8 (32.6)	<0.001
Calcium, mmol/L	2.29 (0.23)	2.40 (0.15)	<0.001
Creatinine, mg/L	15.4 (7.0)	9.5 (3.6)	<0.001
hsCRP, mg/L	1.71 (3.33)	1.32 (1.80)	0.092
TNF-α, μg/L	14.5 (10.9)	12.1 (8.2)	0.006
Current drug use			
Beta-blockers	84.5	46.9	<0.001
Aspirin	20.9	12.9	<0.001
Diuretics	96.4	44.1	<0.001
Lipid-lowering drugs	49.0	23.6	<0.001
Antiarhythmics	55.5	31.7	<0.001
Antihypertensive drugs	74.5	56.6	<0.001

[a] Data are % or mean (SD).

STUDY PROCEDURE

Blood samples were drawn within the first 3 days of hospital admission in the patients with end-stage heart failure or during a regular outpatient visit in other patients. For data analysis, patients with end-stage heart failure were included after the first blood sample was collected. Patients who participated in the 2 randomized trials were included into our data analysis at the last blood drawing, when the active part of the study

was terminated. Blood drawing was not limited to a specific season. We collected fasting blood samples from each patient between 0700 and 0900 after a 10- to 12-h overnight fast. Blood samples were centrifuged at 1500*g*, and aliquots were frozen immediately at −80 °C until analysis. We restricted follow-up to 1 year. In the severely ill patients, we used electronic records to assess their status at the 1-year follow-up. In the remaining patients, we assessed their 1-year follow-up status during outpatient visits or interviewed them or their relatives by telephone. The primary endpoint of this prospective cohort investigation was 1-year total mortality.

BIOCHEMICAL ANALYSIS

We measured 25(OH)D (sum of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2) using the DiaSorin RIA per manufacturer guidelines. Cross-reactivity of the assay is 100% for 25(OH)D_3 and 100% for 25(OH)D_2. We measured calcitriol (sum of 1,25-dihydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_2) using a competitive ELISA with a selected monoclonal antibody recognizing calcitriol (Immundiagnostik). According to the manufacturer, intra- and interassay CVs are <7.0% and <9.0%, respectively. The reference range of the calcitriol assay is considered 17–53 ng/L for healthy adults 20–50 years old. Cross-reactivity for vitamin D metabolites is as follows: 1,25-dihydroxyvitamin D_3, 100%; 1,25-dihydroxyvitamin D_2, 41%; vitamin D_2 and D_3, <0.01%; 24,25-dihydroxyvitamin D_3, <0.1%; 25(OH)D_2, <0.1%; 25(OH)D_3, <0.01%; and alfacalcidol, <0.003%. Because concentrations of 25(OH)D_2 are usually very low in Germany (<1.5 μg/L) *(20)*, the vast majority of circulating calcitriol can be expected to consist of 1,25-dihydroxyvitamin D_3. The limit of detection (LOD) of the calcitriol assay is 1 ng/L. All calcitriol concentrations that were below the detection limit of the assay were recorded as 1 ng/L. We measured calcium, creatinine, and high-sensitivity C-reactive protein (hsCRP) using the Architect autoanalyzer (Abbott) and tumor necrosis factor α (TNF-α) and intact parathyroid hormone (iPTH) using the Immulite autoanalyzer (DPC).

STATISTICS

We report categorical variables using the percentage of observations and express continuous variables using mean values and SD. We used χ^2 test and ANOVA, respectively, to assess group-specific differences in categorical variables and continuous variables. Because several biochemical variables, such as PTH, hsCRP, TNF-α, and 25(OH)D, were non–normally distributed by Kolmogorov–Smirnov tests, these data were normalized by logarithmic transformation. Because reference values that are confirmed by calcitriol-mediated functional alterations in the human body currently do not exist, we divided calcitriol concentrations into quintiles for data analysis. We generated Kaplan–Meier estimates to investigate the association of different calcitriol cutoff groups with survival probability during follow-up as a function of time after blood sampling. The log-rank test was used to test for differences in survival rates between subgroups. We then examined the associations between serum calcitriol and mortality risk using multivariable Cox proportional hazard analysis. We tested the proportional hazards assumption for calcitriol categories in a log minus log plot. This plot demonstrated that the proportional hazards assumption was true. We made adjustments for age, body mass index, smoking, diagnosis, aspirin use, renal function, and inflammation markers. Results are presented as hazard ratios (HRs) with 95% CI. Power calculations were based on the assumption that the follow-up period would be 12 months and the median survival 48 months. The probability was 80% that the study would detect a difference at a 2-sided 5% significance level, if the true hazard ratio between 2 groups was 3 and a total of 213 patients entered the study. The area under the ROC curve was used to assess how clearly circulating calcitriol concentrations could discriminate between survivors and nonsurvivors. We used Pearson's correlation coefficient (*r*) and multiple linear regression analysis to assess interrelationships of circulating calcitriol concentrations with other continuous variables. The software package SPSS, version 14, was used to perform statistical analyses.

Results

A total of 510 patients had a complete follow-up (100%). The mean calcitriol concentration of the study cohort was 29.0 ng/L. Values ranged between 1.0 and 152 ng/L. Quintiles for circulating calcitriol were <16.7 ng/L, 16.7–25.2 ng/L, 25.3–33.2 ng/L, 33.3–43.4 ng/L, and >43.4 ng/L. Table 2 presents concentrations or percentages of selected risk factors across calcitriol quintiles. Patients with low calcitriol concentrations were more likely to have coronary heart disease, heart failure, hypertension, diabetes, and renal failure. These patients also had low 25(OH)D concentrations and high concentrations of iPTH, creatinine, hsCRP, and TNF-α. In addition, they took beta-blockers, diuretics, antiarrhythmics, and antihypertensive drugs more frequently than other patients.

Eighty-two patients died during follow-up; thus, 1-year mortality was 16.0% for the entire study cohort. Almost all patients who died (n = 78) had end-stage heart failure. Causes of death were cardiovascular related in 37 patients and non–cardiovascular related

Table 2. Baseline characteristics of selected covariates by calcitriol quintile.[a]

Characteristic	First quintile	Second quintile	Third quintile	Fourth quintile	Fifth quintile	*P* (trend)
Calcitriol, ng/L	9.9 (5.3)	21.3 (2.4)	29.1 (2.3)	37.9 (2.9)	56.2 (16.2)	
Male	81.4	66.3	71.4	61.2	52.9	<0.001
Age, years	54.7 (11.8)	54.7 (10.1)	54.0 (9.9)	52.4 (10.3)	52.5 (10.8)	0.336
Body mass index, kg/m^2	26.5 (4.4)	27.5 (4.8)	28.7 (5.5)	28.9 (4.9)	29.0 (4.6)	0.001
Current smoker	8.2	23.8	23.5	16.3	27.5	0.006
History of chronic disease						
Coronary heart disease	44.3	45.6	30.6	26.5	23.5	0.001
Heart failure	94.0	77.2	66.3	56.1	46.0	<0.001
Hypertension	75.3	69.3	69.4	51.0	56.7	0.002
Diabetes	35.1	25.7	20.4	8.2	11.8	<0.001
Renal insufficiency	34.0	21.0	15.5	12.4	6.9	<0.001
Biochemical parameters						
25(OH)D, μg/L	12.8 (11.3)	17.3 (12.7)	20.7 (34.8)	22.1 (22.7)	24.0 (18.7)	0.003
iPTH, ng/L	63.0 (56.4)	60.6 (51.9)	71.8 (69.8)	53.4 (47.4)	50.2 (37.8)	0.043
Calcium, mmol/L	2.32 (0.17)	2.37 (0.16)	2.38 (0.13)	2.37 (0.15)	2.34 (0.30)	0.132
Creatinine, mg/L	15.1 (9.3)	12.1 (6.0)	11.8 (4.3)	11.3 (4.6)	10.5 (3.9)	<0.001
hsCRP, mg/L	2.60 (3.60)	1.73 (2.76)	1.02 (2.30)	1.05 (1.75)	0.90 (1.68)	<0.001
TNF-α, μg/L	15.7 (10.9)	14.2 (9.1)	12.5 (7.2)	13.7 (10.2)	10.6 (11.1)	0.004
Current drug use						
Beta-blockers	84.2	68.7	62.2	50.0	54.0	<0.001
Aspirin	19.6	19.8	11.2	19.4	12.7	0.279
Diuretics	93.7	75.8	68.4	55.1	45.6	<0.001
Lipid-lowering drugs	44.1	44.5	30.9	19.6	30.9	<0.001
Antiarrhythmics	55.8	47.5	40.8	35.7	30.4	0.003
Antihypertensive drugs	76.8	70.1	69.4	51.0	57.4	0.001

[a] Data are % or mean (SD).

(sepsis, infections, and renal failure) in 45 patients. In Fig. 1, freedom from 1-year mortality is given according to calcitriol quintile. Patients with low calcitriol concentrations had a significantly increased 1-year mortality risk. Broken down by quintiles, probability of survival was 66.7% in the lowest quintile of calcitriol concentrations, 82.2% in the second quintile, 86.7% in the intermediate quintile, 88.8% in the fourth quintile, and 96.1% in the highest quintile of calcitriol concentrations. None of the patients with a calcitriol concentration >58.5 ng/L (n = 29) died during follow-up.

Table 3 presents HRs of total 1-year mortality across the categories of circulating calcitriol concentrations. In the unadjusted model, patients in the lowest calcitriol quintile had a 1-year mortality risk 10.3 times higher than patients in the highest calcitriol quintile (95% CI 3.6–29.0). After adjustment for age, body mass index, and smoking, the HR was somewhat attenuated but remained statistically significant (HR 7.6; 95%CI 2.7–21.6). After further adjustment for renal function (serum creatinine) and inflammation markers (hsCRP and TNF-α), the HR was 5.5 (95% CI 1.9–15.8). In a final model, we also adjusted for aspirin use and diagnoses such as coronary heart disease, end-stage heart failure, hypertension, and diabetes. Adjusted 1-year mortality risk was still approximately 4 times higher in patients in the lowest calcitriol quintile than in patients in the highest quintile (95% CI 1.3–11.6). Moreover, the results did not change appreciably after excluding the second 6 months of follow-up (HR 3.9; 95%CI 1.1–12.9) but did not reach significance after excluding the first 6 months of follow-up (HR 6.8; 95% CI 0.87–56.1). Circulating 25(OH)D concentrations did not pre-

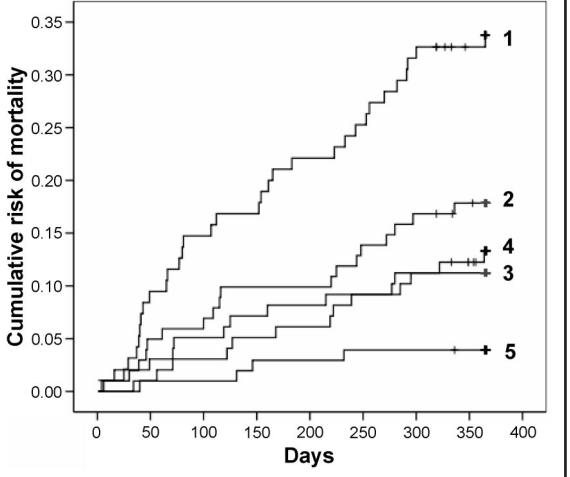

Fig. 1. Cumulative mortality risk according to quintile of circulating calcitriol concentration.

First quintile, calcitriol <16.7 ng/L; second quintile, calcitriol 16.7–25.2 ng/L; third quintile, calcitriol 25.3–33.2 ng/L; fourth quintile, 33.3–43.4 ng/L; fifth quintile, calcitriol >43.4 ng/L.

dict 1-year mortality when used as continuous variable and did not predict mortality when used as categorical variable after inclusion of end-stage heart failure into the adjusted model (Table 4).

In the unadjusted model, the β coefficient for a 1 ng/L increment in circulating calcitriol was −0.050 (SE 0.009), indicating the 1-year mortality risk decreased by 5% per a 1 ng/L increment in circulating calcitriol. In the full multivariable model, the β coefficient for a 1 ng/L increment in circulating calcitriol was −0.027 (SE 0.009), indicating the 1-year mortality risk decreased by 2.7% per a 1 ng/L increment in circulating calcitriol.

Our subset included only a limited number of participants >70 years old (n = 10). Exclusion of these participants did not substantially alter the results (data not shown). Similarly, exclusion of participants with a body mass index >40 kg/m^2 (n = 10) did not affect the results (data not shown).

The area under the ROC curve using circulating calcitriol concentration for the discrimination between survivors and nonsurvivors was 0.72 (95% CI 0.66–0.78). When a cutoff value of 25 ng/L was applied, specificity was 64.1% and sensitivity was 65.5%. Fig. 2 illustrates Kaplan–Meier survival curves using a cutoff value of 25 ng/L.

Compared with various continuous variables, circulating calcitriol was related only to 25(OH)D (r = 0.218; $P < 0.001$), creatinine ($r = -0.220$; $P < 0.001$), hsCRP ($r = -0.186$; $P < 0.001$), and body mass index (0.155; $P = 0.002$). As determined by multiple regression analysis with serum calcitriol as the independent variable, calcitriol was directly associated with 25(OH)D ($P < 0.001$) and body mass index (P = 0.032) and inversely related to creatinine ($P < 0.001$) and hsCRP ($P < 0.001$) (multiple R^2 = 0.108; $P <$ 0.001).

Discussion

This study demonstrates that low circulating calcitriol concentrations are related to higher 1-year mortality risk in patients of a specialized heart center. There was a progressive, statistically significant decrease in 1-year mortality risk across calcitriol quintiles, with lower mortality risk at higher calcitriol concentrations. The association between calcitriol and total mortality remained significant after considering important other risk factors for total mortality such as age, body mass index, smoking, aspirin use, various comorbidities, renal function, and inflammation markers. Our results of

Table 3. Adjusted HR (95% CI) for survival within 1-year follow-up by quintile of serum calcitriol concentration.

Calcitriol	n	1-Year survival, %	Model 1[a]	Model 2	Model 3	Model 4
<16.7 ng/L	102	66.2	10.26 (3.63–29.01)	7.59 (2.66–21.6)	5.46 (1.87–15.80)	3.93 (1.34–11.55)
16.7–25.2 ng/L	102	82.2	4.90 (1.66–14.48)	4.26 (1.44–12.62)	3.41 (1.13–10.27)	2.84 (0.94–8.60)
25.3–33.2 ng/L	102	86.7	3.54 (1.16–10.87)	3.27 (1.07–10.04)	3.20 (1.04–9.83)	2.14 (0.68–6.68)
33.3–43.4 ng/L	102	88.8	2.96 (0.94–9.28)	2.86 (0.92–9.04)	2.96 (0.94–9.30)	2.88 (0.89–9.25)
>43.4 ng/L	102	96.1	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
P (trend)			<0.001	<0.001	0.002	0.013

[a] Model 1, unadjusted data; model 2, adjusted for age, body mass index, and smoking; model 3, adjusted as in model 2 and for renal function and the inflammation markers CRP and TNF-α; model 4, adjusted as in model 3 and for aspirin use and diagnoses such as coronary heart disease, end-stage heart failure, hypertension, and diabetes.

Table 4. Adjusted HR (95% CI) for survival within 1-year follow-up by quintile of serum 25-hydroxyvitamin D concentration.[a]

25(OH)D	n	1-Year survival, %	Model 1	Model 2	Model 3	Model 4
<7.09 μg/L	102	79.8	2.76 (1.21–6.26)	2.95 (1.28–6.79)	2.86 (1.19–6.88)	1.46 (0.57–3.78)
7.09–11.1 μg/L	102	71.6	3.90 (1.78–8.56)	3.94 (1.79–8.66)	3.34 (1.44–7.78)	2.09 (0.83–5.27)
11.2–16.38 μg/L	102	89.9	1.29 (0.51–3.26)	1.23 (0.49–3.13)	1.30 (0.51–3.37)	0.86 (0.31–2.34)
16.39–26.95 μg/L	102	87.0	1.69 (0.70–4.07)	1.73 (0.72–4.18)	1.33 (0.52–3.9)	0.90 (0.34–2.38)
>26.95 μg/L	102	92.0	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
P (trend)			0.001	0.001	0.005	0.069

[a] Model 1, unadjusted data; model 2, adjusted for age, body mass index, and smoking; model 3, adjusted as in model 2 and for renal function and the inflammation markers CRP and TNF-α; model 4, adjusted as in model 3 and for aspirin use and diagnoses such as coronary heart disease, end-stage heart failure, hypertension, and diabetes.

high mortality in patients with low calcitriol concentrations are consistent with earlier findings in patients with chronic kidney disease *(21)* or HIV infection *(13)*, patients scheduled for coronary angiography *(10)*, and patients with hip fracture *(22)*, indicating that our results may be generalizable. Although a causal relationship of calcitriol with mortality has yet to be proven by intervention trials using active vitamin D, data demonstrate that circulating calcitriol is at least an important indicator of total mortality risk.

In this prospective cohort study, patients with circulating calcitriol concentrations <17 ng/L had approximately 4 times higher 1-year mortality risk than patients with calcitriol concentrations >44 ng/L, after multivariable risk adjustments. Discrimination between survivors and nonsurvivors was best, however, when a cutoff value of 25 ng/L was used. All patients with calcitriol concentrations >58.5 ng/L survived. Thus, the study raises the question of how to define a reference range for calcitriol. In earlier studies, calcitriol cutoffs for higher mortality rates were <13 ng/L *(21)*, < 20.9 ng/L *(10)*, and <25 ng/L *(13)*. Taken together, these data indicate that the lower threshold value for calcitriol should probably be higher than 17 ng/L, as recommended by the assay provider, and should be 25 ng/L. By considering total mortality as an endpoint, however, no upper reference value can be defined from our data. Interestingly, some physiologic situations where survival is highly desirable from an evolutionary point of view are associated with high circulating calcitriol concentrations. For example, long-distance runners have mean circulating calcitriol concentrations of 73 ng/L. These concentrations decline to 39 ng/L during prolonged hypokinesia *(23)*. In turn, remobilization of healthy adults immobilized for 16 weeks raises calcitriol concentrations from 23 ng/L to 46 ng/L *(24)*. In pregnant women, calcitriol concentrations are twice as high as in nonpregnant women *(25)*. Mean concentrations rise from 63 ng/L during early pregnancy to 140 ng/L during late pregnancy *(26)*.

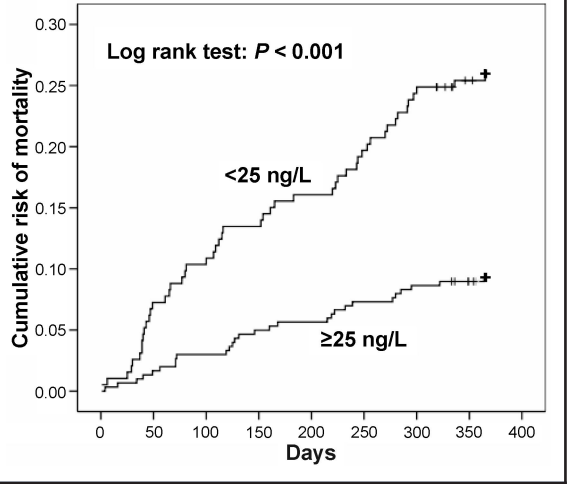

Fig. 2. Cumulative mortality risk according to a calcitriol cutoff value of 25 ng/L.

Because of the inclusion of a large number of patients with end-stage heart failure (n = 228), the 1-year mortality of 16.0% in the entire study cohort was relatively high. This allowed us to assess risk factor analysis with sufficient statistical power. In an earlier study in patients scheduled for coronary angiography *(10)*, the multivariate-adjusted HR for total mortality was 2.08 (95% CI 1.60–2.70) for patients in the lowest 25(OH)D quartile compared with patients in the highest 25(OH)D quartile, but was only 1.61 (95% CI 1.25–2.07) for patients in the lowest calcitriol quartile com-

pared with patients in the highest calcitriol quartile during a median follow-up period of 7.7 years. In that earlier study, median calcitriol concentrations in the lowest quartile were 20.9 ng/L (interquartile range 17.1–23.9 ng/L) and thus higher than in the lowest quintile of our study. Our data indicate that calcitriol is a better predictor of short- and midterm survival than 25(OH)D. This is probably because calcitriol is the active vitamin D hormone and its serum concentration is not only related to substrate availability but also depends on renal function and inflammatory processes. Our study results demonstrate that circulating calcitriol was inversely related to serum creatinine, a marker of renal function, and to the inflammation marker hsCRP. Both renal impairment and inflammatory processes are risk factors frequently found in the aging population of developed countries *(27*, *28)* and may thus influence calcitriol availability in many patients. The actual calcitriol effects on total mortality are therefore probably greater than indicated in the fully adjusted model, since we adjusted for renal function and inflammatory processes. Nevertheless, in our study renal function and inflammatory processes cannot completely explain variations in circulating calcitriol concentrations. There is evidence that other factors, such as fibroblast growth factor 23, also play an important role in regulating circulating calcitriol *(29)*. It may also be that diagnostic sensitivity and specificity for calcitriol was at least in part confounded by assay variability and technologist imprecision. In addition, in some patients with heart failure the intraindividual variation for calcitriol may have been higher during follow-up. Renal impairment and inflammatory processes can result in suppression of calcitriol concentrations within a few weeks *(30)*. Finally, it may be that calcitriol is only one factor of many that contribute to total mortality, serving to modulate mortality risk, but only when other risk factors for mortality are also present.

Although pharmacokinetic studies indicate a very short half-life of calcitriol in the circulation (12–36 h) *(15)*, the concentration of calcitriol appears to be in a relatively steady state. It takes several weeks after a negative stimulus is given for calcitriol concentrations to decrease to a lower value *(23)*. Likewise, it also takes several weeks after a positive stimulus is given for very low calcitriol concentrations to return to the physiological range *(31)*, indicating that 1 blood measurement at a specific time point is sufficient to estimate the range in which circulating calcitriol concentrations will be seen over several months. It is an important finding that low circulating calcitriol concentrations also inhibit uptake of 25(OH)D into extrarenal tissues such as monocytes *(32)*, indicating that extrarenal calcitriol synthesis is also limited if circulating calcitriol concentrations are low.

It is now becoming clear that calcitriol has immunomodulatory properties and can suppress the release of proinflammatory cytokines in monocytes *(33)*. These vitamin D effects probably contribute to the improved survival in subjects with higher calcitriol concentrations and may also explain why many of our severely ill patients died of sepsis and infections. In line with this assumption, no severely immunodeficient HIV-infected patients (CD4 count $<50 \times 10^6$ cells/L) with calcitriol concentrations <25 ng/L survived during 1-year follow-up, whereas 1-year survival was 70% in those HIV-infected patients with calcitriol concentrations >25 ng/L *(13)*. In the less severely immunodeficient HIV patients of that study (CD4 count $<200 \times 10^6$ cells/l), 1-year survival was 34% in the patients with calcitriol concentrations <25 ng/L and 57% ($P < 0.05$) in the patients with calcitriol concentrations >25 ng/L.

Our study has both strengths and limitations. The strengths include the prospective study design, the high follow-up rate of 100%, the multivariable adjustments, the definitive primary endpoint total mortality, and the use of a serum marker which precluded some sources of bias such as recall bias and selection bias. A limitation is that we cannot definitively rule out reverse causation bias, e.g., that more severe disease was associated with lower calcitriol concentrations and independently predicted increased 1-year mortality rates. Therefore, a causal relationship still has to be proven by intervention trials using calcitriol or vitamin D analogs. A second limitation is that the 1 blood draw represents only a snapshot of the patients' calcitriol status, which likely varied throughout their hospital stay as they were being treated. Nevertheless, we were able to use the calcitriol concentration of 1 blood draw as an indicator for mortality risk over the next 12 months. A third limitation is that we included only a small number of elderly patients. Future studies should therefore investigate the association of low calcitriol concentrations with mortality risk in people >70 years old. Another limitation was that the vast majority of heart failure patients were on diuretics, which are known to affect calcium and vitamin D metabolism. This might be an explanation for the low calcitriol concentrations in these patients, and could indicate that calcitriol alone may not be the cause for mortality, but is likely additive. The fact that serum 25(OH)D concentrations did not predict 1-year mortality contradicts some earlier studies *(1*, *8*, *9*, *10*, *21)*. This does not necessarily imply that 25(OH)D is not related to total mortality, however, and may only indicate that in the present study cohort other factors than 25(OH)D were more important and may have masked a potential relationship of 25(OH)D with midterm mortality.

In summary, our data demonstrate that calcitriol is an independent predictor of total mortality in pa-

tients admitted to a heart center. Low calcitriol concentrations should therefore be regarded as a nonclassic risk factor for total mortality. Calcitriol concentrations <25 ng/L were linked to excess midterm mortality.

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