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 (√ < 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 extra-renal 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...
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.

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

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the study cohort at enrollment. a</th>
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<tr>
<td>Characteristic</td>
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<td>Age, years</td>
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<td>Body mass index, kg/m²</td>
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<td><strong>Biochemical parameters</strong></td>
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<td>25(OH)D, ng/L</td>
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* Data are % or mean (SD).
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 1500g, 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 D3 and 25-hydroxyvitamin D2) using the DiaSorin RIA per manufacturer guidelines. Cross-reactivity of the assay is 100% for 25(OH)D3 and 100% for 25(OH)D2. We measured calcitriol (sum of 1,25-dihydroxyvitamin D3 and 1,25-dihydroxyvitamin D2) 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 D3, 100%; 1,25-dihydroxyvitamin D2, 41%; vitamin D2 and D3, <0.01%; 24,25-dihydroxyvitamin D3, <0.1%; 25(OH)D2, <0.1%; 25(OH)D3, <0.01%; and alfacalcidol, <0.003%. Because concentrations of 25(OH)D2 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 D3. 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 χ² 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 (sepsis, infections, and renal failure) in 45 patients. In Fig. 1, freedom from 1-year mortality is given accord-
ing 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 predict 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).

### Table 2. Baseline characteristics of selected covariates by calcitriol quintile.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First quintile</th>
<th>Second quintile</th>
<th>Third quintile</th>
<th>Fourth quintile</th>
<th>Fifth quintile</th>
<th>P (trend)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcitriol, ng/L</td>
<td>9.9 (5.3)</td>
<td>21.3 (2.4)</td>
<td>29.1 (2.3)</td>
<td>37.9 (2.9)</td>
<td>56.2 (16.2)</td>
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<tr>
<td>Male</td>
<td>81.4</td>
<td>66.3</td>
<td>71.4</td>
<td>61.2</td>
<td>52.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>54.7 (11.8)</td>
<td>54.7 (10.1)</td>
<td>54.0 (9.9)</td>
<td>52.4 (10.3)</td>
<td>52.5 (10.8)</td>
<td>0.336</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.5 (4.4)</td>
<td>27.5 (4.8)</td>
<td>28.7 (5.5)</td>
<td>28.9 (4.9)</td>
<td>29.0 (4.6)</td>
<td>0.001</td>
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<td>Current smoker</td>
<td>8.2</td>
<td>23.8</td>
<td>23.5</td>
<td>16.3</td>
<td>27.5</td>
<td>0.006</td>
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<td>History of chronic disease</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Coronary heart disease</td>
<td>44.3</td>
<td>45.6</td>
<td>30.6</td>
<td>26.5</td>
<td>23.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart failure</td>
<td>94.0</td>
<td>77.2</td>
<td>66.3</td>
<td>56.1</td>
<td>46.0</td>
<td>&lt;0.001</td>
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<tr>
<td>Hypertension</td>
<td>75.3</td>
<td>69.3</td>
<td>69.4</td>
<td>51.0</td>
<td>56.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes</td>
<td>35.1</td>
<td>25.7</td>
<td>20.4</td>
<td>8.2</td>
<td>11.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>34.0</td>
<td>21.0</td>
<td>15.5</td>
<td>12.4</td>
<td>6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biochemical parameters</td>
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<tr>
<td>25(OH)D, μg/L</td>
<td>12.8 (11.3)</td>
<td>17.3 (12.7)</td>
<td>20.7 (34.8)</td>
<td>22.1 (22.7)</td>
<td>24.0 (18.7)</td>
<td>0.003</td>
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<tr>
<td>iPTH, ng/L</td>
<td>63.0 (56.4)</td>
<td>60.6 (51.9)</td>
<td>71.8 (69.8)</td>
<td>53.4 (47.4)</td>
<td>50.2 (37.8)</td>
<td>0.043</td>
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<td>Calcium, mmol/L</td>
<td>2.32 (0.17)</td>
<td>2.37 (0.16)</td>
<td>2.38 (0.13)</td>
<td>2.37 (0.15)</td>
<td>2.34 (0.30)</td>
<td>0.132</td>
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<tr>
<td>Creatinine, mg/L</td>
<td>15.1 (9.3)</td>
<td>12.1 (6.0)</td>
<td>11.8 (4.3)</td>
<td>11.3 (4.6)</td>
<td>10.5 (3.9)</td>
<td>&lt;0.001</td>
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<tr>
<td>hsCRP, mg/L</td>
<td>2.60 (3.60)</td>
<td>1.73 (2.76)</td>
<td>1.02 (2.30)</td>
<td>1.05 (1.75)</td>
<td>0.90 (1.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α, μg/L</td>
<td>15.7 (10.9)</td>
<td>14.2 (9.1)</td>
<td>12.5 (7.2)</td>
<td>13.7 (10.2)</td>
<td>10.6 (11.1)</td>
<td>0.004</td>
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<td>Current drug use</td>
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<tr>
<td>Beta-blockers</td>
<td>84.2</td>
<td>68.7</td>
<td>62.2</td>
<td>50.0</td>
<td>54.0</td>
<td>&lt;0.001</td>
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<tr>
<td>Aspirin</td>
<td>19.6</td>
<td>19.8</td>
<td>11.2</td>
<td>19.4</td>
<td>12.7</td>
<td>0.279</td>
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<td>Diuretics</td>
<td>93.7</td>
<td>75.8</td>
<td>68.4</td>
<td>55.1</td>
<td>45.6</td>
<td>&lt;0.001</td>
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<tr>
<td>Lipid-lowering drugs</td>
<td>44.1</td>
<td>44.5</td>
<td>30.9</td>
<td>19.6</td>
<td>30.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antiarrhythmics</td>
<td>55.8</td>
<td>47.5</td>
<td>40.8</td>
<td>35.7</td>
<td>30.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td>76.8</td>
<td>70.1</td>
<td>69.4</td>
<td>51.0</td>
<td>57.4</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Data are % or mean (SD).
In the unadjusted model, the $\beta$ 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 $\beta$ 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 high mortality in patients with low calcitriol concentrations are consistent with earlier findings in patients with chronic kidney disease (21) or HIV infection.

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

* 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.
(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).

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 multivariable-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 compared 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

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

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

* 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.

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

population of developed countries

cesses are risk factors frequently found in the aging

hsCRP. Both renal impairment and inflammatory pro-

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 there-

fore probably greater than indicated in the fully ad-

justed model, since we adjusted for renal function and

inflammatory processes. Nevertheless, in our study re-

nal function and inflammatory processes cannot com-

pletely explain variations in circulating calcitriol con-

centrations. 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 calcit-

riol was at least in part confounded by assay variability

and technologist imprecision. In addition, in some pa-

tients with heart failure the intraindividual variation

for calcitriol may have been higher during follow-up.
Renal impairment and inflammatory processes can re-

sult 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 neg-

ative 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 physio-

logical range (31), indicating that 1 blood measure-

ment 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 in-

hibit uptake of 25(OH)D into extrarenal tissues such as

monocytes (32), indicating that extrarenal calcitriol syn-

thesis is also limited if circulating calcitriol concentra-

tions are low.

It is now becoming clear that calcitriol has immu-

nomodulatory properties and can suppress the release

of proinflammatory cytokines in monocytes (33). These

vitamin D effects probably contribute to the im-

proved survival in subjects with higher calcitriol con-

centrations and may also explain why many of our se-

verely ill patients died of sepsis and infections. In line

with this assumption, no severely immunodeficient

HIV-infected patients (CD4 count <50 × 10^{6} cells/L)

with calcitriol concentrations <25 ng/L survived dur-

ing 1-year follow-up, whereas 1-year survival was 70%

in those HIV-infected patients with calcitriol concen-

trations >25 ng/L (13). In the less severely immuno-

deficient HIV patients of that study (CD4 count

<200 × 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 concen-

trations >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 limita-

tion is that we cannot definitively rule out reverse cau-

sation 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 interven-

tion trials using calcitriol or vitamin D analogs. A sec-

ond 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 mor-

tality risk over the next 12 months. A third limitation is

that we included only a small number of elderly pa-

tients. Future studies should therefore investigate the

association of low calcitriol concentrations with mor-

tality 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 re-

lated 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 concen-

trations should therefore be regarded as a nonclassic

risk factor for total mortality. Calcitriol concentrations

<25 ng/L were linked to excess midterm mortality.
References


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