

## Vitamin D Status and Mortality of German Hemodialysis Patients

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**Abstract.** *Aim: The goal was to analyze the link between blood levels of calcidiol and all-cause, cardiac and infectious diseases, and mortality due to cancer in hemodialysis patients. Patients and Methods: This study retrospectively evaluated a representative sub-cohort (n=6,518) of German hemodialysis patients from the incidence cohorts 1997-2006. Results: Most (58.8%) were found to be vitamin D deficient (25(OH)D<20 ng/ml), with 41.2% being severely deficient (25(OH)D<12.5 ng/ml). All-cause mortality risk more than doubled in patients with severe deficiency (adjusted odds ratio (aOR)=2.67; 95% confidence interval (CI)=2.30–3.10; p<0.0001). Comparable data were obtained for mortality from cardiac disease (aOR=1.57; 95% CI=1.30–1.88; p<0.0001), infectious disease (aOR=1.48; 95% CI=1.15–1.90; p=0.0026), and cancer (aOR=1.51; 95% CI=1.09–2.08; p=0.0121), respectively. Conclusion: These data highlight the need to ensure primarily adequate 25(OH)D levels in dialysis patients for an advantage of survival.*

Adequate vitamin D status is essential for overall health (1, 2). Vitamin D has been identified as an independent determinant of survival (3-6). Blood levels of calcidiol [25-hydroxyvitamin D, 25(OH)D] represent the vitamin D status. Circulating calcidiol represents a summation of both vitamin D intake, and the

natural production of vitamin D in the skin from exposure to the sun (2, 7). Although there is no consensus on optimal levels of 25(OH)D, the target values are generally recommended to be above 30 ng/ml and below 100 ng/ml (7-9).

Various studies have revealed the critical link between vitamin D deficiency and an increased prevalence of hypertension (10), heart failure (11), and cardiovascular events (12). These observations are likely to be of relevance to patients undergoing hemodialysis, where cardiovascular mortality is the most common cause of death (13-15).

Patients with chronic kidney disease frequently exhibit a deficiency of calcidiol and the hormonal active metabolite, calcitriol (16). In recent years, clinical research has shown that low levels of both calcidiol and calcitriol are independently associated with all-cause and cardiovascular mortality (17), and that intake of vitamin D supplements is associated with a reduction in total mortality rates, both in the general population (3) and in patients with chronic kidney disease (18). It is only recently that the pleiotropic effects of 25(OH)D in response to infections, to cancer (19), and other immunological processes have been understood at the molecular level (20, 21).

In view of the growing recognition of the importance of maintaining adequate 25(OH)D levels in patients with chronic kidney disease, more information is needed on the current vitamin D status of high-risk populations (9) and the effect of insufficient vitamin D levels on patient's outcome. The aim of this retrospective analysis was to analyze whether there is a link between blood levels of calcidiol and mortality due to, cardiac disease, infectious diseases, and cancer in the studied patients.

### Patients and Methods

**Study design.** This was a retrospective evaluation with two components: German Renal Registry data organized as a cohort study of dialysis patients, and retrospectively linked external 25(OH)D data (Limbach laboratory).

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**Key Words:** Vitamin D, mortality, hemodialysis, retrospective analysis, calcidiol.

**Study population.** Overall, for 17,291 hemodialysis patients, data from the Renal Registry (QuaSi-Niere) were matched with 73,919 recorded 25(OH)D measurements. The aim of this study was to make a valid analysis of vitamin D deficiency-related death in hemodialysis patients which imposed two restrictions on the subset of patients eligible for analysis. Firstly, as reporting bias in the early months after establishing the German Renal Registry could not be excluded, incident patients for the years 1995 and 1996 were disregarded. Secondly, only patients from dialysis units that had reported continuously over the full observation period were to be included. Thus 6,518 hemodialysis patients from the incidence cohorts 1997-2006 were identified for analysis. A comparison based on age, gender, primary disease, treatment modality and cause of death between this subgroup and the corresponding hemodialysis population revealed no clinical relevant differences, *i.e.* this subgroup can be regarded as a representative subsample.

**Analysis of 25(OH)D levels.** 25-Hydroxyvitamin D (25(OH)D) levels were measured by means of a competitive protein-binding assay which was developed in-house (22), from the start of the study until June 11, 2001. From June 12, 2001, the laboratory replaced the type of assay with the Nichols Advantage 25-hydroxyvitamin D Assay (Nichols Institutes Diagnostics, San Clemente, CA, USA), which was used until December 31, 2006. The previous laboratory values were adjusted by a combined factor of 0.4 multiplied by 0.38 so that the 25(OH)D levels would be comparable with the newly obtained laboratory values. The first factor, with a value of 0.4, was used to convert the units of measurements (from nmol/l to ng/ml). The second factor, with a value of 0.38, was used to adjust for the two different assay standardizations (23). The functional sensitivity for both tests was  $\leq 7$  ng/ml. As a result, all of the 25(OH)D measurements were then analyzed as a single dataset. Reference values used for vitamin D status are in accordance with Holick (8) and Lips *et al.* (24) (Table I). 25(OH)D measurements were irregular with regard to time and frequency for each patient. It was decided to describe the vitamin D status of an individual patient by using her or his last recorded 25(OH)D value, as this measurement most appropriately reflects the vitamin D status from a clinical perspective with respect to risk and cause of death.

**European Dialysis and Transplant Association (EDTA) coding.** The German Renal Registry collected and grouped diagnoses and causes of death according to schemes defined by the EDTA (EDTA code).

**Statistical methods.** Multivariable logistic-regression analysis was performed for all-cause mortality, cardiac mortality, infectious diseases mortality, and cancer mortality. Covariates used for data adjustment included gender, age at incidence and diabetes type I/II as primary renal disease. Patients were regarded as being at risk of death from the start of dialysis until death, or the end of the follow-up period (31/12/2006). Reported outcomes are adjusted odds ratios (aORs) and 95% confidence intervals (95% CIs) for categorized 25(OH)D levels, with the highest level group (vitamin D sufficiency, 25(OH)D level  $\geq 30$   $\mu\text{g/l}$ ) as the reference category. *p*-Values below 0.05 were considered to be significant. Reported *p*-values are descriptive, and no adjustments were made for multiple testing. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Table I. Categories of vitamin D level and their clinical implications.

25(OH) D level (ng/ml)	Clinical implication
<12.5	Severe deficiency
12.5-<20	Deficiency
20-<30	Insufficiency
$\geq 30$	Sufficiency

## Results

**Patient data characteristics.** From 6,518 patients eligible for analysis from the incidence cohorts 1997-2006, 2,701 (41.4%) were female and 3,817 (58.6%) were male. The median age was 71 years, with a range from 19 to 98 years. Diabetes type I/II (EDTA code 80 and 81) was recorded for 34.9% of the patients as the most prominent primary renal disease group. At the end of the observation period (31/12/2006) 3,010 (46.2%) patients had died, of which cardiac death (EDTA codes 11, 14-16) contributed to 1,148 cases (38.1%), infectious diseases (EDTA codes 31-38) contributed to 513 cases (17.0%), and cancer (EDTA code 67) contributed to 289 cases (9.6%).

**Laboratory findings.** Vitamin D levels were divided into four categories: sufficiency, insufficiency, deficiency and severe deficiency, according to the criteria in Table I. Patient characteristics according to categories of vitamin D status are presented in Table II, where the individual 25(OH)D readings were aggregated over time by using the last recorded value for each patient. The majority of patients (59.0%) were vitamin D deficient (25(OH)D <20 ng/ml), with 41.2% being severely deficient, *i.e.* 25(OH)D <12.5 ng/ml (Figure 1, Table III). Severe deficiency was more pronounced in females (49.3%) than in males (35.5%),  $p < 0.0001$ . A total of 47.5% of patients with diabetes type I/II as primary renal disease presented severely deficient levels of vitamin D, whereas in patients with primary diseases other than diabetes type I/II, severe deficiency was lower (37.9%),  $p < 0.0001$ .

**Logistic regression analysis.** Logistic regression was performed on data from hemodialysis patients of the study population. Compared with patients with sufficient vitamin D status (25(OH)D  $\geq 30$  ng/ml), the main findings of the study were (Table III, Figure 2):

- All-cause mortality risk increased with decreasing vitamin D level. For vitamin D insufficiency (25(OH)D levels of 20-<30 ng/ml), a moderate increase in risk was observed (aOR=1.19; 95% CI=0.99-1.43;  $p=0.0582$ ). For vitamin D deficiency (25(OH)D 12.5-<20 ng/ml), this increase was much more pronounced (aOR=1.50; 95% CI=1.25-1.79;  $p < 0.0001$ ), and for severe vitamin D deficiency (25(OH)D

Table II. Incidence cohorts 1997–2006, patients' characteristics (n=6,518).

Characteristic	n	Vitamin D status (last recorded 25(OH)D value [ng/ml])			
		<12.5	12.5-<20	20-<30	≥30
Number of patients (%)	6,518	2,686 (41.2)	1,162 (16.8)	1,097 (17.8)	1,573 (24.1)
Gender (%)					
Male	3,817	35.5	19.0	18.9	26.7
Female	2,701	49.3	16.2	13.9	20.5
Age (years) (at last 25(OH)D value)					
Median (range)	6,518	71 (20-94)	69 (22-98)	70 (19-96)	71 (20-96)
Primary renal disease (%)					
Type I/II diabetes:	4,243	37.9	18.1	17.2	26.9
Other	2,275	47.5	17.4	16.2	18.9
Vital status					
Alive	3,508	30.7	19.5	19.5	30.3
Dead	3,010	53.5	15.9	13.7	16.9
Cause of death					
Cardiac*	1,148	52.5	16.3	13.8	17.4
Infectious**	513	53.8	14.6	14.0	17.5
Cancer***	289	50.2	14.9	15.2	19.7
Other	1,060	55.3	16.4	13.0	15.3

EDTA codes: \*11,14-16, \*\*31-38; \*\*\*67.

Table III. Logistic regression analysis of mortality of the incidence cohorts 1997–2006 (n=6,518).

	Vitamin D status (last recorded 25(OH)D value [ng/ml]) (n=6,518)			
	<12.5	12.5-<20	20-<30	≥30
Number of patients (%)	2,686 (41.2)	1,162 (16.8)	1,097 (17.8)	1,573 (24.1)
All-cause mortality (aOR; 95% CI)	2.67 (2.30-3.10) <i>p</i> <0.0001	1.50 (1.25-1.79) <i>p</i> <0.0001	1.19 (0.99-1.43) <i>p</i> =0.0582	
Cardiac mortality (aOR; 95% CI)	1.57 (1.30-1.88) <i>p</i> <0.0001	1.26 (1.01-1.58) <i>p</i> =0.0416	1.07 (0.85-1.35) <i>p</i> =0.5638	Reference value
Infectious mortality (aOR; 95% CI)	1.07 (0.77-1.47) <i>p</i> =0.0026	1.48 (1.15-1.90) <i>p</i> =0.6952	1.07 (0.77-1.48) <i>p</i> =0.6856	1.0
Cancer mortality (aOR; 95% CI)	1.51 (1.09-2.08) <i>p</i> =0.0121	1.05 (0.70-1.57) <i>p</i> =0.8222	1.13 (0.75-1.69) <i>p</i> =0.5580	

aOR, Adjusted odds ratio; 95% CI, 95% confidence interval. Covariates used for adjustment included gender, year of incidence, age at incidence and diabetes type I/II as primary renal disease.

<12.5 ng/ml), mortality risk, more than doubled (aOR=2.67; 95% CI=2.30-3.10; *p*<0.0001).

- Cardiac mortality risk increased among patients with vitamin D deficiency. In patients with vitamin D deficiency, aOR was 1.26 (95% CI=1.01-1.58; *p*=0.0416). Among those with severe deficiency, the aOR was 1.57 (95% CI=1.30-1.88; *p*<0.0001).
- For deaths due to infectious disease, the highest elevated risk of death was observed in patients with severe vitamin D deficiency (aOR=1.48; 95% CI=1.15-1.90; *p*=0.0026).
- For cancer mortality, the highest mortality risk was also observed in patients with severe vitamin D deficiency (aOR=1.51; 95% CI=1.09-2.08; *p*=0.0121).

## Discussion

The present evaluation is the first of a representative national cohort of hemodialysis patients and reveals that in the cohort studied, more than half of patients with end-stage renal disease undergoing hemodialysis were vitamin D deficient (25(OH)D level of <20 ng/ml), and more than 40% of all patients were severely deficient (25(OH)D level of <12.5 ng/ml). Vitamin D deficiency was more pronounced in females than in males in this study. The data from this study suggest that patients undergoing hemodialysis are at high risk from the adverse health effects associated with vitamin D deficiency (6, 9, 25).

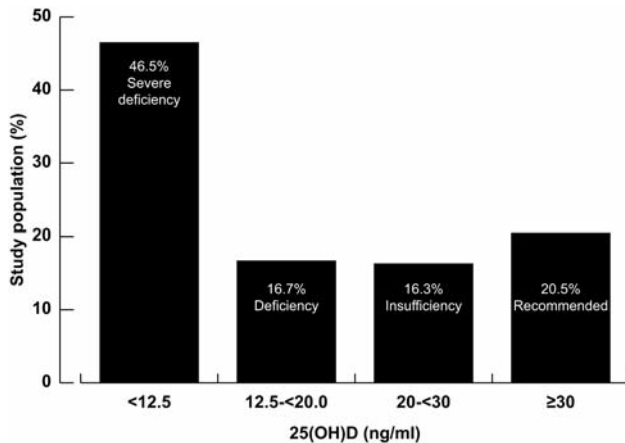


Figure 1. Distribution of 25(OH)D levels in the study population, last recorded values (see also Table II).

The present evaluation indicates that vitamin D deficiency is an independent risk factor for all-cause mortality in German patients on hemodialysis. This is in accordance with data from patients on hemodialysis in the US (6), and data from the general population (3, 4). Moreover, the present evaluation also indicates that vitamin D deficiency is an independent risk factor for cardiac mortality in German patients on hemodialysis. This finding is supported by previous research, which has demonstrated an association between low 25(OH)D levels and an increased risk of cardiovascular disease (4, 13, 26).

Furthermore the present study also demonstrates that severe vitamin D deficiency is linked to an increased risk of death due to infection among patients undergoing hemodialysis. This is in accordance with the evidence that vitamin D is linked to antibacterial defense mechanisms *via* the toll-like receptor and cathelicidin (20, 21, 27).

Within the last decade, there has been growing evidence that vitamin D status and cancer incidence, as well as cancer mortality, are negatively correlated (28). The interactions of the anticancerogenous effects and vitamin D metabolism are multiple: antiangiogenesis, antiapoptotic, antiinflammatory, and immunomodulating (29). Cancer mortality in patients with end-stage kidney disease is well-known to be lower than in the general population (15, 30). Also within this analysis, cancer mortality of German hemodialysis patients is found to be less than 10% of all deaths. Nevertheless, it shows the same relationship with severe vitamin D deficiency as demonstrated for the other causes of mortality.

**Restrictions.** Due to the nature of the retrospective evaluation, the present study has several limitations. Firstly, the data available were restricted to those available both from the German Renal Registry, and the 25(OH)D measurements from Limbach laboratory. Information on whether patients received vitamin D supplementation was not available. Furthermore, during the

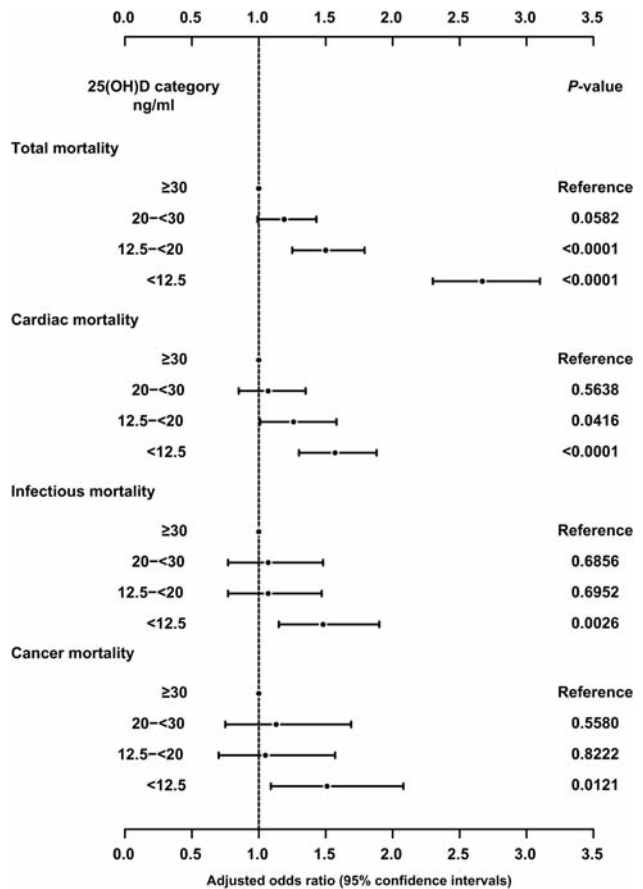


Figure 2. Adjusted odds ratio (with 95% confidence intervals) for mortality according to 25(OH)D level (see also Table III).

observation period, clinical practice changed in line with advances in research on vitamin D and its additional pleiotropic effects, thus promoting revision of clinical practice guidelines.

**Robustness of findings.** Sensitivity analysis was performed to study the robustness of findings. Cox regression was applied to two subsamples of the incidence cohorts 1997-2006 which consistently confirmed the elevated mortality risk according to 25(OH)D deficiency. The analysis was also replicated using the median of the 25(OH)D readings over time instead of the last recorded value for each patient. Again, the original findings were confirmed. The results of the sensitivity analysis are presented elsewhere (31).

## Conclusion

The Institute of Medicine (IOM) classifies patients with chronic kidney disease as a high-risk group for vitamin D deficiency (9). The findings of this evaluation highlight the need to ensure an optimal vitamin D status especially in patients with end-stage kidney disease on hemodialysis. Therefore, this evaluation supports the notion that cholecalciferol supplementation,



according to current clinical guidelines, is an essential part of the treatment of patients undergoing hemodialysis. This could easily be addressed by regular oral intake to ensure pleiotropic effects on cardiovascular and infectious comorbidities and survival advantage (3, 5, 6, 18, 32).

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