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Metabolic alterations in dialysis patients

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CHAPTER

Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events and mortality in haemodialysis patients

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Abstract

Aims: Dialysis patients experience an excess mortality, predominantly of sudden cardiac death (SCD). Accumulating evidence suggests a role of vitamin D for myocardial and overall health. This study investigated the impact of vitamin D status on cardiovascular outcomes and fatal infections in haemodialysis patients.

Methods and Results: 25-hydroxyvitamin D (25[OH]D) was measured in 1109 diabetic haemodialysis patients who participated in the German Diabetes and Dialysis Study (4D Study) and were followed-up for a median of 4 years. By Cox regression analyses, we determined hazard ratios (HR) for pre-specified, adjudicated endpoints according to baseline 25(OH)D levels: SCD (n=146), myocardial infarction (MI, n=174), stroke (n=89), cardiovascular events (CVE; n=414), death due to heart failure (n=37), fatal infection (n=111) and all-cause mortality (n=545). Patients had a mean age of 66±8 years (54% male), and mean 25(OH)D of 18.0±9.8 ng/ml. Patients with severe vitamin D deficiency (25[OH]D ≤ 10ng/ml) had a 3fold higher risk of SCD compared to those with sufficient 25(OH)D levels >30ng/ml (HR 3.0; 95% confidence interval 1.4-6.4). Furthermore, CVE and all-cause mortality were strongly increased (HR 1.8, 95% CI 1.2-2.7, and HR 1.7, 95% CI 1.2-2.5, respectively), all persisting in multivariate models. There was a trend for higher risks of stroke and fatal infection, while MI and deaths due to heart failure were not significantly affected.

Conclusions: Severe vitamin D deficiency was strongly associated with SCD, CVE and mortality, and by trend with stroke and fatal infection in diabetic haemodialysis patients. Whether vitamin D supplementation decreases adverse outcomes, requires further evaluation.

Introduction

Vitamin D deficiency is observed in the vast majority of haemodialysis patients and there is accumulating evidence that vitamin D, beyond its effects on bone and mineral metabolism, is also crucial for cardiovascular health and protection against infectious diseases.¹⁻⁹ In general, vitamin D from either ultraviolet-B induced synthesis in the skin or from nutritional intake is hydroxylated to 25-hydroxyvitamin D (25[OH]D) in the liver. 25(OH)D circulates in up to 1000 fold higher concentrations than the most potent vitamin D metabolite 1,25-dihydroxyvitamin D (1,25[OH]2D) (calcitriol).^{8,9} The renal production of 1,25(OH)2D is tightly controlled by homeostatic mechanisms but becomes significantly dependent on substrate availability when circulating 25(OH)D are low.⁸ In addition, various extrarenal tissues including the myocardium and vasculature have been shown to express 1 α -hydroxylase and are thus capable of producing large amounts of 1,25(OH)2D.^{8,9} Of note, locally produced 1,25(OH)2D, the synthesis of which is dependent on circulating 25(OH)D levels, exerts its effects predominantly in an autocrine and paracrine manner thereby regulating approximately three percent of the human genome.^{8,9,11}

In patients with chronic kidney disease (CKD) limited sunlight exposure and reduced capacity of the skin to synthesize vitamin D as well as loss of vitamin D binding protein in the urine are mainly responsible for the high prevalence of depressed 25(OH)D levels which are used to assess vitamin D status.¹⁻¹⁰ Given that the kidney is the main source for circulating 1,25(OH)2D, which is crucial for calcium and phosphorus as well as parathyroid hormone (PTH) homeostasis, 1,25(OH)2D and its analogues are routinely supplemented in many end-stage CKD patients and this therapy is associated with improved survival.¹¹ Beside the use of this therapy with active vitamin D, little attention has been paid in the past to 25(OH)D levels which can be raised by supplementation with the precursor vitamin D. Recent studies have, however, shown that in CKD low 25(OH)D levels are a significant risk factor for cardiovascular diseases and mortality.¹⁻⁷ In a nested case control study among 1000 incident haemodialysis patients there was a significantly increased 90 day mortality in the group with the lowest 25(OH)D

levels.⁴ However, data on Long-term mortality and specific cardiovascular events such as SCD or stroke, being significantly associated with vitamin D deficiency in patients undergoing coronary angiography,^{13,14} are lacking for haemodialysis patients. In view of the particularly high incidence of SCD in dialysis patients, accounting for one quarter of all deaths, such data are needed to gain a better understanding of the diagnostic and probably therapeutic implications of vitamin D in these patients. Hence we investigated the effect of Vitamin D levels on SCD in relation to other cardiac, vascular, and infection-related outcomes in a large well-characterized cohort of haemodialysis patients.¹⁵

Materials and methods

Study Design and Participants

The 4D study methodology has previously been reported in detail.¹⁵ Briefly, the 4D study was a prospective randomized controlled trial (RCT) including 1255 patients with type 2 diabetes mellitus, aged 18 – 80 years and on haemodialysis for less than 2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis centres in Germany. After a period of 4 weeks, patients were randomly assigned to double-blinded treatment with either 20 mg atorvastatin (n=619) or placebo (n=636) once daily. Study visits took place three times before randomization (visit 1-3), at randomization (visit 4), and at four weeks (visit 5) and every six months (visit 6 etc.) after randomization until the date of death, censoring, or end of the study in March 2004. The primary endpoint of the 4D study was defined as a composite of death from cardiac causes, fatal or nonfatal stroke and nonfatal myocardial infarction (MI), whichever occurred first (composite cardiovascular endpoint; CVE). Death from cardiac causes comprised SCD, fatal MI, death due to congestive heart failure (CHF), death due to coronary heart disease during or within 28 days after an intervention, and all other deaths ascribed to coronary heart disease. SCD was considered as: death verified by terminal rhythm disorders in an electrocardiogram; by witnesses observed death within one hour after the onset of cardiac symptoms; confirmed by autopsy;

unexpected death, presumably or possibly of cardiac origin and in the absence of a potassium level greater or equal to 7.5 mmol per liter before the start of the three most recent sessions of haemodialysis. MI was diagnosed when two of the following three criteria were met: typical symptoms, elevated levels of cardiac enzymes (i.e. a level of creatine kinase MB above 5 percent of the total level of creatine kinase, a level of lactic dehydrogenase 1.5 times the upper limit of normal, or a level of troponin T greater than 2 ng per milliliter), or diagnostic changes on the electrocardiogram. 4D Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria.

For the present analysis, SCD, MI (fatal and nonfatal), stroke (fatal and nonfatal), the primary endpoint (CVE), death due to CHF, death due to infection and all-cause mortality were all chosen to be separate outcome measures. The study complies with the Declaration of Helsinki, was approved by the medical ethical committee, and all patients gave their written informed consent before inclusion.

Data collection

Information on age, gender and smoking status was obtained through patient interviews. Smoking status was classified as never, former or current. Comorbidities, including the presence of coronary artery disease (CAD) and CHF, as well as the duration of diabetes mellitus and dialysis treatment were reported by the patients' nephrologists. CAD was defined by the history of MI, coronary artery bypass grafting surgery; percutaneous coronary intervention; and the presence of coronary heart disease, as documented by coronary angiography. Blood pressure was measured in sitting position. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Levels of 25(OH)D were measured in blood samples taken at baseline at study visit 3 (1 week before randomization) and stored at -80°C. Determinations in serum were performed by means of a chemiluminescence assay (IDS, iSYS 25-hydroxyvitamin D; Immunodiagnostic systems Ltd, Boldon, England) on an IDS-iSYS multi-discipline automated analyser. Within-day coefficients of variation (CV) were 5.5 to 12.1 %, and inter-day CV were 8.9 to 16.9 %, respectively. All blood samples were taken before the start of dialysis sessions and administration of drugs.

Statistical Analysis

Continuous variables were expressed as mean with standard deviation or median with interquartile range (IQR) as appropriate, and categorical variables were expressed as percentages.

The study population was divided into three groups according to their 25(OH)D status at baseline. In line with widely used cut-off values, patients were grouped into severely vitamin D deficient (≤ 10.0 ng/ml), moderately vitamin D deficient ($>10 \leq 30$ ng/ml), and vitamin D sufficient (>30 ng/ml).^{4,8,13} First, we assessed the association of baseline 25(OH)D with SCD, both as continuous and as categorical variable. For the latter, the patients with sufficient 25(OH)D levels were used as the reference group. Absolute (incidence) rates were calculated, and relative risks derived from Cox regression analyses, i.e. hazard ratios (HRs) and corresponding 95% confidence intervals. The Cox regression analyses were adjusted for the confounders age, sex, atorvastatin treatment, CAD, CHF, systolic blood pressure, smoking status, duration of dialysis, ultrafiltration volume, BMI, levels of LDL-cholesterol, HDL-cholesterol, C-reactive protein and glycohaemoglobin A1c. To account for the seasonal variation of vitamin D, we furthermore adjusted our analyses for the season of blood draw. We therefore used a binary variable reflecting the months October to March and April to September, respectively. Second, in order to explore possible pathways, we performed additional analyses with inclusion of potential intermediate conditions including levels of calcium, PTH and phosphate. The use of active vitamin D treatment was furthermore considered in the additional multivariate analyses. Third, we investigated vitamin D and the risk of other adverse cardiac and vascular outcomes including MI, stroke, the combined primary endpoint and death due to CHF. Furthermore, we evaluated the association of 25(OH)D levels with all-cause mortality and fatal infections. In addition, we used an alternative approach to account for the seasonal fluctuation of vitamin D and calculated z-values of logarithmically transformed 25(OH)D levels based on their means and standard deviation values within each month of blood sampling (formula for z-values: $X\text{-mean}/\text{standard deviation}$). Finally, to exclude potential interaction by atorvastatin treatment, we repeated our analyses stratified by medication.

All p-values are reported two-sided. Analyses were performed using SPSS version 16.0.

Results

Patient characteristics

Between March 1998 and October 2002, a total of 1255 patients were included into the 4D study, of whom 1109 had a measurement of 25(OH)D at baseline. The mean follow-up period was 3.96 years (median 4.0 years) on atorvastatin and 3.91 years (median 4.08 years) on placebo. During follow-up, 414 out of the 1109 patients reached the primary endpoint of CVE. A total of 545 patients died, of whom 146 patients died of SCD. Furthermore, 37 patients died due to CHF and 111 patients died due to infection. A total of 174 patients experienced a MI (fatal or non-fatal), and 89 patients experienced a stroke (fatal or non-fatal).

In the study population (n=1109), the mean (standard deviation) age was 66 (8) years and 54% of the patients were male. In general, the mean (standard deviation) level of 25(OH)D at baseline was 18.0 (9.8) ng/ml. As expected, we observed a seasonal variation of 25(OH)D in our patients, with the lowest concentrations in February (13.8 (10.8) ng/ml) and the highest concentrations in August (23.0 (13.0) ng/ml). The patient characteristics are shown in Table 1. Patients with severe vitamin D deficiency were more likely to be female, and had higher levels of glycohaemoglobin A_{1c}. Furthermore, the burden of left ventricular hypertrophy was higher, as were the levels of LDL-cholesterol and NT-pro-BNP in patients with severe vitamin D deficiency compared to patients with sufficient vitamin D levels.

Table 1: Baseline patient characteristics, presented per Vitamin D category; study population n=1109

Characteristic	Vitamin D		
	≤ 10 (n=177)	> 10 ≤ 30 (n=818)	> 30 (n=114)
Age <i>years</i>	66 (8)	66 (8)	65 (8)
Gender % <i>men</i>	49.2	53.6	67.5
25-hydroxyvitamin D <i>ng/mL</i>	8.0 (1.5)	17.1 (5.2)	39.8 (10.0)
Atorvastatin treatment %	52.0	49.6	49.1
Use of active Vitamin D %	22.6	17.9	14.9
Systolic BP* <i>mmHg</i>	146 (22)	146 (22)	147 (22)
Diastolic BP <i>mmHg</i>	76 (11)	76 (11)	77 (10)
BMI* <i>kg/m²</i>	27.4 (5.3)	27.6 (4.7)	27.0 (4.1)
Duration of diabetes <i>years</i>	19.9 (8.4)	17.7 (8.8)	17.4 (9.0)
Time on dialysis <i>months</i>	8.3 (7.0)	8.2 (6.8)	9.4 (7.4)
Smoker / Ex-smoker %	42.9	39.2	44.7
History of			
CAD* %	28.8	28.7	36.8
CHF* %	37.9	34.6	40.4
Presence of LVH* %	14.1	11.6	12.4
Ultrafiltration volume <i>kg</i>	2.16 (1.17)	2.27 (1.19)	2.22 (1.25)
Laboratory parameters			
LDL* cholesterol <i>mg/dL</i>	131 (29)	125 (30)	122 (28)
HDL* cholesterol <i>mg/dL</i>	37 (14)	36 (13)	38 (12)
Triglycerides <i>mg/dL</i>	244 (165-322)	224 (153-324)	185 (135-304)
Hemoglobin <i>g/dL</i>	11.0 (1.4)	10.9 (1.4)	10.9 (1.2)
Albumin <i>g/dL</i>	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)
C-reactive protein <i>mg/L</i>	4.9 (2.5-10.9)	5.2 (2.5-13.6)	4.5 (2.1-10.3)
HbA1c %	6.8 (1.3)	6.7 (1.3)	6.5 (1.2)
Calcium <i>mmol/L</i>	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)
Phosphate <i>mmol/L</i>	6.2 (1.5)	6.0 (1.6)	5.7 (1.6)
NT-pro-BNP <i>pg/mL</i>	3690 (1479-8080)	3411 (1447-9568)	2608 (1087-7026)

Values are presented as means (SD) or median (interquartile range) or %.

*Abbreviations: HbA1c = haemoglobin A1c; BP = blood pressure;

BMI = body mass index; CAD = coronary artery disease,

CHF = congestive heart failure; LVH = left ventricular hypertrophy

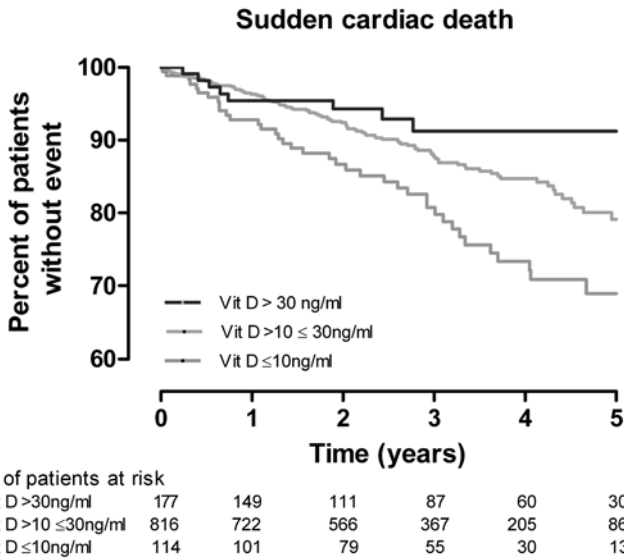
LDL = low density lipoprotein, HDL = high density lipoprotein,

NT-pro-BNP = N-terminal-pro-B-type natriuretic peptide

Vitamin D status and risk of sudden cardiac death

Vitamin D status at baseline was strongly associated with the risk of SCD (see Figure 1A). By Cox regression analyses, the unadjusted hazard to experience SCD was 3 fold higher in patients with severe vitamin D deficiency as compared to those with sufficient vitamin D levels (HR 3.0, 95% CI 1.4-6.4), Table 2). This association was virtually unchanged after controlling for potential confounders and seasonal variation of vitamin D (HR 2.9, 95% CI 1.3-6.3). Additional adjustment for markers of mineral metabolism including PTH, calcium and phosphate also did not materially change the results (HR 2.9, 95% CI 1.3-6.4), which persisted even after further adjustment for the use of active Vitamin D treatment (HR 3.1, 95% CI 1.4-6.7). When vitamin D was analysed as a continuous variable, the hazard to die suddenly increased by 60% per unit decrease in 25(OH)D levels (Table 3). This association persisted in multivariate analyses. To strengthen our results, we performed additional analyses using z-values as an alternative approach to account for the seasonal variation of vitamin D. The results were similar, confirming a strong association of 25(OH)D levels with the risk of SCD (data not shown).

A



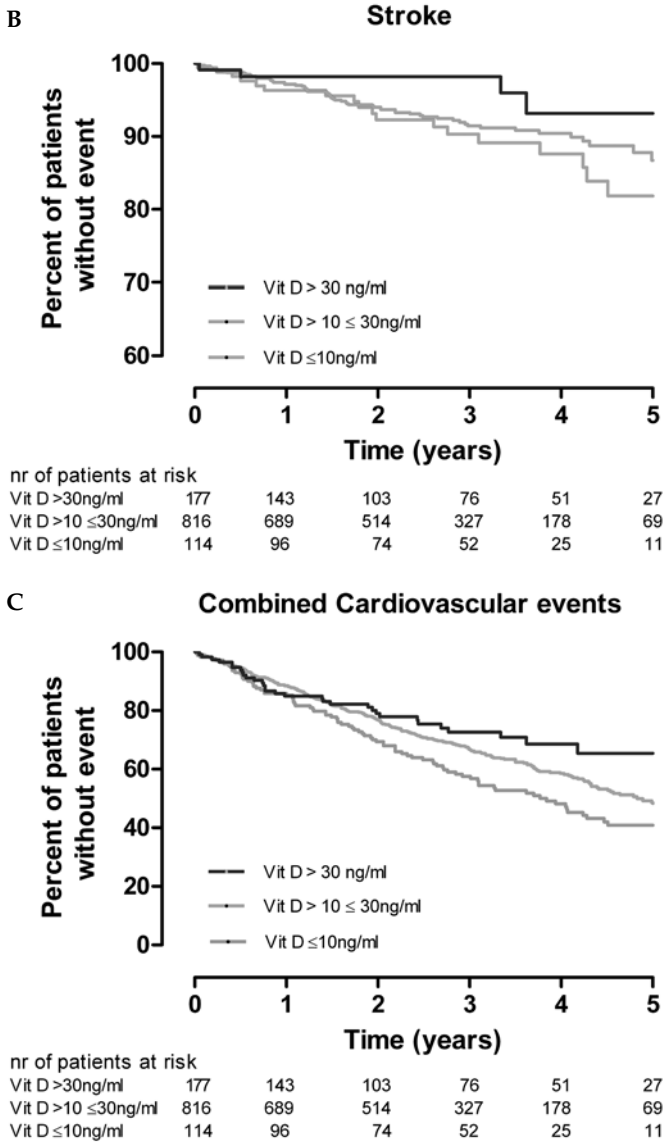


Figure 1: Kaplan-Meier curves for the time to A) sudden cardiac death, B) stroke, C) combined cardiovascular events in subgroups of patients according to 25(OH)Vitamin D levels at baseline (severely vitamin D deficient (≤ 10.0 ng/ml), moderately vitamin D deficient ($>10 \leq 30$ ng/ml), and vitamin D sufficient (>30 ng/ml))

Vitamin D status and risk of myocardial infarction, stroke, death due to heart failure, and combined cardiovascular events

There was a trend for higher risks of stroke with lower vitamin D levels (see Figure 1B). Per unit decrease in vitamin D, the risk of stroke increased by 30% after adjustment for confounders. In categorical analyses, patients with severe vitamin D deficiency had an almost 3 fold increased risk of stroke compared to those with normal levels (adjusted HR 2.9, 95% CI 0.9-10.0). In contrast, no association of vitamin D status with MI was found. Both in continuous (adjusted HR 1.1, 95% CI 0.8-1.5) and in categorical analyses, the risk of MI did not increase at lower levels of vitamin D (Tables 2 and 3). When non-fatal and fatal MI were analyzed separately, the results were similar showing no relation to vitamin D status. The number of deaths due to CHF was small in the present study (n=37). These deaths similarly were not meaningfully affected by vitamin D status.

Finally, the primary endpoint of combined CVE was markedly increased with lower levels of 25(OH)D (Table 2 and Figure 1C). Patients with severe vitamin D deficiency had an adjusted 80% higher risk of experiencing a CVE as compared to patients with sufficient vitamin D status (Table 3).

Vitamin D status and risk of death due to infection and all-cause mortality

Deaths due to infection were almost 2 fold increased in patients suffering from severe vitamin D deficiency (adjusted HR 1.9, 95% CI 0.8-4.6). Per unit decrease in log transformed vitamin D, the rate of fatal infections rose by 50% (adjusted HR 1.5, 95% CI 1.0-2.3). Furthermore, deaths due to all causes increased significantly by 40% per unit decrease in log vitamin D. Patients with levels below 10 ng/ml had an adjusted 90% higher risk of death as compared to patients with sufficient 25(OH)D levels (Tables 2 and 3).

To strengthen our results, we repeated all analyses in the placebo group only. The results were similar, supporting the use of the complete data.

Table 2: Absolute incidence rates, and hazard ratios with 95% confidence intervals (HR, 95% CI) for sudden cardiac death, stroke, myocardial infarction and death due to heart failure according to levels of 25-hydroxyvitamin D at baseline; n=1109

Outcome	Vitamin D <i>ng/mL</i>		
	≤10 (n=177)	>10 ≤30 (n=818)	>30 (n=114)
<i>Sudden cardiac death</i>			
Incidence rate / 100py	7.4	4.3	2.5
Crude HR (95% CI)	3.0 (1.4-6.4)	1.8 (0.9-3.6)	1
Adj. ¹ HR (95% CI)	2.9 (1.3-6.3)	1.7 (0.8-3.5)	1
<i>Stroke</i>			
Incidence rate / 100py	3.8	2.9	1.2
Crude HR (95% CI)	3.0 (1.0-8.8)	2.3 (0.9-6.4)	1
Adj. ¹ HR (95% CI)	2.9 (0.9-10.0)	2.3 (0.7-7.5)	1
<i>Myocardial infarction</i>			
Incidence rate / 100py	6.7	5.7	5.2
Crude HR (95% CI)	1.3 (0.7-2.4)	1.1 (0.7-1.8)	1
Adj. ¹ HR (95% CI)	1.4 (0.8-2.7)	1.2 (0.7-2.0)	1
<i>Death due to heart failure</i>			
Incidence rate / 100py	1.6	1.1	1.2
Crude HR (95% CI)	1.3 (0.4-4.2)	0.9 (0.3-2.5)	1
Adj. ¹ HR (95% CI)	1.5 (0.4-5.0)	0.8 (0.3-2.4)	1
<i>Cardiovascular events</i>			
Incidence rate / 100py	18.2	13.8	10.2
Crude HR (95% CI)	1.8 (1.2-2.7)	1.4 (0.9-2.0)	1
Adj. ¹ HR (95% CI)	1.8 (1.2-2.8)	1.4 (0.9-2.0)	1
<i>Death due to infection</i>			
Incidence rate / 100py	4.3	3.5	2.2
Crude HR (95% CI)	1.9 (0.8-4.5)	1.7 (0.8-3.7)	1
Adj. ¹ HR (95% CI)	1.9 (0.8-4.6)	1.6 (0.7-3.4)	1
<i>All-cause mortality</i>			
Incidence rate / 100py	22.9	16.6	13.0
Crude HR (95% CI)	1.7 (1.2-2.5)	1.3 (0.9-1.8)	1
Adj. ¹ HR (95% CI)	1.9 (1.3-2.7)	1.3 (0.9-1.8)	1

¹ model 1: adjusted for age, sex, atorvastatin treatment, season, coronary artery disease, congestive heart failure, systolic blood pressure, smoking, duration of dialysis, ultrafiltration volume, body mass index, levels of LDL, HDL cholesterol, C-reactive protein, HbA1c

Table 3: Risk of cardiovascular events, sudden cardiac death, stroke, myocardial infarction, death due to heart failure, death due to infection and all-cause mortality per unit decrease in 25-hydroxyvitamin D (continuous variable, log transformed); study population n=1109

Outcome	Hazard ratio (HR) and 95% CI		
	crude	adjusted ¹	adjusted ²
<i>Sudden cardiac death</i>	1.6 (1.1-2.2) p=0.007	1.5 (1.1-2.2) p=0.019	1.6 (1.1-2.2) p=0.016
<i>Stroke</i>	1.4 (0.9-2.2) p=0.09	1.3 (0.8-2.1) p=0.23	1.3 (0.8-2.1) p=0.26
<i>Myocardial infarction</i>	1.0 (0.7-1.3) p=0.96	1.1 (0.8-1.5) p=0.71	1.0 (0.8-1.4) p=0.83
<i>Death due to heart failure</i>	1.0 (0.5-2.0) p=0.90	1.1 (0.5-1.9) p=0.83	1.0 (0.5-2.0) p=0.94
<i>Cardiovascular events</i>	1.2 (1.0-1.4) p=0.11	1.2 (1.0-1.5) p=0.12	1.2 (0.9-1.4) p=0.20
<i>Death due to infection</i>	1.4 (1.0-2.1) p=0.06	1.5 (1.0-2.3) p=0.05	1.4 (1.0-2.2) p=0.08
<i>All-cause mortality</i>	1.4 (1.2-1.6) p<0.001	1.4 (1.2-1.7) p<0.001	1.4 (1.2-1.7) p<0.001

¹ model 1: adjusted for age, sex, atorvastatin treatment, season, coronary artery disease, congestive heart failure, systolic blood pressure, smoking, duration of dialysis, ultrafiltration volume, body mass index, levels of LDL, HDL cholesterol, C-reactive protein, HbA1c

² model 2: additional adjustments for PTH, Calcium and Phosphate

Discussion

We have shown that vitamin D deficiency is an independent risk factor for SCD, CVE and all-cause mortality in diabetic haemodialysis patients. These associations were independent of common known risk factors. In patients with severe vitamin D deficiency there was also a remarkable trend towards increased risks of stroke and fatal infections.

Our study is the first to highlight the role of vitamin D deficiency as a risk factor of adverse long-term outcomes in diabetic haemodialysis patients. These data are in line with observations in other cohorts of CKD patients,²⁻⁷ in patients referred for coronary angiography,^{13,14} as well as in population based cohorts.¹⁶⁻¹⁹ These latter studies showed an increased risk of mortality and/or CVE in individuals with low 25(OH)D levels.^{2-7,13,14,16-19} Given that most haemodialysis patients are vitamin D deficient, we believe that our findings might have significant clinical implications when considering that natural vitamin D supplementation is considered a relatively safe, easy and cheap therapy.²⁰ We are aware of a history of promising data on risk factors of mortality in haemodialysis patients leading to the initiation of RCTs which failed to show significant effects of targeted treatments.²¹ Without claiming causality for our findings we want to stress that vitamin D exerts various effects which might, in a causal manner, underlie harmful consequences of vitamin D deficiency.¹¹

First, we want to point out that classic effects of vitamin D related to calcium and phosphorus homeostasis as well as PTH regulation might of course play an important role for cardiovascular risk in CKD.²² Reduced vitamin D metabolites lead to hypocalcaemia and secondary hyperparathyroidism, which is associated with increased mortality risk.^{12,23} In this context, previous studies indicate that natural vitamin D or 25(OH)D supplementation might have beneficial effects on mineral metabolism including reductions in PTH levels.^{12,24} Results on this latter topic are, however, inconsistent and further studies are required.^{12,24} Interestingly, our prospective results did not materially change even after adjustments for various parameters of mineral metabolism suggesting that other mechanisms might have mainly driven the association of low 25(OH)D and adverse outcomes in the 4D study. Associations of vitamin D deficiency with cardiovascular risk factors including type 2 diabetes mellitus,²⁵ arterial hypertension,²⁶ malnutrition and inflammation may hypothetically explain the increased mortality risk in patients with low 25(OH)D levels. Adjustments for these latter risk factors had, however, only little impact on our prospective analyses. Hence other mechanisms may be relevant. Data from the Multi-Ethnic Study of Atherosclerosis suggest that vitamin D deficiency is prospectively associated with increased risk of

coronary artery calcification.²⁷ This relationship seemed to be stronger for patients with lower estimated GFR and there was no significant association of 1,25(OH)₂D and coronary artery calcification.²⁷ Vitamin D might exert direct anti-atherosclerotic effects on endothelial and vascular smooth muscle cells as well as on macrophages whose foam cell formation was inhibited by 1,25(OH)₂D.^{28,29} The strong association of vitamin D deficiency with SCD but not with MI might, however, suggest that atherosclerosis related to vitamin D deficiency might not be the main pathophysiological link for our findings. Direct vitamin D effects on the myocardium, which expresses the VDR as well as 1 α -hydroxylase,^{30,31} may therefore be of importance. Experimental animal studies revealed myocardial hypertrophy and dysfunction with a hypercontractile state in both conditions of vitamin D deficiency as well as in VDR knockout models,^{11,32,33} even if VDR knockout was exclusively performed in cardiomyocytes.³⁴ Clinical studies confirmed associations of vitamin D deficiency with CHF and in particular with diastolic dysfunction but our results regarding heart failure deaths, which are limited by relatively low numbers of events, do not support an important role of vitamin D in this context.^{30,35,36} Furthermore, altered myocardial calcium flux and increased risk of SCD related to a poor vitamin D status suggest a link to cardiac arrhythmias.^{13,30} This notion is in line with observations in haemodialysis patients showing that calcitriol reduced a prolonged QT interval, which is a risk factor for SCD, the single largest cause of death in dialysis patients.^{37,38} Apart from this, detrimental consequences of vitamin D deficiency might also be mediated by an increased risk of infections, which is supported by our data showing an important trend for fatal infections in patients with severe vitamin D deficiency.³⁹ Our data should be viewed in light of a meta-analysis, which showed significantly improved survival of natural vitamin D supplementation in individuals without end-stage renal failure.⁴⁰ We are aware that findings from RCTs among patients free of advanced CKD cannot be uncritically extrapolated to haemodialysis patients.^{12,21} We also want to underline that physicians, impressed by previous data in favour of multiple health benefits of natural vitamin D and by the magnitude of the present observed associations should not abstain from future RCTs, which are urgently needed. Unless these trial results are published our vitamin D

prescription among haemodialysis patients should be guided by considerations that weight the probable benefit versus the probable risks and costs of this therapy. Supplementation of natural vitamin D to reach proposed optimal 25(OH)D levels of 30 to 60 ng/mL is considered safe and it should be kept in mind that sunbathing can produce up to 20,000 IU vitamin D per day.^{8,9,20} This latter dose is much higher than required to reach 25(OH)D target levels by using the rule of thumb that 1,000 IU vitamin D can increase 25(OH)D levels by approximately 10 ng/mL in patients without severe CKD.^{8,9,20}

Our data are limited due to the observational nature of our study which precludes any conclusion regarding cause and effect relationships of our observed results. Despite extensive adjustments we cannot exclude residual confounding. Another drawback of our work is that due to lack of data we were not able to study interactions and confounding by levels of 1,25(OH)₂D or fibroblast growth factor-23 (FGF-23) which suppresses 1,25(OH)₂D synthesis.⁴¹ Associations of 25(OH)D and outcome measures were, however, independent of parameters of mineral metabolism and the use of active vitamin D treatment.

In conclusion, we observed that low 25(OH)D levels are associated with increased risks of SCD, CVE and mortality, and there was furthermore a trend for higher risks of stroke and fatal infections. The magnitude of the observed associations, as well as previous data in favour of multiple health benefits of natural vitamin D, point to the urgent need for an RCT. Such study can clarify whether the relatively easy, safe and cheap supplementation therapy with vitamin D can decrease adverse outcomes, in particular SCD, in haemodialysis patients.

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