

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

Christiane Drechsler^{1,2*†}, Stefan Pilz^{3†}, Barbara Obermayer-Pietsch³, Marion Verduijn², Andreas Tomaschitz³, Vera Krane¹, Katharina Espe⁴, Friedo Dekker², Vincent Brandenburg⁵, Winfried März^{6,7}, Eberhard Ritz⁸, and Christoph Wanner¹

¹Department of Internal Medicine 1, Division of Nephrology, University of Würzburg, Oberdürrbacher Str. 6, D-97080 Würzburg, Germany; ²Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; ³Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria; ⁴Institute of Nutritional Science, University of Potsdam, Potsdam, Germany; ⁵Department of Cardiology, University Hospital of the RWTH Aachen, Aachen, Germany; ⁶Synlab Centre of Laboratory Diagnostics, Heidelberg, Germany; ⁷Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; and ⁸Ruperto Carola University Heidelberg, Division of Nephrology, Nierenzentrum, Heidelberg, Germany

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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 1108 diabetic haemodialysis patients who participated in the German Diabetes and Dialysis 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 median 25(OH)D of 39 nmol/L (interquartile range: 28–55). Patients with severe vitamin D deficiency [25(OH)D of ≤ 25 nmol/L] had a 3-fold higher risk of SCD compared with those with sufficient 25(OH)D levels >75 nmol/L [HR: 2.99, 95% confidence interval (CI): 1.39–6.40]. Furthermore, CVE and all-cause mortality were strongly increased (HR: 1.78, 95% CI: 1.18–2.69, and HR: 1.74, 95% CI: 1.22–2.47, respectively), all persisting in multivariate models. There were borderline non-significant associations with stroke and fatal infection while MI and deaths due to heart failure were not meaningfully affected.

Conclusion

Severe vitamin D deficiency was strongly associated with SCD, CVE, and mortality, and there were borderline associations with stroke and fatal infection. Whether vitamin D supplementation decreases adverse outcomes requires further evaluation.

Keywords

Vitamin D • Sudden cardiac death • Mortality • Dialysis • Kidney • Cardiovascular

* Corresponding author. Tel: +49 931 201 39030, Fax: +49 931 201 639300, Email: c.drechsler@gmx.net

† Both authors contributed equally to this work.

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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 3% of the human genome.^{8–10}

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.^{1–9,11} 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.¹⁰ Besides 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 (CVDs) 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 (CVE), such as sudden cardiac death (SCD) or stroke, being significantly associated with vitamin D deficiency in patients undergoing coronary angiography,^{12,13} 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 25(OH)D levels on SCD in relation to other cardiac, vascular, and infection-related outcomes in a large well-characterized cohort of haemodialysis patients.¹⁴

Methods

Study design and participants

The German Diabetes and Dialysis Study (4D study) methodology has previously been reported in detail.¹⁴ 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 <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 of atorvastatin ($n = 619$) or placebo ($n = 636$) once daily. Study visits took place three times before randomization (Visits 1–3), at randomization (Visit 4), and at 4 weeks (Visit 5), and every 6 months (Visit 6) 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 non-fatal stroke, and non-fatal 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. Sudden cardiac death was considered as: death verified by terminal rhythm disorders in an electrocardiogram; by witnesses observed death within 1 h 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 ≥ 7.5 mmol/L before the start of the three most recent sessions of haemodialysis. Myocardial infarction 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% 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 >2 ng/mL), or diagnostic changes on the electrocardiogram. German Diabetes and Dialysis Study endpoints were centrally adjudicated by three members of the endpoint committee blinded to study treatment and according to pre-defined criteria.

In the present analysis, SCD, MI (fatal and non-fatal), stroke (fatal and non-fatal), 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 Ethics 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. Coronary artery disease 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. Left ventricular hypertrophy (LVH) was based on ECG criteria. 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 serum samples taken at baseline at study Visit 3 (1 week before randomization) and stored without repeated freeze thaw cycles at -80°C until measurement in November 2009. Determinations in serum were performed by means of a chemiluminescence assay (IDS, iSYS 25(OH)D; Immunodiagnostic systems Ltd, Boldon, UK) on an IDS-iSYS multi-discipline automated analyser. Within-day coefficients of variation (CV) were 5.5–12.1% and inter-day CV were 8.9–16.9%, 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 as appropriate, and categorical variables were expressed as percentages.

The study population was divided into four 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 (≤ 25 nmol/L), moderately vitamin D deficient (> 25 and ≤ 50 nmol/L), vitamin D insufficient (> 50 and ≤ 75 nmol/L), and vitamin D sufficient (> 75 nmol/L, to convert nanomolar to nanogram per millilitre divide by 2.496).^{4,8,12} For comparisons across baseline vitamin D groups, we used ANOVA and χ^2 test, as appropriate. In our prospective analyses, 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 the relative risks derived from Cox regression analyses, i.e. hazard ratios (HR) and corresponding 95% confidence intervals (CI). The Cox regression analyses were adjusted for the confounders age, sex, atorvastatin treatment, use of angiotensin-converting enzyme inhibitors, beta-blockers, and diuretics, CAD, CHF, systolic blood pressure, smoking status, duration of dialysis, ultrafiltration volume, BMI, levels of low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, C-reactive protein, and glycohaemoglobin A1c. To account for the seasonal variation of 25(OH)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. Second, to explore the 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 additional multivariate analyses. Third, we investigated 25(OH)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 25(OH)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, we tested for interactions of statin use (yes/no), active vitamin D treatment (yes/no), prior CVD (yes/no), and event rates in our prospective analyses by adding product terms to our Cox proportional hazards models.

All *P*-values are reported two-sided, and a *P*-value < 0.05 was considered statistically significant. 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 1108 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 of the 1108 patients reached the primary endpoint of CVE. In our primary analyses, there was no significant effect of atorvastatin treatment on CVE.¹⁴ 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 an MI (fatal or non-fatal) and 89 patients experienced a stroke (fatal or non-fatal).

In the study population ($n = 1108$), the mean (standard deviation) age was 66 (8) years and 54% of the patients were male. In general, the median (interquartile) level of 25(OH)D at baseline was 39 nmol/L (28–55). As expected, we observed a seasonal variation of 25(OH)D in our patients, with the lowest median concentrations of 31 nmol/L in February and the highest median concentrations of 50 nmol/L in August. 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 A1c. Furthermore, the burden of LVH was higher, as were the levels of LDL-cholesterol and N-terminal-pro-B-type natriuretic peptide in patients with severe vitamin D deficiency compared with patients with sufficient 25(OH)D levels.

Vitamin D status and risk of sudden cardiac death

Vitamin D status at baseline was strongly associated with the risk of SCD (*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 with those with sufficient 25(OH)D levels (HR: 2.99, 95% CI: 1.39–6.40, *Table 2*). This association was virtually unchanged after controlling for potential confounders and seasonal variation of 25(OH)D (HR: 2.95, 95% CI: 1.35–6.46). Additional adjustment for markers of mineral metabolism including PTH, calcium, and phosphate also did not materially change the results (HR: 3.00, 95% CI: 1.36–6.60), which persisted even after further adjustment for the use of active vitamin D treatment (HR: 3.04, 95% CI: 1.38–6.70). When 25(OH)D was analysed as a continuous variable, the hazard to die suddenly increased by 59% 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 25(OH)D. The results were similar, confirming a strong association of low 25(OH)D levels with the risk of SCD (data not shown).

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 25(OH)D levels (*Figure 1B*). Per unit decrease in 25(OH)D, the risk of stroke increased by 30% after adjustment for confounders. In categorical analyses, patients with severe vitamin D deficiency had a 2.8-fold increased risk of stroke compared with those with normal levels (adjusted HR: 2.83, 95% CI: 0.82–9.80). In contrast, no association of vitamin D status with MI was found. Both in continuous (adjusted HR: 1.04, 95% CI: 0.76–1.44) and in categorical analyses, the risk of MI did not increase at lower levels of 25(OH)D (*Tables 2 and 3*). When non-fatal and fatal MI were analysed 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.

Table 1 Baseline patient characteristics, presented per vitamin D category; study population (n = 1108)

Characteristic	Vitamin D category [25(OH)D levels in nmol/L]				P-value
	≤25 (n = 177)	>25 and ≤50 (n = 607)	>50 and ≤75 (n = 210)	>75 (n = 114)	
Age (years)	66 (8)	66 (8)	65 (8)	65 (8)	0.395
Gender (% (men))	49.2	49.1	66.2	67.5	<0.001
25(OH)D (nmol/L)	20 (17–23)	35 (30–42)	61 (56–67)	94 (83–104)	<0.001
Atorvastatin treatment (%)	52.0	49.8	49.0	49.1	0.940
Use of active vitamin D (%)	22.6	16.8	21.0	14.9	0.175
Systolic BP (mmHg)	146 (22)	146 (23)	144 (20)	147 (22)	0.772
Diastolic BP (mmHg)	76 (11)	76 (11)	76 (11)	77 (10)	0.495
BMI (kg/m ²)	27.4 (5.3)	27.7 (4.7)	27.5 (4.6)	27.0 (4.1)	0.505
Duration of diabetes (years)	19.9 (13.5–25.7)	18.0 (11.4–23.8)	16.3 (10.0–23.2)	18.3 (9.9–22.5)	0.003
Time on dialysis (months)	5.7 (3.2–12.1)	6.0 (2.9–11.3)	5.8 (3.1–11.7)	7.3 (3.9–12.1)	0.336
Ultrafiltration volume (kg)	2.0 (1.2–3.0)	2.0 (1.1–3.0)	2.0 (2.0–3.0)	2.0 (1.0–3.0)	0.420
Smoker/ex-smoker (%)	42.9	37.8	43.3	44.7	0.247
History of					
CAD (%)	28.8	27.3	32.4	36.8	0.159
CHF (%)	37.9	35.9	31.0	40.4	0.320
Presence of LVH (%)					
NYHA class I (%)	66.1	72.0	76.1	61.4	0.039
NYHA class II (%)	27.7	23.4	17.6	31.6	
NYHA class III (%)	6.2	4.6	5.7	7.0	
NYHA class IV (%)	0.0	0.0	0.5	0.0	
Beta-blockers (%)	26.0	35.3	44.3	48.2	<0.001
ACE inhibitors (%)	41.8	45.8	52.4	57.0	0.027
Diuretics (%)	80.8	81.2	77.6	75.4	0.418
Laboratory parameters					
LDL-cholesterol (mg/dL)	128 (111–145)	122 (104–145)	121 (103–141)	118 (101–142)	0.023
HDL-cholesterol (mg/dL)	35 (28–44)	33 (27–42)	33 (26–42)	36 (30–44)	0.083
Triglycerides (mg/dL)	244 (165–322)	225 (148–325)	222 (160–328)	185 (135–302)	0.110
Haemoglobin (g/dL)	11.0 (1.4)	10.9 (1.4)	11.0 (1.3)	10.9 (1.2)	0.533
Albumin (g/dL)	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)	0.063
C-reactive protein (mg/L)	4.9 (2.5–10.9)	5.2 (2.5–13.3)	5.3 (2.4–14.2)	4.5 (2.1–10.3)	0.489
HbA1c (%)	6.8 (1.3)	6.8 (1.3)	6.6 (1.1)	6.5 (1.2)	0.051
Calcium (mmol/L)	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)	0.161
Phosphate (mmol/L)	6.2 (1.5)	6.0 (1.6)	6.1 (1.6)	5.7 (1.6)	0.060
NT-pro-BNP (pg/mL)	3690 (1479–8080)	3523 (1524–10 396)	3036 (1171–7659)	2608 (1087–7026)	0.010

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

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.

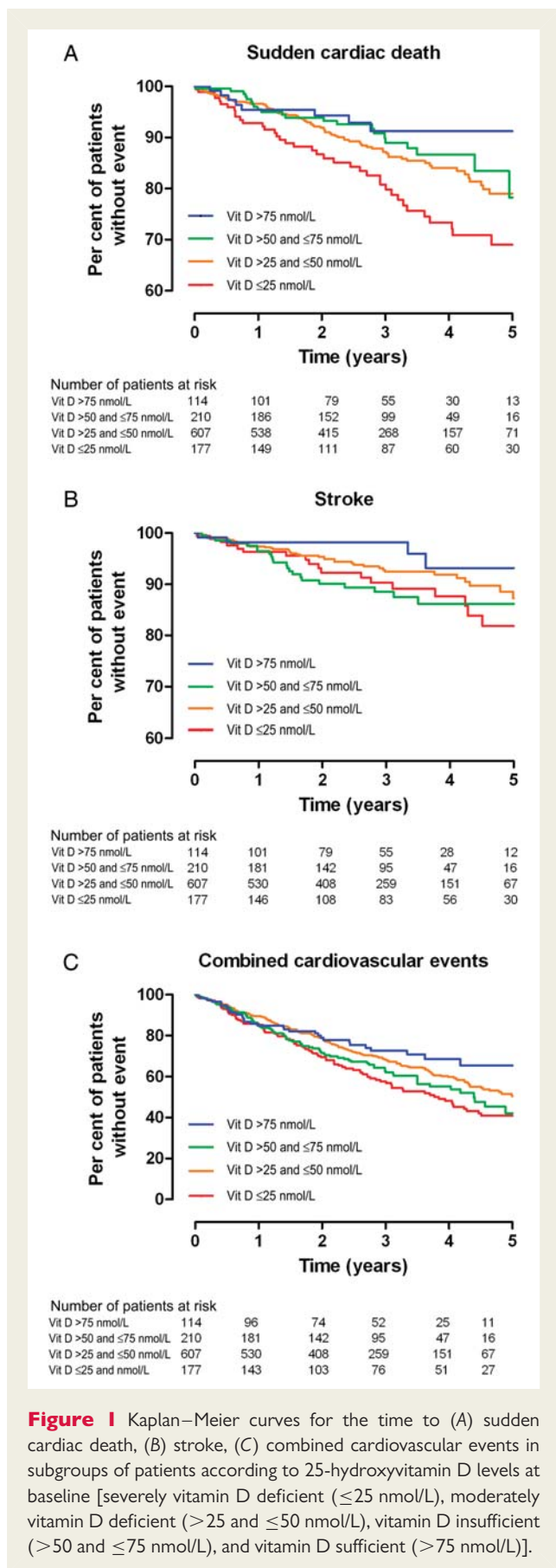


Figure 1 Kaplan–Meier curves for the time to (A) sudden cardiac death, (B) stroke, (C) combined cardiovascular events in subgroups of patients according to 25-hydroxyvitamin D levels at baseline [severely vitamin D deficient (≤ 25 nmol/L), moderately vitamin D deficient (> 25 and ≤ 50 nmol/L), vitamin D insufficient (> 50 and ≤ 75 nmol/L), and vitamin D sufficient (> 75 nmol/L)].

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 with 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: 1.92, 95% CI: 0.82–4.51). Per unit decrease in log transformed 25(OH)D, the rate of fatal infections rose by 46% (adjusted HR: 1.46, 95% CI: 0.96–2.23). Furthermore, deaths due to all causes increased significantly by 39% per unit decrease in log 25(OH)D. Patients with levels < 25 nmol/L had an adjusted 79% higher risk of death as compared with patients with sufficient 25(OH)D levels (Tables 2 and 3).

There were no significant interactions of statin use, active vitamin D treatment as well as prior CVD in our prospective analyses indicating that the association of 25(OH)D levels with event rates is not different in patients with and without statin or active vitamin D treatment as well as prior CVD.

Linearity assumptions for all Cox regression analyses were evaluated by log-minus-log survival plots and partial (Schönfeld) residuals vs. survival time plots and found valid.

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. There were also borderline non-significant associations of severe vitamin D deficiency with increased risk 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. Strengths of our study are the large sample size, the well-characterized cohort of diabetic haemodialysis patients, and the detailed long-term follow-up. Our data are in line with observations in other cohorts of CKD patients,^{2–7} inpatients referred for coronary angiography,^{12,13} as well as in population-based cohorts.^{15–18} These latter studies showed an increased risk of mortality and/or CVE in individuals with low 25(OH)D levels.^{2–7,12,13,15–18} Previous observations on specifically strong associations of vitamin D deficiency with MI and heart failure could, however, not be clearly confirmed in the 4D study.^{12,16} 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.¹⁹ 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.²⁰ Without claiming causality for our findings, we want to stress that vitamin D exerts various effects that might, in a causal manner, underlie harmful consequences of vitamin D deficiency.¹⁰

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 = 1108)

Outcome	Vitamin D (nmol/L)			
	≤25 (n = 177)	>25 and ≤50 (n = 607)	>50 and ≤75 (n = 210)	>75 (n = 114)
Sudden cardiac death				
Incidence rate/100 persons-years py	7.4	4.5	3.7	2.5
Crude HR (95% CI)	2.99 (1.39–6.40)	1.83 (0.89–3.79)	1.49 (0.66–3.35)	1
Adjusted ^a HR (95% CI)	2.95 (1.35–6.46)	1.71 (0.82–3.60)	1.54 (0.68–3.50)	1
Adjusted ^b HR (95% CI)	3.00 (1.36–6.60)	1.74 (0.83–3.65)	1.54 (0.68–3.51)	1
Stroke				
Incidence rate/100 py	3.8	2.6	3.8	1.2
Crude HR (95% CI)	2.97 (1.01–8.75)	2.08 (0.75–5.78)	3.11 (1.07–9.03)	1
Adjusted ^a HR (95% CI)	2.83 (0.82–9.80)	1.92 (0.58–6.27)	3.53 (1.04–11.92)	1
Adjusted ^b HR (95% CI)	2.58 (0.74–8.98)	1.79 (0.55–5.90)	3.26 (0.96–11.07)	1
Myocardial infarction				
Incidence rate/100py persons-years	6.7	5.2	7.1	5.2
Crude HR (95% CI)	1.29 (0.71–2.34)	1.00 (0.59–1.71)	1.37 (0.76–2.45)	1
Adjusted ^a HR (95% CI)	1.40 (0.76–2.59)	1.07 (0.62–1.84)	1.44 (0.80–2.60)	1
Adjusted ^b HR (95% CI)	1.27 (0.68–2.36)	1.01 (0.58–1.74)	1.34 (0.74–2.42)	1
Death due to heart failure				
Incidence rate/100py persons-years	1.6	1.0	1.2	1.2
Crude HR (95% CI)	1.27 (0.38–4.23)	0.85 (0.29–2.50)	0.95 (0.28–3.25)	1
Adjusted ^a HR (95% CI)	1.26 (0.36–4.43)	0.72 (0.23–2.22)	0.89 (0.25–3.12)	1
Adjusted ^b HR (95% CI)	0.96 (0.27–3.41)	0.61 (0.20–1.90)	0.67 (0.19–2.44)	1
Cardiovascular events				
Incidence rate/100py persons-years	18.2	13.0	16.1	10.2
Crude HR (95% CI)	1.78 (1.18–2.69)	1.28 (0.88–1.87)	1.59 (1.05–2.40)	1
Adjusted ^a HR (95% CI)	1.75 (1.14–2.69)	1.24 (0.84–1.83)	1.65 (1.08–2.51)	1
Adjusted ^b HR (95% CI)	1.59 (1.03–2.45)	1.18 (0.80–1.75)	1.53 (1.01–2.34)	1
Death due to infection				
Incidence rate/100py persons-years	4.3	3.6	3.5	2.2
Crude HR (95% CI)	1.92 (0.82–4.51)	1.67 (0.76–3.65)	1.66 (0.71–3.91)	1
Adjusted ^a HR (95% CI)	1.85 (0.77–4.43)	1.51 (0.68–3.36)	1.61 (0.68–3.84)	1
Adjusted ^b HR (95% CI)	1.55 (0.64–3.73)	1.35 (0.61–3.02)	1.34 (0.56–3.23)	1
All-cause mortality				
Incidence rate/100py persons-years	22.9	17.1	15.1	13.0
Crude HR (95% CI)	1.74 (1.22–2.47)	1.32 (0.96–1.83)	1.17 (0.81–1.69)	1
Adjusted ^a HR (95% CI)	1.79 (1.24–2.59)	1.26 (0.90–1.76)	1.25 (0.86–1.81)	1
Adjusted ^b HR (95% CI)	1.65 (1.14–2.38)	1.20 (0.86–1.68)	1.16 (0.80–1.70)	1

^aModel 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, use of beta-blockers, ACE inhibitors, and diuretics.

^bModel 2: additionally adjusted for levels of parathyroid hormone, calcium, and phosphate.

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.²¹ Reduced vitamin D metabolites lead to hypocalcaemia and secondary hyperparathyroidism, which is associated with increased mortality risk.^{22–24} 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.^{22,25} Results on this latter topic are, however, inconsistent and further studies are required.^{22,25} Interestingly, our prospective results did not materially change even after adjustments for various parameters of mineral metabolism

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 = 1108)

Outcome	Hazard ratio (HR) and 95% CI		
	Crude	Adjusted ^a	Adjusted ^b
Sudden cardiac death	1.59 (1.14–2.23) P = 0.007	1.55 (1.08–2.23) P = 0.017	1.58 (1.09–2.27) P = 0.015
Stroke	1.45 (0.94–2.23) P = 0.09	1.30 (0.81–2.07) P = 0.27	1.27 (0.79–2.03) P = 0.32
Myocardial infarction	0.99 (0.73–1.35) P = 0.96	1.04 (0.76–1.44) P = 0.79	1.02 (0.74–1.40) P = 0.92
Death due to heart failure	0.96 (0.50–1.85) P = 0.90	0.97 (0.47–2.00) P = 0.94	0.93 (0.45–1.92) P = 0.84
Cardiovascular events	1.18 (0.96–1.43) P = 0.11	1.15 (0.93–1.42) P = 0.19	1.12 (0.91–1.38) P = 0.29
Death due to infection	1.44 (0.98–2.12) P = 0.06	1.46 (0.96–2.23) P = 0.07	1.42 (0.93–2.15) P = 0.10
All-cause mortality	1.38 (1.16–1.64) P < 0.001	1.39 (1.15–1.68) P = 0.001	1.36 (1.13–1.64) P = 0.001

^aModel 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, use of beta-blockers, ACE inhibitors, and diuretics.

^bModel 2: additional adjustments for PTH, calcium, and phosphate.

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,²⁶ arterial hypertension,²⁷ 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.²⁸ This relationship seemed to be stronger for patients with lower estimated glomerular filtration rate, and there was no significant association of 1,25(OH)2D and coronary artery calcification.²⁸ This latter result does not necessarily argue against important vascular protective actions of 1,25(OH)2D because circulating levels of 1,25(OH)2D are not necessarily correlated with its tissue levels. Previous study results suggest 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.^{29,30} 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 vitamin D receptor (VDR) as well as 1 α -hydroxylase,^{31,32} 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,^{10,33,34} even if VDR knockout was exclusively performed in cardiomyocytes.³⁵ Clinical studies confirmed the 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.^{31,36,37} Furthermore, altered myocardial calcium flux and increased risk of SCD related to a poor vitamin D status suggest a link to cardiac arrhythmias.^{12,31,38} 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.^{39,40} 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 a borderline non-significant association of vitamin D deficiency with fatal infections.⁴¹ In line with previous data, we also found a non-significant association of low 25(OH)D levels with increased risk of strokes.¹³ With reference to strokes, vitamin D might not only be useful for the prevention but also for the treatment when considering its neuromuscular and osteoprotective effects.^{8–10}

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.⁴² We are aware that findings from RCTs among patients free of advanced CKD cannot be uncritically extrapolated to haemodialysis patients.^{20,22} 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 vs. the probable risks and costs of this therapy. Supplementation of natural vitamin D to reach proposed optimal 25(OH)D levels of 75–150 nmol/L is considered safe (intoxication starts at >500 nmol/L),⁴³ and it should be kept in mind that sunbathing can produce up to 20 000 IU vitamin D per day.^{8,9,19} This latter dose is much higher than required to reach 25(OH)D target levels by using the rule of thumb that 1000 IU vitamin D can increase 25(OH)D levels by ~25 nmol/L in patients without severe CKD.^{8,9,19}

It should, however, also be pointed out that reversed causation might be mainly responsible for our prospective results. Hence, we cannot rule out that vitamin D deficiency is simply a consequence of a poor health status and not the cause of an adverse health outcome. In this context, it should be mentioned that our data are limited due to the observational nature of our study, which precludes any conclusion regarding cause and effect relationships of our results. Despite the extensive adjustments, we cannot exclude residual confounding. A further limitation 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, which suppresses 1,25(OH)₂D synthesis.⁴⁴ Associations of 25(OH)D and outcome measures were, however, independent of parameters of mineral metabolism and the use of active vitamin D treatment. Another drawback of our work is missing data on left ventricular ejection fraction and severity of CAD.

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 borderline non-significant association of vitamin D deficiency with increased risk of strokes 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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References

- London GM, Guerin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ, Metivier F. Mineral metabolism and arterial function in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol* 2007;**18**: 613–620.
- Mehrotra R, Kermah DA, Salusky IB, Wolf MS, Thadhani RI, Chiu YW, Martins D, Adler SG, Norris KC. Chronic kidney disease, hypovitaminosis D, and mortality in the United States. *Kidney Int* 2009;**76**:977–983.
- Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, Mallamaci F, Zoccali C. Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int* 2009;**75**:88–95.
- Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA Jr, Tonelli M, Thadhani R. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* 2007;**72**:1004–1013.
- Pecovnik-Balon B, Jakopin E, Bevc S, Knehtl M, Gorenjak M. Vitamin D and mortality as a novel nontraditional risk factor for mortality in hemodialysis patients. *Ther Apher Dial* 2009;**13**:268–272.
- Barreto DV, Baretto FC, Liabeuf S, Temmar M, Boitte S, Choukroun G, Fournier A, Massy ZA. Vitamin D affects survival independently of vascular calcification in chronic kidney disease. *Clin J Am Soc Nephrol* 2009;**4**:1128–1135.
- Wang AY, Lam CW, Sanderson JE, Wang M, Chan IH, Lui SF, Sea MM, Woo J. Serum 25-hydroxyvitamin D status and cardiovascular outcomes in chronic peritoneal dialysis patients: a 3-y prospective cohort study. *Am J Clin Nutr* 2008;**87**: 1631–1638.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;**357**:266–281.
- Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest* 2005;**35**:290–304.
- Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008;**29**:726–776.
- Jacob AI, Sallman A, Santz Z, Hollis BW. Defective photoproduction of cholecalciferol in normal and uremic humans. *J Nutr* 1984;**114**:1313–1319.
- Pilz S, März W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cohort of patients referred for coronary angiography. *J Clin Endocrinol Metab* 2008;**93**:3927–3935.
- Pilz S, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, März W. Low vitamin D levels predict stroke in patients referred for coronary angiography. *Stroke* 2008;**39**:2611–2613.
- Wanner C, Krane V, März W, Olschewski M, Mann JF, Ruf G, Ritz E, for the German Diabetes, Dialysis Study Investigators. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005;**353**: 238–248.
- Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008;**168**:1629–1637.
- Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 2008;**168**: 1174–1180.
- Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;**117**:503–511.
- Pilz S, Dobnig H, Nijpels G, Heine RJ, Stehouwer CD, Snijder MB, van Dam RM, Dekker JM. Vitamin D and mortality in older men and women. *Clin Endocrinol (Oxf)* 2009;**71**:666–672.
- Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr* 2007;**85**:6–18.
- Kaisar MD, Isbel NM, Johnson DW. Recent clinical trials of pharmacologic cardiovascular interventions in patients with chronic kidney disease. *Rev Recent Clin Trials* 2008;**3**:79–88.
- Rostand SG, Crüeke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. *Kidney Int* 1999;**56**:383–392.
- Kalantar-Zadeh K, Kovesdy CP. Clinical outcomes with active versus nutritional vitamin D compounds in chronic kidney disease. *Clin J Am Soc Nephrol* 2009;**4**: 1529–1539.
- Drechsler C, Krane V, Grootendorst DC, Ritz E, Winkler K, März W, Dekker F, Wanner C, German Diabetes, Dialysis Study Investigators. The association between parathyroid hormone and mortality in dialysis patients is modified by wasting. *Nephrol Dial Transplant* 2009;**24**:3151–3157.
- Pilz S, Tomaschitz A, Drechsler C, Ritz E, Boehm BO, Grammer TB, März W. Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography. *Eur Heart J* 2010;**31**: 1591–1598. doi:10.1093/eurheartj/ehq109.
- Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C. Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: effects on mineral metabolism and bone markers. *Nephrol Dial Transplant* 2008;**23**:3670–3676.
- Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007;**92**:2017–2029.
- Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol* 2009;**6**:621–630.
- de Boer IH, Kestenbaum B, Shoben AB, Michos ED, Sarnak MJ, Siscovick DS. 25-hydroxyvitamin D levels inversely associate with risk of developing coronary artery calcification. *J Am Soc Nephrol* 2009;**20**:1805–1812.
- Zittermann A, Schleithoff SS, Koerfer R. Vitamin D and vascular calcification. *Curr Opin Lipidol* 2007;**18**:41–46.
- Oh J, Weng S, Felton SK, Bhandare S, Riek A, Butler B, Proctor BM, Petty M, Chen Z, Schechtman KB, Bernal-Mizrachi L, Bernal-Mizrachi C. 1,25(OH)₂ vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. *Circulation* 2009;**120**:687–698.
- Tishkoff DX, Nibelink KA, Holmberg KH, Dandu L, Simpson RU. Functional vitamin D receptor (VDR) in the t-tubules of cardiac myocytes: VDR knockout cardiomyocyte contractility. *Endocrinology* 2008;**149**:558–564.
- Chen S, Glenn DJ, Ni W, Grigsby CL, Olsen K, Nishimoto M, Law CS, Gardner DG. Expression of the vitamin D receptor is increased in the hypertrophic heart. *Hypertension* 2008;**52**:1106–1112.

33. Nibelink KA, Tishkoff DX, Hershey SD, Rahman A. 1,25(OH)₂-vitamin D₃ actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol* 2007;**103**:533–537.
34. Green JJ, Robinson DA, Wilson GE, Simpson RU, Westfall MV. Calcitriol modulation of cardiac contractile performance via protein kinase C. *J Mol Cell Cardiol* 2006;**41**:350–359.
35. Gardner DG, Glenn D, Nei W, Grigsby C, Law C, Olsen K, Chen S. Cardiomyocyte-specific vitamin D receptor gene knockout causes cardiac hypertrophy. *Fourteenth Workshop on Vitamin D. Abstract, Brugge, Belgium, 4–8 October, 2009*. p194
36. Zittermann A, Schleithoff SS, Koerfer R. Vitamin D insufficiency in congestive heart failure: why and what to do about it? *Heart Fail Rev* 2006;**11**:25–33.
37. Bodyak N, Ayus JC, Achinger S, Shivalingappa V, Ke Q, Chen YS, Rigor DL, Stillman I, Tamez H, Kroeger PE, Wu-Wong RR, Karumanchi SA, Thadhani R, Kang PM. Activated vitamin D attenuates left ventricular abnormalities induced by dietary sodium in Dahl salt-sensitive animals. *Proc Natl Acad Sci USA* 2007;**104**:16810–16815.
38. Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W. Vitamin D deficiency and myocardial diseases. *Mol Nutr Food Res* doi:10.1002/mnfr.200900474.
39. Kim HW, Park CW, Shin YS, Kim YS, Shin SJ, Kim YS, Choi EJ, Chang YS, Bang BK. Calcitriol regresses cardiac hypertrophy and QT dispersion in secondary hyperparathyroidism on hemodialysis. *Nephron Clin Pract* 2006;**102**:21–29.
40. Yamshchikov AV, Desai NS, Blumberg H, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. *Endocrin Pract* 2009;**15**:438–449.
41. de Bie MK, van Dam B, Gaasbeek A, van Buren M, van Erven L, Bax JJ, Schalij MJ, Rabelink TJ, Jukema JW. The current status of interventions aiming at reducing sudden cardiac death in dialysis patients. *Eur Heart J* 2009;**21**:1559–1564.
42. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 2007;**167**:1730–1737.
43. Zittermann A, Gummert JF, Börgermann J. Vitamin D deficiency and mortality. *Curr Opin Clin Nutr Metab Care* 2009;**12**:634–639.
44. Gutierrez OM, Mannstadt M, Isakov T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee K, Thadhani R, Jüppner H, Wolf M. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 2008;**359**:584–592.

CARDIOVASCULAR FLASHLIGHT

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Acute myocardial infarction secondary to direct myocardial infiltration by a malignant neoplasia

David Vivas*, Borja Ruiz-Mateos, and Eduardo Franco

Cardiovascular Institute, San Carlos University Hospital, Professor Martin Lagos Street, Madrid 28040, Spain

*Corresponding author. Tel: +34 91 330 30 00, Fax: +34 91 330 31 82, Email: dvivas@secardiologia.es

A 24-year-old male, with a previous history of osteosarcoma with cerebral and pulmonary metastasis, presented to the emergency room with severe chest pain, dyspnoea, and diaphoresis. Physical examination revealed an apical S4 gallop, abolition of left breath sounds, and right hemiparesis. On electrocardiogram, persistent lateral ST-segment elevation was noted (*Panel A*). Echocardiogram showed lateral akinesis with global left ventricular ejection fraction preserved, severe pericardial effusion probably related to the malignant neoplasia, and infiltration of mediastinum by the pulmonary metastasis (arrows, *Panel B*). He was referred for urgent coronary angiography, which revealed no significant stenosis (*Panels C and D*). Interestingly, ventriculography showed a great distance between epicardic coronary arteries and left ventricular cavity (*Panel E*). Magnetic resonance imaging was performed and confirmed the direct infiltration of the neoplasia in the pericardium and myocardium (arrows, *Panel F*). The patient was treated with anti-ischaemic and opiate analgesic drugs and died a few days later by respiratory failure.

Malignant cardiac neoplasias are an extremely unusual cause of acute myocardial infarction, usually a consequence of an external compression of large coronary arteries by infiltrative processes or coronary tumour embolisms. To the best of our knowledge, this is the first report of an acute myocardial infarction caused by direct myocardial neoplastic infiltration.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

