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RESEARCH ARTICLE

1,25-Dihydroxyvitamin D to PTH(1–84) Ratios Strongly Predict Cardiovascular Death in Heart Failure

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Abstract

Objectives

Vitamin D deficiency and hyperparathyroidism are common in patients with heart failure (HF). There is a growing body of evidence supporting the role of vitamin D and parathyroid hormone (PTH) in cardiac remodeling and worsening of HF. Lack of reliable automated testing of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the biologically active metabolite of vitamin D, has limited its contribution to the prognostic assessment of HF. Here, the association of $1,25(OH)_2D$ and PTH(1–84) levels was evaluated for prediction of cardiovascular death in chronic HF patients.

Methods

We conducted a single center prospective cohort including 170 chronic HF patients (females n = 36; males n = 134; NYHA II-IV; mean age: 67 years; etiology: ischemic n = 119, dilated cardiomyopathy n = 51; mean LVEF: 23%). The primary outcome was cardiovascular death.

Results

Serum levels of $1,25(OH)_2D$ decreased markedly with increased HF severity. Medians were 33.3 pg/mL for NYHA-II patients, 23.4 pg/mL for NYHA-III, and 14.0 pg/mL for NYHA-IV patients ($p<0.001$). Most patients had levels of 25(OH)D below 30ng/mL, and stratification by NYHA functional class did not show significant differences ($p = 0.249$). The $1,25(OH)_2D$ to PTH(1–84) ratio and the $(1,25(OH)_2D)^2$ to PTH(1–84) ratio were found to be the most significantly related to HF severity. After a median follow-up of 4.1 years, 106 out of 170

Competing Interests: Claudia Zierold, Frank Blocki, and Fabrizio Bonelli are employees of DiaSorin Inc., a provider of *in vitro* diagnostic products. For the current study, their contribution was to provide advice for the best use and monitoring of novel assays and scientific input for results interpretation as well as editing of manuscript. The authors are also working on a provisional patent. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

patients reached the primary endpoint. Cox proportional hazard modeling revealed 1,25 (OH)$_2$D and the 1,25(OH)$_2$D to PTH(1–84) ratios to be strongly predictive of outcomes.

Conclusions

1,25(OH)$_2$D and its ratios to PTH(1–84) strongly and independently predict cardiovascular mortality in chronic HF.

Background

Cardiovascular (CV) diseases remain a leading cause of death around the world [1]. Among CV diseases, heart failure (HF) represents a major health concern because of increasing prevalence worldwide with major human, societal and economic impacts [2–7]. The need for biomarkers for the prognosis of HF is well established, and different biomarkers from several pathophysiological pathways have been evaluated in this setting [8–13].

There is a growing body of evidence supporting the role of vitamin D and parathyroid hormone (PTH) in cardiac remodeling and worsening HF [14–17]. Furthermore, PTH together with aldosterone and fibroblast growth factor 23 (FGF-23), may also be part of a vicious and deleterious cycle which compromises CV function [18]. Markedly elevated levels of FGF-23 and PTH were observed in patients with CV disorders and HF, and have been related to adverse CV events [14;15;19–21].

Like PTH and FGF-23, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D, calcitriol) is an important regulator of calcium and phosphate homeostasis [21–23]. Recently, a novel fully-automated 1,25(OH)$_2$D assay with improved analytical performance, sensitivity, and reliability has emerged [22;24]. The imprecision at low levels of existing 1,25(OH)$_2$D measurement has precluded the ability to identify meaningful clinical correlates of HF progression so far. The aim of this study, therefore, was to assess the impact of sensitive, precise, accurate 1,25(OH)$_2$D measurement and its ratios to PTH(1–84) on CV survival in HF patients.

Methods

Study population

We prospectively assessed CV death of 170 consecutive fully treated patients with chronic HF and reduced left ventricular ejection fraction (LVEF) followed at the Cliniques Universitaires Saint-Luc, an academic hospital of Brussels, Belgium, between March 30th 2004 and June 16th 2006. Patients with left ventricular systolic dysfunction and ejection fraction of 35% or less were eligible for the study. Ejection fraction was measured by radionuclide technique or contrast ventriculography, the latter being associated with coronary arteriography to confirm ischemic etiology. Exclusion criteria were age <18 years, LVEF higher than 35%, abnormal liver function test (AST/ALT 2 times the upper limit of the reference interval), anaemia or iron reserve deficiencies, genetic hypertrophic cardiopathy, severe pulmonary diseases (COPD gold 3–4), patients under dialysis and primary hyperparathyroidism. Survival status was obtained by phone contact with patients, their relatives, or their physicians.

Ethics statement

The research protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and all participants gave verbal informed consent regarding the goals of the study and their

willingness to participate. The ethics committee of the Catholic University of Louvain approved this study as well as the consent procedure.

Clinical outcomes

Patient history and treatment was retrieved from medical files and review of hospital visitation records. Follow-up events including CV mortality and cardiac transplantation were 100% complete. Cardiac death was defined as death attributable to congestive HF, myocardial infarction, sudden death, or death occurring pursuant to revascularization procedures.

Laboratory measurements

Routine laboratory measurements and blood samples for biomarker analyses were obtained at hospital admission. Venous blood samples were obtained at enrollment, processed, and stored at -80°C until time of assay. Levels of $1,25(OH)_2D$ were determined at baseline with a fully automated and sensitive immunoassay that uses a recombinant fusion construct of the vitamin D receptor ligand binding domain for specific capture of $1,25(OH)_2D$ (DiaSorin, Saluggia, Italy). The limit of quantitation for this $1,25(OH)_2D$ assay is 5 pg/mL and the reference interval determined in healthy volunteers ranged between 25.0 and 86.5 pg/mL with a median of 48.1 pg/mL. Plasma Ct-FGF23 concentrations were determined with a second-generation C-terminal human enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). Levels of 25(OH)D, PTH(1–84) (both DiaSorin), B-type natriuretic peptide (BNP, Beckman Coulter, Fullerton, CA, USA; Alere reagents), N-terminal proBNP (NT-proBNP, Roche Diagnostics, Mannheim, Germany), Chromogranin A (CgA, Dako, Glostrup, Denmark), and Galectin-3 (Gal-3, BG Medicine, Waltham, MA, USA) were also determined. Glomerular filtration rate (eGFR) was estimated by the Modification of Diet in Renal Disease formula.

Statistical analysis

Independent determinants of baseline $1,25(OH)_2D$ levels were assessed using multivariable linear regression methods with Log-transformed levels of $1,25(OH)_2D$ as the dependent variable. The discriminatory power between biomarkers was assessed by fitting Cox proportional hazard (CPH) models using each of them, along with patient characteristics of age, sex, clinical variables, etiology of the disease, previous admission to hospital and treatment to predict outcomes. Biomarkers with highly skewed distributions – $1,25(OH)_2D$, PTH(1–84), NT-proBNP, 25(OH)D, FGF-23, and creatinine were Log-transformed for use in the CPH models. The strength of association of each of these predictors can be summarized by the chi-squared statistic of the fitted CPH. Along with the individual markers and patient characteristics, $1,25(OH)_2D$ to PTH(1–84) ratios were also determined.

Several of these predictors have large chi-squared values, indicating strong associations with survival. For these biomarkers, receiver operating curve (ROC) analyses were performed to explore the trade-off between sensitivity and specificity as assessed at the seven-year mark, and to locate a cut point suitable for use in dichotomizing the patients into high- and low-risk groups. This cut point was defined as that value maximizing the difference between sensitivity and false positive rates. The Kaplan-Meier survival curve of patients split by this dichotomy was found and tested using the Log-Rank test. P-values < 0.05 were considered significant. Statistical analysis was performed using R version 3.1.0 (The R Foundation for Statistical Computing) and JMP software version 11.00 (SAS institute, NC, USA). It may be mentioned that the CPH and the ROC analyses involve somewhat different perspectives. The ROC calculations use a snapshot of the patients at the end of the monitoring period and do not distinguish

between earlier and later deaths. The CPH and Kaplan-Meier analyses, on the other hand, use the entire survival function and thereby do distinguish earlier from later deaths.

Results

Baseline characteristics

Table 1 shows the baseline characteristics and laboratory values of the study population, according to 25(OH)D and $1,25(OH)_2D$ levels. 170 chronic HF patients were included (mean age 67±14 years; females n = 36; males n = 134; New York Heart Association (NYHA) II-IV; etiology: ischemic n = 119, dilated cardiomyopathy n = 51; mean LVEF 23±7%).

Most patients had levels of 25(OH)D below 30 ng/mL (58% of HF patients were below 15 ng/mL, 21% between 15 and 20 ng/mL, 15% between 20 and 30 ng/mL, and only 6% were higher than 30 ng/mL, and stratification by NYHA functional class did not disclose significant differences (p = 0.249). In contrast, median serum levels of $1,25(OH)_2D$ decreased markedly according to HF severity: 33.3 pg/mL in NYHA class II (n = 60), 23.4 pg/mL in NYHA class III (n = 94), and 14.0 pg/mL in NYHA class IV (n = 16; $p<0.001$). The median $1,25(OH)_2D$ level for all patients was 25.4 pg/mL (range: 5.0–100.0 pg/mL). Participants with $1,25(OH)_2D$ levels below the median 25.4 pg/mL value were more likely to have higher circulating levels of BNP, NT-proBNP, FGF-23, CgA and Galectin-3.

The median PTH(1–84) levels by severity were as follows: NYHA class II 27.6 pg/mL, NYHA class III 41.4 pg/mL, and NYHA class IV 41.0 pg/mL ($p<0.01$ between Class II and III). The median for PTH(1–84) for all patients was 38.0 pg/mL (range: 4.0–244 pg/mL). Because $1,25(OH)_2D$ and PTH(1–84) are physiologically interrelated (23), we examined the ratio of these two hormones and found them to be significantly related to HF severity: NYHA class II ratio = 1.14, NYHA class III ratio = 0.47, and NYHA class IV ratio = 0.38 ($p<0.001$). The median ratio for all patients was 0.62 (range: 0.02–9.3). The $1,25(OH)_2D$ to PTH(1–84) ratios were higher in patients with $1,25(OH)_2D$ levels above the median, while PTH was not different between the two groups.

In multiple regression analyses including age, LVEF, clinical variables, eGFR, $1,25(OH)_2D$, PTH(1–84), NT-proBNP, 25(OH)D, FGF-23, CgA and Gal-3, the independent determinants of baseline $1,25(OH)_2D$ were age, eGFR, PTH(1–84), and Galectin-3. The levels of $1,25(OH)_2D$ were not significantly different between patients with an ischemic origin of the disease (26.6 pg/mL) in comparison to dilated cardiopathy (31.5 pg/mL; p = 0.057). The $1,25(OH)_2D$ concentrations were not significantly different between patient receiving β-blockers (28.0 pg/mL) in comparison to those not receiving β-blockers (28.1 pg/mL; p = 0.981). Levels of $1,25(OH)_2D$ were correlated to eGFR ($\rho = 0.42$, $p<0.0001$). In HF patients with normal kidney function (eGFR>60 mL/min/1.73m^2, n = 74), the median $1,25(OH)_2D$ level was 31.5 pg/mL, which remains clearly lower than the median of healthy individuals (49.6 pg/mL, data not shown); in this group of patients the $1,25(OH)_2D$ to PTH(1–84) ratios remain significantly correlated to BNP, NT-proBNP, CgA, Gal-3 and FGF-23, while $1,25(OH)_2D$ is not significantly correlated to NT-pro-BNP in patients with eGFR>60 mL/min/1.73m^2. HF patients with a history of previous admission to hospital have higher levels of $1,25(OH)_2D$ (29.7 pg/mL) in comparison to those without previous admission to hospital wards (24.4 pg/mL; p = 0.037).

$1,25(OH)_2D$ level and $1,25(OH)_2D$ to PTH(1–84) ratio outcomes in chronic HF

Over a median follow-up time of 4.1 years (occurrence of a first endpoint and maximum follow-up times were 7 days and 7.4 years, respectively), 106 HF patients met the endpoint of

Table 1. Baseline characteristics and biomarkers of the study population according to median levels of 25(OH)D and $1,25(OH)_2D$.

Characteristics	Entire cohort	25(OH)D less than median	25(OH)D greater than median	P-value	$1,25(OH)_2D$ less than median	$1,25(OH)_2D$ greater than median	P-value
Age (years)	69 [21–89]	66 [33–89]	70 [21–87]	0.559	70 [33–89]	67 [21–86]	0.116
Sex (M/F)	134/36	64/21	70/15	0.808	67/18	67/18	0.785
Dilated cardiomyopathy (%)	30	26	34	0.016	26	34	0.016
Ischemic cardiomyopathy (%)	70	74	66	0.022	74	66	0.022
EF (%)	24 [8–35]	23 [8–35]	24 [9–35]	0.119	23 [9–35]	23 [8–35]	0.970
Heart Rate	79 [46–135]	79 [50–135]	78 [46–124]	0.893	79 [51–135]	78 [46–130]	0.795
Diabetes (%)	29	31	26	0.095	38	20	0.038
Hypertension (%)	55	49	61	0.031	45	55	0.047
Previous admission to hospital (%)	32	27	34	0.045	38	41	0.112
Smoker C/F/N (%)	16/25/59	21/20/59	12/31/57	0.108	15/27/58	18/24/58	0.467
Treatment							
ACE inhibitors (%)	79	70	85	0.037	76	77	0.324
β-blockers (%)	83	83	81	0.134	86	80	0.112
Diuretics (%)	72	76	74	0.250	85	64	<0.001
Aldosterone antagonists (%)	62	60	75	0.027	73	61	0.030
Angiotensin II receptor blockers (%)	20	25	17	0.017	26	18	0.023
Vitamin D (%)	3	2	4	0.346	5	1	0.076
Anticoagulant (%)	38	40	35	0.332	41	35	0.276
Antiplatelet (%)	67	73	60	0.030	68	66	0.649
Antidiabetic drug (%)	28	30	25	0.112	36	20	0.024
eGFR (mL/min/1.73m^2)	56.1 [9.4–144.6]	55.6 [9.4–144.6]	58.5 [11.0–114.7]	0.958	51.6 [9.4–107.8]	61.6 [22.2–144.6]	0.001
Calcium, total (mg/dL)	8.9 [6.2–10.8]	8.9 [6.2–10.8]	8.9 [7.8–10.4]	0.320	8.8 [6.2–10.4]	9.0 [7.8–10.8]	0.007
LDL-Cholesterol (mg/dL)	97 [16–220]	99 [16–185]	97 [32–220]	0.434	91 [16–180]	100 [32–220]	0.459
Triglycerides (mg/dL)	90 [14–345]	90 [14–345]	91 [28–282]	0.522	85 [14–231]	99 [39–345]	0.256
25(OH)D (ng/mL)	12.3 [4.3–46.1]	8.5 [4.3–12.1]	18.8 [12.5–46.1]	< 0.001	10.9 [4.3–34.0]	15.0 [5.0–46.1]	0.024
$1,25(OH)_2D$ (pg/mL)	25.4 [5.0–100]	22.5 [5.0–74.1]	28.4 [7.5–100]	0.025	17.4 [5.0–25.2]	35.8 [25.6–100]	<0.001
PTH 1–84 (pg/mL)	45 [4–244]	52 [12–244]	37 [4–201]	<0.001	44 [8.7–244]	45 [4–201]	0.024
BNP (ng/L)	455 [17–5017]	756 [21–5017]	308 [17–4408]	<0.001	687 [43–5017]	299 [17–4408]	<0.001
Nt-proBNP (ng/L)	2157 [66–33020]	3385 [66–33020]	1571 [95–21295]	<0.001	3276 [71–33020]	929 [66–29925]	<0.001
CgA (UI/L)	35.1 [4.9–422]	34.8 [6.8–384]	34.4 [4.9–422]	0.889	46.8 [7.9–422]	26.7 [4.9–384]	< 0.001
Gal-3 (ng/mL)	18.0 [7.8–49.6]	18.0 [7.9–45.5]	17.4 [9.8–49.6]	0.453	20.1 [9.9–49.6]	14.9 [7.9–45.5]	< 0.001
FGF-23 (RU/mL)	1126 [23–15000]	228 [23–13906]	148 [32–15000]	<0.001	300 [32–15000]	101 [23–10301]	<0.001
$1,25(OH)_2D$/PTH(1–84)	0.62 [0.02–9.33]	0.45 [0.02–3.76]	0.89 [0.12–9.33]	<0.001	0.38 [0.02–2.84]	1.15 [0.15–9.33]	<0.001
$(1,25(OH)_2D)^2$/PTH(1–84)	15.3 [0.10–357]	9.7 [0.10–248]	23.7 [1.6–357]	<0.001	6.5 [0.10–70.1]	45.5 [4.8–357]	<0.001

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Table 2. Clinical variables and biomarkers among stable HF patients and HF patients who developed the outcome.

Variables	Stable patients (n = 64)	Patients with outcomes (n = 106)	P-value
Age (years)	60.4	68.6	0.001
Sex (M/F)	47/17	87/19	0.153
Dilated cardiomyopathy (%)	42	22	0.046
Ischemic cardiomyopathy (%)	58	78	0.042
EF (%)	23.7	20.3	0.003
Heart Rate	79	79	0.897
Diabetes (%)	28.8	37.6	0.303
Hypertension (%)	55.3	62.4	0.358
Previous admission to hospital (%)	31.8	62.4	<0.001
Treatment			
ACE inhibitors (%)	73	77	0.203
β-blockers (%)	63.0	77.8	0.048
Diuretics (%)	63.0	75.6	0.076
Aldosterone antagonists (%)	63.0	64.4	0.521
Angiotensin II receptor blockers (%)	20.7	24.0	0.655
Vitamin D (%)	1	3	0.487
Anticoagulant (%)	37.6	38.2	0.196
Antiplatelet (%)	67.1	62.4	0.633
Antidiabetic drug (%)	28.2	37.6	0.366
eGFR (mL/min/1.73m^2)	61.7	49.2	0.002
Calcium, total (mg/dL)	8.9	8.8	0.955
BNP (ng/L)	227	666	< 0.001
NT-proBNP (ng/L)	1012	2992	< 0.001
Gal-3 (ng/mL)	16.3	19.5	0.001
CgA (UI/L)	26.6	47.2	< 0.001
FGF-23 (RU/mL)	149	413	< 0.001
PTH (1–84)(pg/mL)	28	41	0.001
25(OH)D (ng/mL)	13.1	12.4	0.488
1,25(OH)$_2$D (pg/mL)	31.7	20.2	< 0.001
1,25(OH)$_2$D / PTH(1–84)	1.12	0.49	< 0.001
(1,25(OH)$_2$D)^2 / PTH(1–84)	35.38	9.91	< 0.001

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which 94 died, and 12 underwent heart transplant. Biomarker concentrations as well as clinical variables between patients that developed the outcome and those who remained stable are presented in Table 2.

CPH analysis predicting survival from the Logs of 1,25(OH)$_2$D and PTH(1–84) show that each of these biomarkers is highly significant even when the other is used (their p-values in the CPH using both being $1.2x10^{-7}$ and $1.5x10^{-3}$); in other words, both contribute separate, highly significant, predictive power. The coefficient of Log 1,25(OH)$_2$D is roughly -2 times that of Log PTH(1–84), suggesting that the two might be summarized by a score function -2 Log 1,25 (OH)$_2$D + Log PTH(1–84), which is the negative Log of the ratio of the square of the 1,25 (OH)$_2$D assay to the PTH. This composite score marker is assessed in Table 3 as "Square ratio", and indeed it has the second-highest chi-squared value of all the biomarkers, being outperformed only (and very modestly) by BNP. A simpler approach using the 1,25(OH)$_2$D to PTH(1–84) ratio, without the doubling on the 1,25(OH)$_2$D, is assessed under the label "Ratio". As Table 3 might lead one to expect, this simpler ratio does not perform quite as well, but

Table 3. Strength of biomarkers determined by CPH fits and respective chi-square.

Biomarker	Chi-square
BNP	39.2
Square ratio	38.9
NT-proBNP	37.3
Ratio	36.0
FGF-23	35.0
NYHA	31.0
$1,25(OH)_2D$	29.1
Galectin-3	15.6
LVEF	13.8
PTH(1–84)	12.3
Age	10.7
Creatinin	9.1
Etiology	8.3
Sex	1.1
Calcium, total	1.0

The biomarkers with highly skewed distributions were Log-transformed (Square ratio, Ratio, NT-proBNP, $1,25(OH)_2D$, PTH(1–84), FGF-23, and creatinine). Square ratio = $(1,25(OH)_2D)^2$/PTH(1–84), Ratio = $1,25(OH)_2D$/PTH(1–84).

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nevertheless is beaten only by BNP and NT-proBNP. Thus both the simple $1,25(OH)_2D$/PTH (1–84) ratio and the slightly more complex $(1,25(OH)_2D)^2$/PTH(1–84) ratio are highly competitive risk scores.

Going from the CPH to the ROC approach and focusing just on the seven-year survival, in ROC analysis the area under the curve (AUC), criteria defined as CV death at the end of the follow-up, for $1,25(OH)_2D$ and the ratios of $1,25(OH)_2D$/PTH(1–84) and $(1,25(OH)_2D)^2$/PTH (1–84) were 0.722 (95% CI: 0.648–0.788), 0.741 (95% CI: 0.668–0.805), and 0.749 (95% CI: 0.677–0.812) respectively, which was similar to BNP (AUC 0.744 [(95% CI: 0.671–0.808]), but clearly higher than 25(OH)D (AUC 0.529 [(95% CI: 0.451–0.606]; $p<0.01$) (Fig 1). Clinically accepted biomarkers had AUCs as follows: NT-proBNP (AUC 0.730 [(95% CI: 0.657–0.796]), Gal-3 (AUC 0.660 [(95% CI: 0.583–0.731]), FGF-23 (AUC 0.702 [(95% CI: 0.624–0.773]), CgA (AUC 0.663 [(95% CI: 0.587–0.733]), and PTH(1–84) (AUC 0.641 [(95% CI: 0.564–0.713]).

Youden's index and the associated criterion based on the ROC curve analysis were used for the stratification of the Kaplan-Meier curves (35.4 pg/mL for $1,25(OH)_2D$, 1.06 for the ratio, 32.0 pg/mL for the Square ratio). Kaplan Meier survival curves for patients stratified by $1,25(OH)_2D$ levels diverged significantly (Log-rank test: $p<0.001$; Fig 2A). Survival curves for patients stratified by the $1,25(OH)_2D$ to PTH(1–84) ratios also diverged significantly (Log-rank test: $p<0.001$; Fig 2B and 2C).

Table 4 shows the coefficients and the statistical significance of each term in a multivariate CPH model including all the variables listed in the table. In this table, the contribution of each predictor is adjusted for the common predictive information of all other predictors. The Square ratio has a highly significant p-value (0.011) showing that this predictor is significant even after all other predictors are taken into account; its information therefore is largely unique and additive to that of the other predictors.

In HF patients with normal kidney function (eGFR>60 mL/min/1.73m^2; n = 74) the ratios of $1,25(OH)_2D$/PTH(1–84) and $(1,25(OH)_2D)^2$/PTH(1–84) remain predictive of CV death

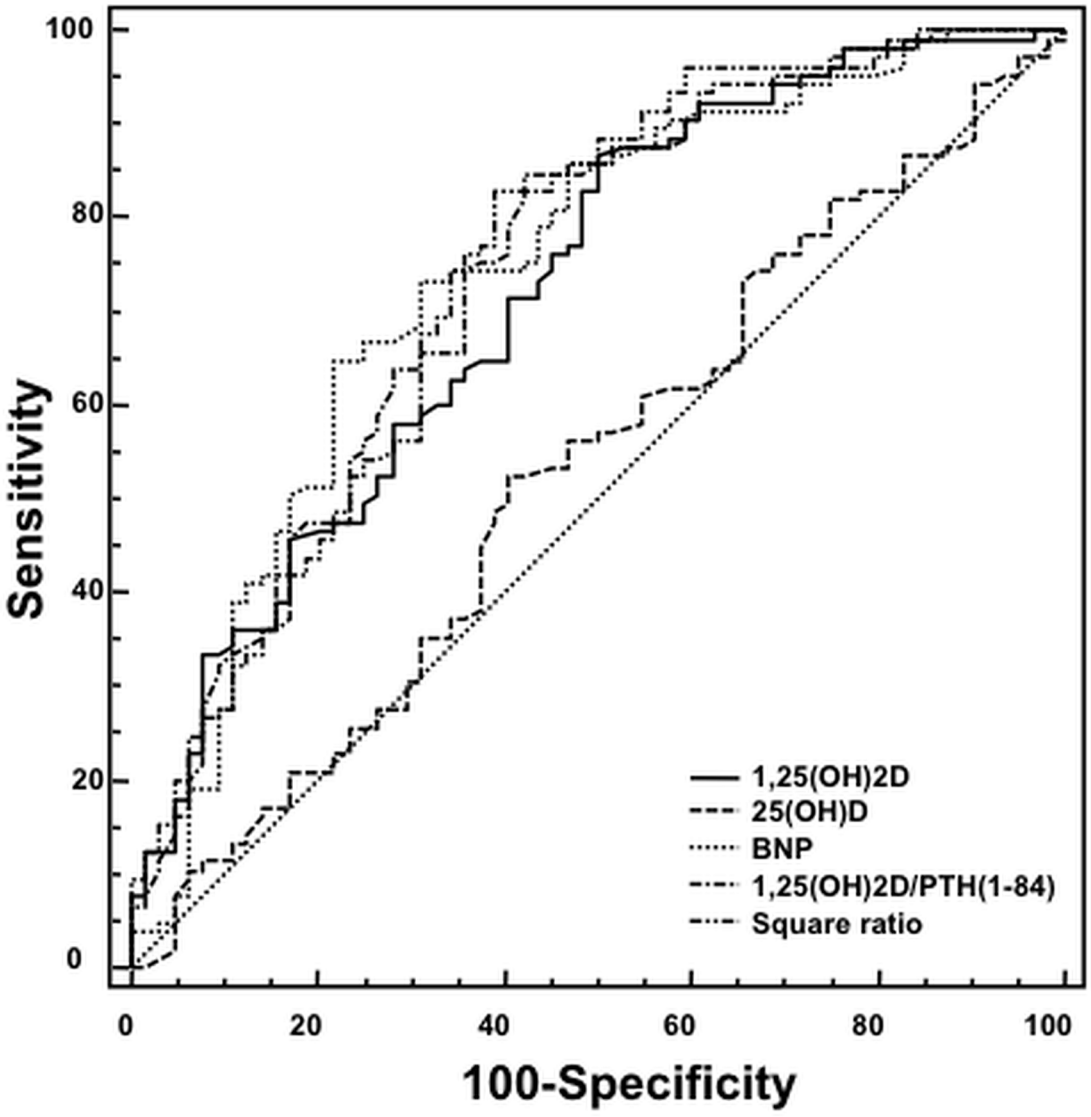

Fig 1. Receiver operating characteristic (ROC) analysis was performed for CV death at the end of the follow-up for 25(OH)D, BNP, $1,25(OH)_2D$ and its ratios to PTH(1–84).

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($p<0.01$; data not showed). In HF patients with a history of previous admission to hospital the ratios of $1,25(OH)_2D$/PTH(1–84) and $(1,25(OH)_2D)^2$/PTH(1–84) remain predictive of CV death ($p<0.03$; data not showed).

Discussion

The main objective of our study was to investigate the prognostic value of $1,25(OH)_2D$, measured with the new generation assay, and its ratios to PTH(1–84) for long-term CV death in patients with chronic HF. These results clearly demonstrate the relationship between decreased levels of $1,25(OH)_2D$ and long-term CV death in HF, and most importantly, the novel use of the $1,25(OH)_2D$ to PTH(1–84) ratios as superior biomarkers for the prognosis of HF patients.

Vitamin D deficiency was previously shown to be associated with cardiovascular diseases [25;26], however the biologically active metabolite $1,25(OH)_2D$ was not routinely assessed due

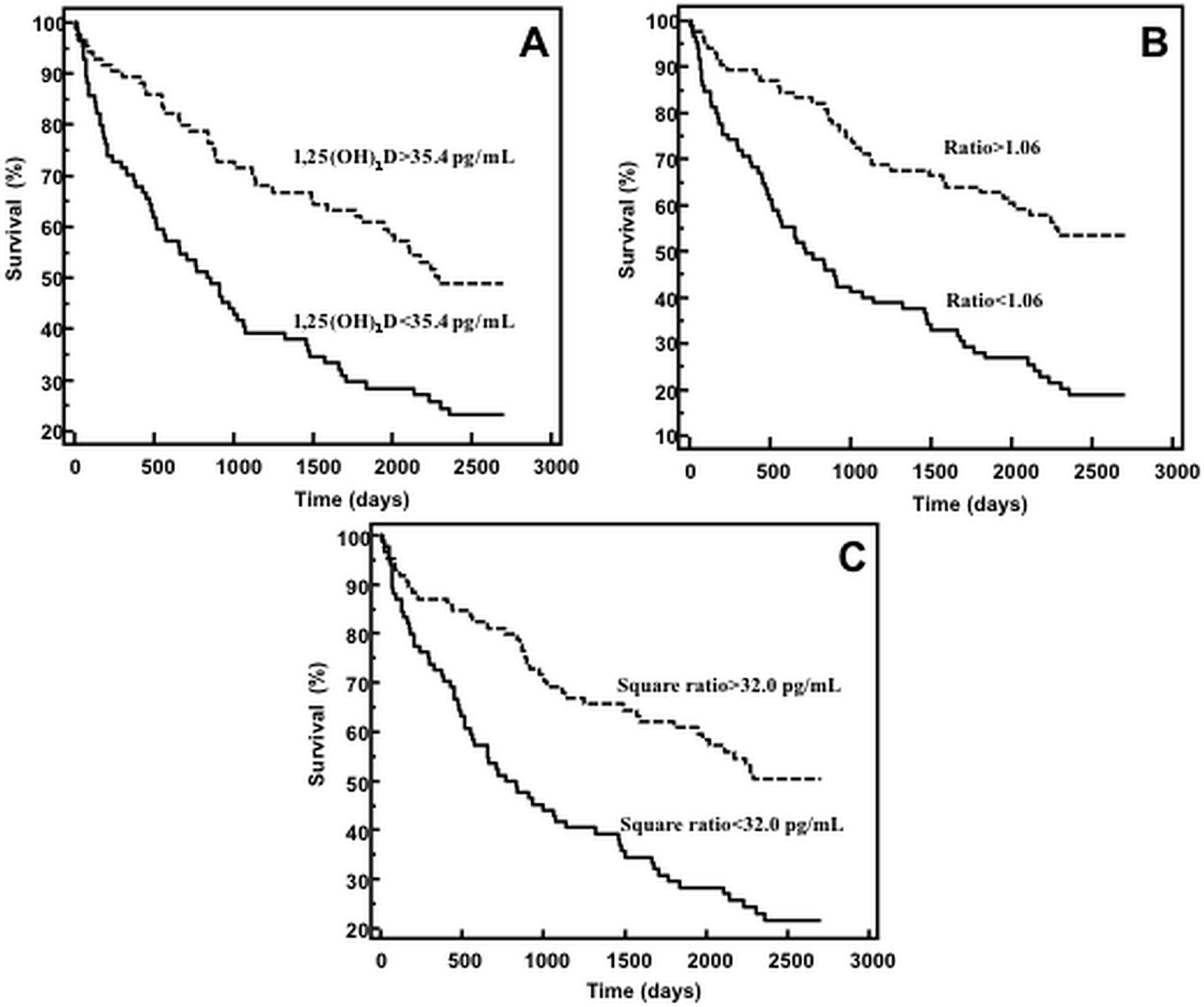

Fig 2. Kaplan-Meier survival curves stratified by (A) $1,25(OH)_2D$ levels, (B) the ratio of $1,25(OH)_2D$ to PTH(1–84) (Ratio), and (C) the ratio of $(1,25(OH)_2D)^2$ to PTH(1–84) (Square ratio).

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in part to the lack of assays that are accurate and precise at low $1,25(OH)_2D$ concentrations. Decreased $1,25(OH)_2D$ levels in HF patients were previously reported [25;27]; once again, the concentrations were obtained with less sensitive and reliable assays. The development of a novel automated, extraction-free $1,25(OH)_2D$ immunoassay, with precision and sensitivity superior to LC-MS/MS, has allowed the exploration of new biological relationships that until now were beyond the reach of current methodologies [22]. The $1,25(OH)_2D$ test used here, in contrast to the tests for existing biomarkers, offers several advantages: automation, standardization, small sample size and short turn-around-time. In addition, the pre-analytical extraction step, on samples routinely in excess of 1 mL that is normally required for other $1,25(OH)_2D$ assays LC-MS/MS inclusive, is not required. This greatly diminishes the imprecision of the assay, especially at low concentrations [22]. The effect on imprecision that results from

Table 4. Multivariate CPH analysis encompassing most variables.

Covariate	b	SE	z	p
Square ratio	-0.311	0.122	-2.556	0.011
Age	0.024	0.010	2.402	0.016
BNP	0.271	0.127	2.138	0.033
β-blockers	-0.636	0.2594	-1.5295	0.039
Etiology	0.5191	0.2818	1.684	0.065
NYHA	0.408	0.228	1.79	0.073
LVEF	-0.034	0.020	-1.703	0.089
25(OH)D	0.359	0.249	1.441	0.150
eGFR	0.008	0.006	1.28	0.200
FGF23	0.125	0.102	1.232	0.220
Previous admission to hospital	0.170	0.241	0.546	0.384
Sex	-0.254	0.296	-0.859	0.390
PTH(1–84)	0.002	0.003	1.03	0.5099
Calcium, total	0.078	0.179	0.439	0.660

Clinical variables and biomarkers with low statistical significance indicate the use of the same predictive information. The high significance of the square ratio indicates contribution of information distinct from the other predictors. The biomarkers with highly skewed distributions were Log-transformed (Square ratio, 25(OH)D, BNP, FGF-23). Square ratio = $(1,25(OH)_2D)^2$/PTH(1–84).

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combining values from two assays into a single ratio was also examined by running two different lots each of 1,25(OH)$_2$D and PTH(1–84) kits, and determining the ratio for the four resulting kit combinations. The coefficient of variation of the ratio was on average 2.8% for ratios >0.5 with a maximum CV of 6.3% (data not shown).

These data clearly demonstrate the relationship between decreased levels of 1,25(OH)$_2$D and long-term CV death in HF. Most importantly these data indicate that the relationship between the 1,25(OH)$_2$D and PTH(1–84) hormones afford potent biomarkers for the prognosis of HF patients, as depicted by the Kaplan Meier curves, which disclose markedly early changes in survival according to the 1,25(OH)$_2$D to PTH(1–84) ratios. The association of 1,25(OH)$_2$D levels and the risk of adverse outcomes in CV diseases was previously observed [25;27], however the 1,25(OH)$_2$D to PTH(1–84) ratios are novel biomarkers not previously investigated. The predictive value of 1,25(OH)$_2$D to PTH(1–84) ratios were equivalent to established biomarkers of HF severity such as BNP, NT-proBNP and FGF-23 as evidenced by the AUC. These are all the more remarkable given the modest sample size. In contrast, 1,25(OH)$_2$D to PTH(1–84) ratios were superior to Gal-3, PTH(1–84) and 25(OH)D. In comparison to the other biomarkers, 25(OH)D levels were not predictive of the outcome. Several studies have related 25(OH)D levels to survival in HF patients [15;16;28]. The differences might be related to a lower median level of 25(OH)D with a high number of HF patients below 15ng/mL but also to length of follow-up which was longer in our study in comparison to the other reports. However, our results are in agreement with some other reports showing that 25(OH)D deficiency was not related to HF in contrast to PTH or FGF-23 [29;30].

In addition, that the 1,25(OH)$_2$D to PTH(1–84) ratios were shown to be statistically superior to 1,25(OH)$_2$D and PTH(1–84) alone allowed for the integration of interrelated confounders. This now presents the possibility for treatment based on two modulable factors. Though the square ratio appears to be slightly better than the simple ratio, future studies with larger cohorts are needed to determine which of the two ratios will provide the stronger biomarker. The use of squared ratios, like that employed in the calculation of the widely accepted Body

Mass Index, is not without precedent [31]. The $1,25(OH)_2D$ to PTH(1–84) ratios are potent biomarkers that combine the contribution of the active form of vitamin D, $1,25(OH)_2D$, with circulating PTH(1–84), which was previously shown to be associated with mortality [16;32]. In addition, studies have shown that PTH contributes to the pathophysiology and worsening of HF [14;33]. Secondary hyperparathyroidism was previously observed in patients with untreated and treated HF with reduced left ventricular ejection fraction with second and third generation immunoassays [33–35]. In addition, PTH was shown to have several negative direct and indirect effects on the heart and cardiac cells [18;36]. Furthermore, increased circulating concentrations of PTH might stimulate adrenal aldosterone synthesis, initiating a vicious cycle between hyperparathyroidism and hyperaldosteronism leading to more pro-inflammatory, pro-oxidant and pro-fibrotic actions [37–39].

Like $1,25(OH)_2D$ and PTH(1–84), FGF-23 is a key regulator of mineral and phosphorus homeostasis [40;41]. Interestingly, a significant relationship between FGF-23 and PTH was previously documented in chronic kidney disease and HF patients [42–45]. Previous studies have found significantly higher mortality in HF patients with FGF-23 levels >172 RU/mL [45]. FGF-23 and FGF receptors are both expressed in the myocardium, and it was hypothesized that FGF-23 may have a direct effect on the heart and participate in the physiopathology of CV diseases and HF [46;47]. As $1,25(OH)_2D$ participates in the regulation of bone and mineral metabolism with PTH and FGF-23, the potential for significant diagnostic interrelations between $1,25(OH)_2D$, PTH and FGF-23 in the physiopathology of the cardio-renal syndrome related to HF is increasing. Our data demonstrate that decreased $1,25(OH)_2D$ levels are significantly related to HF severity and to the rise of serum PTH and FGF-23 levels. The decrease of the biologically active $1,25(OH)_2D$ concentrations according to NYHA classes might be related to the worsening HF and associated related worsening renal failure which can decrease the 1α hydroxylase activity and therefore leading to a reduction of the $1,25(OH)_2D$ levels. The fall of $1,25(OH)_2D$ might trigger the rise of PTH and FGF-23 contributing to a vicious cycle for cardiovascular function. On the other hand, worsening of HF and renal failure in HF patients leads to secondary hyperparathyroidism and increases of circulating FGF-23, both of which impact the synthesis of $1,25(OH)_2D$. Measurement of the ratios appears therefore as an efficient tool to assess these interrelated players independently of the kidney function.

Moreover, we observed a significant relationship between $1,25(OH)_2D$ and CgA ($\rho = -0.42$, $p<0.0001$). Like $1,25(OH)_2D$, CgA exerts a crucial role in calcium homeostasis as dense intracellular core granules involving CgA represent the major intracellular calcium reservoir [48;49]. Furthermore, CgA plays a role for in CV diseases and HF [50].

The therapeutic rational for testing $1,25(OH)_2D$ and its ratios to PTH in HF patients is that, not only could it enable more reliable risk stratification, it also could serve to guide treatment selection and monitor the efficiency of other medical devices [51]. Previous studies showed that $1,25(OH)_2D$ supplementation has protective effects on myocardial fibrosis of diabetic rats [52], that it is effective in preserving endothelial function in hypertension [53], and it improved cardiac function in patients on hemodialysis that had controllable hyperparathyroidism [54]. Indeed, more tailored treatment selection might be guided by aiming for higher $1,25(OH)_2D$/PTH(1–84) ratios. Lower levels of $1,25(OH)_2D$ in HF patients could be increased with calcitriol supplementation (or other active metabolite). On the other hand, PTH could be decreased with other pharmacological treatments such as aldosterone antagonists, known to prevent hyperparathyroidism and its consequences [55;56].

In conclusion, based on the present data, $1,25(OH)_2D$ to PTH(1–84) ratios are novel biomarkers that strongly and independently predict CV mortality in chronic HF, stronger than $1,25(OH)_2D$ alone. In addition, the $1,25(OH)_2D$ to PTH(1–84) ratios are comparable or better than currently used biomarkers such as BNP, NT-proBNP, Galectin-3, CgA and FGF-23 for

prognosis, but with the potential additional advantage of providing determinants for therapy guidance and patient monitoring.

Supporting Information

S1 Dataset. data set of the studied HF patients.
(XLSX)

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Author Contributions

Conceived and designed the experiments: DG DH F. Bonelli CZ F. Blocki MR. Performed the experiments: DG BF SA DH MR. Analyzed the data: DG SA DH F. Bonelli CZ F. Blocki MR. Contributed reagents/materials/analysis tools: DG BF SA DH F. Bonelli CZ F. Blocki MR. Wrote the paper: DG SA DH F. Bonelli CZ F. Blocki MR.

References

1. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014 Nov 7; 35(42):2950–9. doi: 10.1093/eurheartj/ehu299 PMID: 25139896
2. Liu LC, Damman K, Lipsic E, Maass AH, Rienstra M, Westenbrink BD. Heart failure highlights in 2012–2013. Eur J Heart Fail 2014 Feb; 16(2):122–32. doi: 10.1002/ejhf.43 PMID: 24464645
3. Braunwald E, Bristow MR. Congestive heart failure: fifty years of progress. Circulation 2000 Nov 14; 102(20 Suppl 4):IV14–IV23. PMID: 11080127
4. Yancy CW, Jessup M, Bozkurt B, Masoudi FA, Butler J, McBride PE, et al. 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013 Jun 5;(13:):10.
5. Januzzi JL Jr., Chen-Tournoux AA, Moe G. Amino-terminal pro-B-type natriuretic peptide testing for the diagnosis or exclusion of heart failure in patients with acute symptoms. Am J Cardiol 2008 Feb 4; 101 (3A):29–38. doi: 10.1016/j.amjcard.2007.11.017 PMID: 18243855
6. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014 Aug;%19. pii: ehu299.:ehu299.
7. Mueller C. Cost-effectiveness of B-type natriuretic peptide testing. Congest Heart Fail 2008 Jul; 14(4 Suppl 1):35–7. PMID: 18772637
8. Roubille F, Delseny D, Cristol JP, Merle D, Salvetat N, Larue C, et al. Depletion of proBNP1-108 in patients with heart failure prevents cross-reactivity with natriuretic peptides. PLoS One 2013 Sep 17; 8 (9):e75174. doi: 10.1371/journal.pone.0075174 PMID: 24069392
9. Savarese G, Trimarco B, Dellegrottaglie S, Prastaro M, Gambardella F, Rengo G, et al. Natriuretic peptide-guided therapy in chronic heart failure: a meta-analysis of 2,686 patients in 12 randomized trials. PLoS One 2013; 8(3):e58287. doi: 10.1371/journal.pone.0058287 PMID: 23472172
10. Maisel A, Mueller C, Adams K Jr., Anker SD, Aspromonte N, Cleland JG, et al. State of the art: using natriuretic peptide levels in clinical practice. Eur J Heart Fail 2008 Sep; 10(9):824–39. doi: 10.1016/j.ejheart.2008.07.014 PMID: 18760965
11. Emdin M, Vittorini S, Passino C, Clerico A. Old and new biomarkers of heart failure. Eur J Heart Fail 2009 Apr; 11(4):331–5. doi: 10.1093/eurjhf/hfp035 PMID: 19329823
12. Volpe M, Rubattu S, Burnett J Jr. Natriuretic peptides in cardiovascular diseases: current use and perspectives. Eur Heart J 2013 Nov 13.
13. de Boer RA, Lok DJ, Jaarsma T, Van Der Meer P, Voors AA, Hillege HL, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. Ann Med 2011 Feb; 43(1):60–8. doi: 10.3109/07853890.2010.538080 PMID: 21189092
14. Gruson D, Buglioni A, Burnett JC Jr. PTH: Potential role in management of heart failure. Clin Chim Acta 2014 Jun 10; 433:290–6. doi: 10.1016/j.cca.2014.03.029 Epub;%2014 Apr 1.:290–6. PMID: 24704306

15. Tunon J, Cristobal C, Tarin N, Acena A, Gonzalez-Casaus ML, Huelmos A, et al. Coexistence of low vitamin D and high fibroblast growth factor-23 plasma levels predicts an adverse outcome in patients with coronary artery disease. PLoS One 2014 Apr 18; 9(4):e95402. doi: 10.1371/journal.pone.0095402 PMID: 24748388

16. Schierbeck LL, Jensen TS, Bang U, Jensen G, Kober L, Jensen JE. Parathyroid hormone and vitamin D—markers for cardiovascular and all cause mortality in heart failure. Eur J Heart Fail 2011 Jun; 13 (6):626–32. doi: 10.1093/eurjhf/hfr016 PMID: 21415099

17. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008 Jan 29; 117(4):503–11. doi: 10.1161/CIRCULATIONAHA.107.706127 PMID: 18180395

18. Tomaschitz A, Ritz E, Pieske B, Fahrleitner-Pammer A, Kienreich K, Horina JH, et al. Aldosterone and parathyroid hormone: a precarious couple for cardiovascular disease. Cardiovasc Res 2012 Apr 1; 94 (1):10–9. doi: 10.1093/cvr/cvs092 PMID: 22334595

19. Silver J, Rodriguez M, Slatopolsky E. FGF23 and PTH—double agents at the heart of CKD. Nephrol Dial Transplant 2012 May; 27(5):1715–20. doi: 10.1093/ndt/gfs050 PMID: 22447519

20. Udell JA, Morrow DA, Jarolim P, Sloan S, Hoffman EB, O'Donnell TF, et al. Fibroblast growth factor-23, cardiovascular prognosis, and benefit of angiotensin-converting enzyme inhibition in stable ischemic heart disease. J Am Coll Cardiol 2014 Jun 10; 63(22):2421–8. doi: 10.1016/j.jacc.2014.03.026 PMID: 24727254

21. Scialla JJ, Wolf M. Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease. Nat Rev Nephrol 2014 May; 10(5):268–78. doi: 10.1038/nrneph.2014.49 PMID: 24686452

22. van HJ, Weiskirchen R. Experience with the first fully automated chemiluminescence immunoassay for the quantification of 1alpha, 25-dihydroxy-vitamin D. Clin Chem Lab Med 2014 Sep 30;j/cclm-print/cclm.

23. Renkema KY, Alexander RT, Bindels RJ, Hoenderop JG. Calcium and phosphate homeostasis: concerted interplay of new regulators. Ann Med 2008; 40(2):82–91. doi: 10.1080/07853890701689645 PMID: 18293139

24. Keyzer CA, Riphagen IJ, Joosten MM, Navis G, Muller Kobold AC, Kema IP, et al. Associations of 25 (OH) and 1,25(OH) Vitamin D with Long-Term Outcomes in Stable Renal Transplant Recipients. J Clin Endocrinol Metab 2014 Oct 31;jc20143012.

25. Zittermann A, Schleithoff SS, Frisch S, Gotting C, Kuhn J, Koertke H, et al. Circulating calcitriol concentrations and total mortality. Clin Chem 2009 Jun; 55(6):1163–70. doi: 10.1373/clinchem.2008.120006 PMID: 19359534

26. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab 2010 Feb; 95(2):471–8. doi: 10.1210/jc.2009-1773 PMID: 20133466

27. Zittermann A, Schleithoff SS, Gotting C, Dronow O, Fuchs U, Kuhn J, et al. Poor outcome in end-stage heart failure patients with low circulating calcitriol levels. Eur J Heart Fail 2008 Mar; 10(3):321–7. doi: 10.1016/j.ejheart.2008.01.013 PMID: 18304873

28. Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, et al. Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. Eur J Heart Fail 2012 Apr; 14(4):357–66. doi: 10.1093/eurjhf/hfr175 PMID: 22308011

29. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup PH, Sattar N. Elevated parathyroid hormone, but not vitamin D deficiency, is associated with increased risk of heart failure in older men with and without cardiovascular disease. Circ Heart Fail 2014 Sep; 7(5):732–9. doi: 10.1161/CIRCHEARTFAILURE.114.001272 PMID: 25104043

30. di Giuseppe R, Buijsse B, Hirche F, Wirth J, Arregui M, Westphal S, et al. Plasma fibroblast growth factor 23, parathyroid hormone, 25-hydroxyvitamin D3, and risk of heart failure: a prospective, case-cohort study. J Clin Endocrinol Metab 2014 Mar; 99(3):947–55. doi: 10.1210/jc.2013-2963 PMID: 24423292

31. Eknoyan G. Adolphe Quetelet (1796–1874)—the average man and indices of obesity. Nephrol Dial Transplant 2008 Jan; 23(1):47–51. PMID: 17890752

32. Gruson D, Lepoutre T, Ahn SA, Ketelslegers JM, Rousseau MF. Comparison between intact and bioactive parathyroid hormone assays in patients with severe heart failure. Clin Biochem 2013 Mar; 46(4–5):391–4. doi: 10.1016/j.clinbiochem.2012.12.002 PMID: 23246538

33. Altay H, Zorlu A, Binici S, Bilgi M, Yilmaz MB, Colkesen Y, et al. Relation of serum parathyroid hormone level to severity of heart failure. Am J Cardiol 2012 Jan 15; 109(2):252–6. doi: 10.1016/j.amjcard.2011.08.039 PMID: 21996143

34. Khouzam RN, Dishmon DA, Farah V, Flax SD, Carbone LD, Weber KT. Secondary hyperparathyroidism in patients with untreated and treated congestive heart failure. Am J Med Sci 2006 Jan; 331(1):30–4. PMID: 16415661

35. Gruson D, Lepoutre T, Ahn SA, Ketelslegers JM, Rousseau MF. Increased circulating concentrations of bioactive PTH 1–84 in patients with heart failure. J Endocrinol Invest 2012 Dec; 35(11):987–91. doi: 10.3275/8286 PMID: 22391109

36. Saleh FN, Schirmer H, Sundsfjord J, Jorde R. Parathyroid hormone and left ventricular hypertrophy. Eur Heart J 2003 Nov; 24(22):2054–60. PMID: 14613742

37. Chhokar VS, Sun Y, Bhattacharya SK, Ahokas RA, Myers LK, Xing Z, et al. Hyperparathyroidism and the calcium paradox of aldosteronism. Circulation 2005 Feb 22; 111(7):871–8. PMID: 15710759

38. Rutledge MR, Farah V, Adeboye AA, Seawell MR, Bhattacharya SK, Weber KT. Parathyroid hormone, a crucial mediator of pathologic cardiac remodeling in aldosteronism. Cardiovasc Drugs Ther 2013 Apr; 27(2):161–70. doi: 10.1007/s10557-012-6378-0 PMID: 22373564

39. Vidal A, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC, Weber KT. Calcium paradox of aldosteronism and the role of the parathyroid glands. Am J Physiol Heart Circ Physiol 2006 Jan; 290(1):H286–H294. PMID: 16373592

40. Lysaght AC, Yuan Q, Fan Y, Kalwani N, Caruso P, Cunnane M, et al. FGF23 deficiency leads to mixed hearing loss and middle ear malformation in mice. PLoS One 2014 Sep 22; 9(9):e107681. doi: 10.1371/journal.pone.0107681 PMID: 25243481

41. Razzaque MS. The FGF23-Klotho axis: endocrine regulation of phosphate homeostasis. Nat Rev Endocrinol 2009 Nov; 5(11):611–9. doi: 10.1038/nrendo.2009.196 PMID: 19844248

42. Bernheim J, Benchetrit S. The potential roles of FGF23 and Klotho in the prognosis of renal and cardiovascular diseases. Nephrol Dial Transplant 2011 May 4.

43. Parker BD, Schurgers LJ, Brandenburg VM, Christenson RH, Vermeer C, Ketteler M, et al. The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med 2010 May 18; 152(10):640–8. doi: 10.7326/0003-4819-152-10-201005180-00004 PMID: 20479029

44. Gutierrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, et al. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. Circulation 2009 May;%19; 119 (19):2545–52. doi: 10.1161/CIRCULATIONAHA.108.844506 PMID: 19414634

45. Gruson D, Lepoutre T, Ketelslegers JM, Cumps J, Ahn SA, Rousseau MF. C-terminal FGF23 is a strong predictor of survival in systolic heart failure. Peptides 2012 Oct; 37(2):258–62. doi: 10.1016/j.peptides.2012.08.003 PMID: 22902597

46. Liu S, Guo R, Simpson LG, Xiao ZS, Burnham CE, Quarles LD. Regulation of fibroblastic growth factor 23 expression but not degradation by PHEX. J Biol Chem 2003 Sep 26; 278(39):37419–26. PMID: 12874285

47. Li G, Oparil S, Kelpke SS, Chen YF, Thompson JA. Fibroblast growth factor receptor-1 signaling induces osteopontin expression and vascular smooth muscle cell-dependent adventitial fibroblast migration in vitro. Circulation 2002 Aug 13; 106(7):854–9. PMID: 12176960

48. Yoo SH, Huh YH, Hur YS. Inositol 1,4,5-trisphosphate receptor in chromaffin secretory granules and its relation to chromogranins. Cell Mol Neurobiol 2010 Nov; 30(8):1155–61. doi: 10.1007/s10571-010-9564-2 PMID: 21046461

49. D'amico MA, Ghinassi B, Izzicupo P, Manzoli L, Di Baldassarre A. Biological function and clinical relevance of chromogranin A and derived peptides. Endocr Connect 2014 Apr 29; 3(2):R45–R54. doi: 10.1530/EC-14-0027 PMID: 24671122

50. Angelone T, Mazza R, Cerra MC. Chromogranin-A: a multifaceted cardiovascular role in health and disease. Curr Med Chem 2012; 19(24):4042–50. PMID: 22834795

51. Wu C, Kato TS, Pronschinske K, Qiu S, Naka Y, Takayama H, et al. Dynamics of bone turnover markers in patients with heart failure and following haemodynamic improvement through ventricular assist device implantation. Eur J Heart Fail 2012 Dec;%2012 Sep 18.

52. Wang L, Yuan T, Du G, Zhao Q, Ma L, Zhu J. The impact of 1,25-dihydroxyvitamin D3 on the expression of connective tissue growth factor and transforming growth factor-beta1 in the myocardium of rats with diabetes. Diabetes Res Clin Pract 2014 May; 104(2):226–33. doi: 10.1016/j.diabres.2014.01.031 PMID: 24613393

53. Dong J, Wong SL, Lau CW, Lee HK, Ng CF, Zhang L, et al. Calcitriol protects renovascular function in hypertension by down-regulating angiotensin II type 1 receptors and reducing oxidative stress. Eur Heart J 2012 Dec; 33(23):2980–90. doi: 10.1093/eurheartj/ehr459 PMID: 22267242

54. Lemmila S, Saha H, Virtanen V, Ala-Houhala I, Pasternack A. Effect of intravenous calcitriol on cardiac systolic and diastolic function in patients on hemodialysis. Am J Nephrol 1998; 18(5):404–10. PMID: 9730564

55. Selektor Y, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Cinacalcet and the prevention of secondary hyperparathyroidism in rats with aldosteronism. Am J Med Sci 2008 Feb; 335(2):105–10. doi: 10.1097/MAJ.0b013e318134f013 PMID: 18277117

56. Carbone LD, Cross JD, Raza SH, Bush AJ, Sepanski RJ, Dhawan S, et al. Fracture risk in men with congestive heart failure risk reduction with spironolactone. J Am Coll Cardiol 2008 Jul 8; 52(2):135–8. doi: 10.1016/j.jacc.2008.03.039 PMID: 18598893