ORIGINAL ARTICLE

Disorders of calcium and phosphate metabolism in patients with significant mitral regurgitation

Olga Możeńska[1], Jacek Bil[2], Agnieszka Segiet[1], Dariusz A. Kosior[3]

1 Department of Cardiology and Hypertension, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland
2 Department of Invasive Cardiology, Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland
3 Department of Applied Physiology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

KEY WORDS

hypocalcemia, valvular heart disease, vitamin D

ABSTRACT

INTRODUCTION Although there are several known risk factors of cardiovascular diseases (CVDs), the search for new factors continues. In recent years, clinical trials have reported vitamin D and other calcium (Ca) and phosphate (P) metabolism disorders as potential new cardiovascular risk factors, but literature data on this association are limited.

OBJECTIVES We aimed to assess the extent of Ca and P metabolism disorders in patients with mitral regurgitation (MR) and potential role of these disorders as risk factors of CVD.

PATIENTS AND METHODS We enrolled adult patients with significant MR (vena contracta >3 mm, effective orifice area >0.2 cm^2, and MR volume >30 ml/s) hospitalized in our department between July and September 2013. Anthropometric data were collected. Moreover, all patients underwent blood and urine analysis, transthoracic echocardiography, and 6-minute walking test.

RESULTS A total of 99 patients were enrolled (median age, 75 years; [Q1–Q3, 66.0–81.5]; women, 35.4%). The median serum Ca level corrected by albumin was 3.22 mmol/l [Q1–Q3, 3.14–3.27]. The mean (SD) serum ionized Ca level corrected by pH was 1.05 (0.08) mmol/l. The median levels of parathyroid hormone (PTH) and 25(OH)D_3 were 63.10 pg/ml [Q1–Q3, 40.95–88.55] and 14.80 ng/ml [Q1–Q3, 9.93–20.12], respectively. Patients with a history of heart failure (HF) with reduced ejection fraction (New York Heart Association class IV), shorter distance in the 6-minute walking test, lower left ventricular ejection fraction, and larger left ventricular end-diastolic diameter had significantly higher probability of elevated PTH levels.

CONCLUSIONS Disorders of Ca and P metabolism in patients with significant MR are a noteworthy clinical problem. Our study is the first to systematically describe these disorders in patients with CVD. However, larger studies are needed to confirm the significance of our results.

Correspondence to:
Olga Możeńska, MD, PhD, Klinika Kardiologii i Nadciśnienia Tętniczego, Centralny Szpital Kliniczny Ministerstwa Spraw Wewnętrznych i Administracji, ul. Woloska 137, 02-507 Warszawa, Poland, phone: +48 22 508 16 70, email: olgamozenska@gmail.com
Received: November 20, 2017.
Revision accepted: January 26, 2018.
Published online: January 26, 2018.
Conflict of interest: none declared.
Pol Arch Intern Med. 2018; 128 (4): 222-227
doi:10.20452/pamw.4191

INTRODUCTION Although there are several known risk factors associated with cardiovascular diseases (CVDs), the search for new factors continues. In recent years, clinical trials have reported vitamin D and other calcium (Ca) and phosphate (P) metabolism disorders as potential new cardiovascular risk factors.[1-13] Valvular heart disease is the third most common cause of heart failure (HF) after coronary artery disease and hypertension.[14] In Europe, mitral regurgitation (MR) is the second most frequent acquired valvular disease in adults.[14] The coexistence of Ca and P metabolism disorders and valvular heart disease has been well described only in the context of aortic stenosis. In contrast, data on Ca accumulation in the mitral annulus in patients with MR and chronic kidney disease are limited.[15-18] Only one study has examined the frequency of valvular heart disease (defined as the need for mitral valve [MV] repair or replacement and at least moderate stenosis or regurgitation with no distinction between the valves) in patients with vitamin D deficiency.[19] Therefore, we aimed to assess the extent of Ca and P metabolism disorders in patients with MR and potential role of these disorders as risk factors of CVD.

PATIENTS AND METHODS

Study population

The study population included 99 patients hospitalized (both elective and emergent hospitalization) in our cardiology department between July 1, 2013, and September 30, 2013. The study was conducted in the summer months to ensure adequate exposition to sunlight. The inclusion criteria were age above 18 years and significant MR confirmed by transthoracic echocardiography (vena contracta >3 mm, effective orifice area >0.2 cm^2, and MR volume >30 ml/s). The exclusion criteria were a lack of written consent, medical history of invasive MV treatment (surgical or percutaneous MV repair or surgical MV replacement), MV stenosis of any type, chronic kidney disease requiring dialysis, history of thyroid or parathyroid disorders (including thyroidectomy or parathyroidectomy), any clinical condition requiring temporary (at least 2 weeks) or long-term immobilization during the 6 months prior to the study, and chronic Ca or vitamin D supplementation. On admission, all patients underwent physical examination for anthropometric measurements, and detailed medical history was collected regarding valvular heart disease, Ca and P metabolism, vitamin D deficiency, dietary regimens, physical activity, and vitamin supplementation. Each patient underwent standard hospitalization with respect to their individual symptoms, clinical status, initial diagnosis, and comorbidities.

Biochemical parameters

Blood samples were collected in the morning, after at least 12 hours of fasting and after 30-minute rest in a supine position in a quiet, environmentally controlled room, using a standardized protocol. Urine samples were collected in the morning after at least 12 hours of fasting and then after 24 hours. Serum parathyroid hormone (PTH) levels were determined with the Elecsys PTH (1–84) test (CobasÒ, Roche Diagnostics GmbH, Mannheim, Germany). Vitamin D [25(OH)D_3] was measured using the LIAISON test (DiaSorin Inc., Stillwater, Minnesota, United States). To calculate Ca levels corrected by albumin ($[Ca]_{alb}$) and pH ($[Ca^{2+}]_{pH}$), the following formulations were used[20-25]:

$[Ca]_{alb}$ (mmol/l) = Ca (mmol/l) + {[40 g/l – albumins (g/l)] × 0.025}, and

$[Ca^{2+}]_{pH}$ (mmol/l) = Ca^{2+} (mmol/l) × [1 – 0.53 × (7.4 – pH)].

Laboratory calibration references for Ca and P metabolism parameters were as follows: $[Ca]_{alb}$ (serum), 2.19–2.54 mmol/l; $[Ca^{2+}]_{pH}$ (serum), 1.13–1.32 mmol/l; daily urinary Ca secretion, 2.5–6.25 mmol/24 h (females) and 3.75–7.5 mmol/24 h (males); P (serum) 0.9–1.5 mmol/l and P (urine) 40–136 mg/dl; daily urinary P secretion, 0.4–1.3 g/24 h; PTH (serum), 10–65 pg/ml; and 25(OH)D_3, 30–80 ng/ml.

Echocardiographic parameters

A standard 2-dimensional and Doppler echocardiography examination was performed, using a commercially available diagnostic ultrasound system (iE 33, Philips Medical System, Best, the Netherlands). All measurements were performed by an experienced cardiologist according to the guidelines of the European Association of Cardiovascular Imaging.[26-28]

The study was conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent to participate in the study. The local institutional review board approved the study protocol (approval number 63/2013).

Statistical methods

The Shapiro–Wilk test of normality was used to assess the distribution of continuous variables. Continuous variables were reported as mean (SD) for normally distributed variables and median (interquartile range [IQR]) for variables deviating from normal distribution. Categorical variables were reported as frequencies and percentages. To compare continuous variables, the *t* test was used for normally distributed variables, and the Mann–Whitney test was applied for variables deviating from normal distribution. To compare categorical variables, the Fisher exact test was used. In the regression analysis, data were corrected for age, sex, and renal function impairment. The significance level was set at a *P* value of less than 0.05. The analysis was performed using the R 3.1.2 statistical package (R Core Team. [2014] R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The study included 99 patients at a median age of 75 years (IQR, 66.0–81.5). All patients had a history of HF; 64.6% had hypertension, and 46.5% had chronic kidney disease. The clinical characteristics of the patients are summarized in TABLE 1. Echocardiographic and biochemical parameters are presented in TABLE 2. All patients had elevated serum Ca levels corrected by albumin (median [IQR], 3.22 mmol/l [3.14–3.27]). The serum P level was also elevated in all patients (mean [SD], 3.51 [0.62] mmol/l). The median serum PTH level was 63.10 pg/ml (IQR, 40.95–88.55); it was elevated in 48.42% of the patients. Vitamin D deficiency was detected in 92.71% of the patients. Parameters of Ca and P metabolism, as well as the frequency of the disorders, are summarized in TABLE 3. Patients with a history of HF with reduced ejection fraction were more likely to have elevated PTH levels (odds ratio [OR], 3.361; 95% confidence interval [CI], 1.331–9.187; $P = 0.013$] than the remaining patients. Elevated PTH levels were also more likely in patients with New York Heart Association (NYHA) class IV (OR, 7.079; 95% CI, 1.908–30.154; $P = 0.001$) and in patients on diuretics (OR, 7.572; 95% CI, 2.245–35.160; $P = 0.003$) than in the remaining patients. The probability of elevated PTH levels was lower in patients with longer distance in the 6-minute walking test (OR, 0.996; 95% CI, 0.0993–0.998; $P = 0.003$).

TABLE 1 Characteristics of the study population (n = 99)

Parameter		Value
Age, y, median (Q1–Q3)		75.0 (66.0–81.5)
Sex, n (%)	Female	35 (35.4)
	Male	64 (64.6)
Weight, kg, median (Q1–Q3)		80.0 (67.0–87.8)
BMI, kg/m^2, median (Q1–Q3)		26.5 (24.2–30.2)
BSA, m^2, mean (SD)		1.89 (0.25)
Distance in 6-MWT, min, median (Q1–Q3)		150.0 (40.0–440.0)
Comorbidities, n (%)		
HF		99 (100)
HF-PEF		32 (32.3)
HF-REF		44 (44.4)
Right ventricular HF		25 (25.3)
DCM		36 (36.4)
Hypertension		64 (64.6)
Diabetes		32 (32.3)
CKD		46 (46.5)
CKD according to stage	I	19 (19.4)
	II	41 (41.8)
	III	33 (33.7)
	IV	5 (5.1)
AF		39 (63.9)
HF according to NYHA class	I	36 (36.4)
	II	34 (34.3)
	III	11 (11.1)
	IV	18 (18.2)
Medical therapy, n (%)		
β-blockers		85 (87.6)
ACEIs		78 (80.4)
Angiotensin receptor blockers		11 (11.3)
Calcium channel blockers		20 (20.6)
Diuretics		76 (78.4)
Mineralocorticoid receptor antagonists		35 (36.1)

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; BMI, body mass index; BSA, body surface area; CKD, chronic kidney disease; DCM, dilated cardiomyopathy; HF, heart failure; HF-PEF, heart failure with preserved ejection fraction; HF-REF, heart failure with reduced ejection fraction; NYHA, New York Heart Association; 6-MWT, 6-minute walking test

Moreover, it decreased with an increase in left ventricular ejection fraction (OR, 0.965, 95% CI, 0.747–0.943; P = 0.01). Finally, the probability of elevated PTH levels was higher with an increase in the left ventricular end-diastolic diameter (OR, 1.049; 95% CI, 1.002–1.102; P = 0.048).

DISCUSSION To our knowledge, our study is the first to systematically describe disorders of Ca and P metabolism in patients with significant MR. All our patients had a history of HF, in addition to significant MR. Therefore, due to the lack of published data concerning the type and frequency of Ca and P metabolism disorders in patients with significant MR, our results are discussed in comparison with those available for patients with HF.

In the study cohort, the mean (SD) serum Ca level was 2.30 (0.12) mmol/l, which is comparable with the values reported by Zittermann et al[29] (2.30 [0.02] mmol/l and 2.27 [0.02] mmol/l) as well as with the value reported by Cubbon et al[30] (2.29 mmol/l) in a cohort of 713 patients with HF. Similar results on serum Ca levels were published by Miura et al[31] and Rozentryt et al.[32] We did not identify any studies that would describe in detail the frequency of hypocalcemia or hypercalcemia or that would analyze ionized Ca or serum Ca levels in patients with CVD.

In our study, the mean (SD) serum P level was 3.51 (0.62) mmol/l. A mean (SD) P level of 1.1 (0.2) mmol/l was reported by Rozentryt et al[32,33] in a group of 722 patients. Moreover, in a study by Myrda et al,[34] including a cohort of 412 patients, the reported values were 1.02 (0.2) mmol/l, 1.06 (0.2) mmol/l, 1.12 mmol/l, and 1.24 mmol/l for patients in the NYHA classes I, II, III, and IV, respectively (P <0.001). Furthermore, Miura et al[31] reported a mean (SD) P level of 1.23 (0.42) mmol/l (in 152 patients) in the group with normal or elevated Ca levels and 1.16 (0.26) mmol/l (in 32 patients) in the group with reduced Ca levels.

The discrepancies between our results and those of the other studies are difficult to explain. A potential reason might be the exact time when the laboratory analyses of P levels were performed. Previous investigators extensively discussed hyperphosphatemia and its potential causes and effects in their study cohorts. They reported the significance of increased catabolic processes in cases of HF exacerbation. In all cited studies, the laboratory determination of P levels was performed in patients with no signs or symptoms of HF decompensation; N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were 480 pg/ml, 917 pg/ml, 2021 pg/ml, and 3063 pg/ml for patients in NYHA classes I, II, III, and IV, respectively (P <0.001).[32,33] In the group with normal or elevated Ca levels, the mean level was 469.3 pg/ml,[34] and in the hypocalcemic group, it was 415.0 pg/ml.[31] In our study, despite attempts to achieve a state of no signs or symptoms of HF decompensation in patients, the median NT-proBNP level was 1815 pg/ml (IQR, 583.50–3483.25). This may indicate an insufficient switch from catabolic into anabolic metabolism in patients with CVD and, at the same time, it may indicate that the analysis might have been performed too early. However, other potential reasons for elevated NT-proBNP levels should be considered. A potential cause may have been the presence of valvular heart disease, including MR.[35-37] No data regarding the frequency and degree of MR could be found in the reviewed literature; therefore, it is possible that the presence of hemodynamically significant MR could explain the inconsistent results. There is evidence that the presence of MR in patients with HF significantly affects mortality.[38] Similar results have been reported for the presence

TABLE 2 Echocardiographic and biochemical parameters of the study population

Parameter	Value
LVEF, %	50.0 (29.0–62.0)
LVDd, mm	56.0 (49.5–65.0)
LVEDV, ml	111.5 (79.0–182.3)
LVEDV/BSA	58.4 (43.2–91.4)
IVSDd, mm	10.0 (10.0–11.8)
PWDd, mm	10.0 (9.0–11.0)
LAV, ml	100.0 (71.3–126.0)
LAV/BSA	53.0 (38.2–65.5)
RVSP, mm Hg	43.0 (39.0–53.5)
TAPSE, mm, mean (SD)	22.0 (6.8)
MV annulus, mm, mean (SD)	37.4 (6.4)
VC, mm	6.0 (5.0–7.0)
PISA radius, mm	6.0 (5.0–7.0)
MR volume, ml/beat	23.0 (17.0–31.0)
ERO area, cm^2	0.15 (0.10–0.22)
Creatinine, mmol/l	1.03 (0.83–1.22)
eGFR, ml/min/1.73 m^2	67.11 (24.09)
Albumin, g/l	4.00 (3.72–4.22)
Total protein, g/l	6.52 (0.69)
C-reactive protein, mg/l	2.80 (1.10–8.47)
NT-proBNP, pg/ml	1815.0 (583.5–3483.3)

Data are presented as median (Q1–Q3) unless stated otherwise.

Abbreviations: BSA, body surface area; eGFR, estimated glomerular filtration rate; ERO, effective regurgitant orifice; IVSDd, intraventricular septum diastolic diameter; LAV, left atrial volume; LVDd, left ventricular diastolic diameter; LVEDV, left ventricular end--diastolic volume; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; MV, mitral valve; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PISA, proximal isovolumetric surface area; PWDd, posterior wall diastolic diameter; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion; VC, vena contracta; others, see **TABLE 1**

TABLE 3 Parameters of calcium and phosphate metabolism in the study group

Parameter	Study group	Deficiency, %	Excess, %
pH	7.40 (7.38–7.43)	–	–
Albumins, g/l	4.00 (3.72–4.22)	–	–
Serum $[Ca]_{alb,}$ mmol/l	3.22 (3.14–3.27)	0	100
Serum $[Ca^{2+}]_{pH,}$ mmol/l, mean (SD)	1.05 (0.08)	84.3	0
24-hour urinary calcium excretion, mmol/24 h	2.25 (1.28–2.92)	56.6	4.0
Serum phosphates, mmol/l, mean (SD)	3.51 (0.62)	0	100
Urinary phosphates, mg/dl	30.00 (18.15–37.65)	76.0	2.7
24-hour urinary phosphate excretion, g/24 h	0.47 (0.32–0.75)	36.0	2.7
Serum PTH, pg/ml	63.10 (40.95–88.55)	0	48.4
$25(OH)D_3$, ng/ml	14.80 (9.93–20.12)	92.7	0

Data are presented as median (Q1–Q3) unless stated otherwise.

Abbreviations: $[Ca_{alb}]$, calcium level corrected by albumin; $[Ca^{2+}]_{pH}$, calcium level corrected by pH; PTH, parathyroid hormone

of Ca and P metabolism disorders.[39-43] However, there are no data describing the direct effect of these disorders on mortality and morbidity in patients only with MR or in those with MR and HF. Moreover, there are no data on the frequency of Ca and P metabolism disorders in patients with CVD. Therefore, further studies are needed in this field.

Disturbances in serum PTH levels were analyzed. The median serum PTH level was 63.10 pg/ml (IQR, 40.95–88.55), which is in line with the results of other studies.[29,31,44] Moreover, our findings on the associations between the probability of elevated PTH levels and NYHA class, use of diuretics, 6-minute walking test results, and an increase in left ventricular ejection fraction and left ventricular end-diastolic diameter are also in line with the cited data and support the concept that disorders of Ca and P metabolism are involved in the development of HF.[31,42,45,46]

In our study, we analyzed also serum vitamin D levels. The median levels of $25(OH)D_3$ in our study cohort were 14.80 ng/ml (IQR, 9.93–20.12). In a study by Witte et al,[44] the mean (SD) $25(OH)D_3$ level was 14.82 (8.81) ng/ml, and in a study by Zittermann et al,[29] it was 13.62 (0.8) ng/ml (259 patients) and 9.21 (1.6) ng/ml (46 patients). In a study by Linefsky et al,[15] Ca and P metabolism disorders in patients with degenerative AV disease and mitral annular calcifications were investigated in 1938 patients at advanced age (mean age, 73.5 years) (73.23 [11.16] years in our population) and no history of CVD; the mean (SD) $25(OH)D_3$ level was 25.8 (11.7) ng/ml. These results are in line with the available data for HF cohorts.[32,47-49] Interestingly, the serum vitamin D concentrations in our study were lower than those observed in the POLSENIOR study,[50] which included 2899 men and 2796 women aged over 65 years. The mean (SD) levels in POLSENIOR were 40.4 (6.2) ng/µl for men and 35.2 (18.5) for women, respectively. This may support our hypothesis that additional factors must play an important role in Ca and P metabolism in patients with significant MR. Our study has several limitations. First, the study sample was small and included only 38 patients with severe MR. Second, Ca and P metabolism parameters were measured only once during the study period. Furthermore, we could not determine some parameters, such as different vitamin D metabolites, so a more detailed analysis was not possible. In addition, there was a lack of a long-term follow-up, including a follow-up after introducing Ca and vitamin D supplementation. Finally, there are no published data for comparison with our results.

In conclusion, disorders of Ca and P metabolism in patients with significant MR are a noteworthy clinical problem. Our study is the first in the literature to systematically describe these disturbances in patients with CVD. However, larger studies are required to confirm the significance of our results.

CONTRIBUTION STATEMENT OM conceived the concept of the study. OM, JB, and DK contributed to the design of the research. All authors were involved in data collection. OM, JB, and AS analyzed the data. All authors edited and approved the final version of the manuscript.

REFERENCES

1 Bell NH, Greene A, Epstein S, et al. Evidence for alteration of the vitamin D-endocrine system in blacks. J Clin Invest. 1985; 76: 470-473.

2 Watson KE, Abrolat ML, Malone LL, et al. Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation. 1997; 96: 1755-1760.

3 Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000; 72: 690-693.

4 Arunabh S, Pollack S, Yeh J, et al. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab. 2003; 88: 157-161.

5 DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004; 80 (6 Suppl): 1689S-1696S.

6 Bischoff-Ferrari HA, Giovannucci E, Willett WC, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006; 84: 18-28.

7 Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med. 2007; 167: 1730-1737.

8 Nibbelink KA, Tishkoff DX, Hershey SD, et al. 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.

9 Lee JH, O'Keefe JH, Bell D, et al. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol. 2008; 52: 1949-1956.

10 Lee P, Greenfield JR, Seibel MJ, et al. Adequacy of vitamin D replacement in severe deficiency is dependent on body mass index. Am J Med. 2009; 122: 1056-1060.

11 Stechschulte SA, Kirsner RS, Federman DG. Vitamin D: bone and beyond, rationale and recommendations for supplementation. Am J Med. 2009; 122: 793-802.

12 Krela-Kazmierczak I, Szymczak A, Tomczak M, et al. Calcium and phosphate metabolism in patients with inflammatory bowel diseases. Pol Arch Med Wewn. 2015; 125: 588-590.

13 Guligowska AR, Piglowska M, Smigielski J, et al. Inappropriate pattern of nutrient consumption and coexistent cardiometabolic disorders in elderly people from Poland. Pol Arch Med Wewn. 2015; 125: 521-531.

14 Vahanian A, Baumgartner H, Bax J, et al. Guidelines on the management of valvular heart disease: The Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology. Eur Heart J. 2007; 28: 230-268.

15 Linefsky JP, O'Brien KD, Katz R, et al. Association of serum phosphate levels with aortic valve sclerosis and annular calcification: the cardiovascular health study. J Am Coll Cardiol. 2011; 58: 291-297.

16 Movva R, Murthy K, Romero-Corral A, et al. Calcification of the mitral valve and annulus: systematic evaluation of effects on valve anatomy and function. J Am Soc Echocardiogr. 2013; 26: 1135-1142.

17 Fulkerson PK, Beaver BM, Auseon JC, et al. Calcification of the mitral annulus: etiology, clinical associations, complications and therapy. Am J Med. 1979; 66: 967-977.

18 Muddassir SM, Pressman GS. Mitral annular calcification as a cause of mitral valve gradients. Int J Cardiol. 2007; 123: 58-62.

19 Vacek JL, Vanga SR, Good M, et al. Vitamin D deficiency and supplementation and relation to cardiovascular health. Am J Cardiol. 2012; 109: 359-363.

20 Orrell DH. Albumin as an aid to the interpretation of serum calcium. Clin Chim Acta. 1971; 35: 483-489.

21 Berry EM, Gupta MM, Turner SJ, et al. Variation in plasma calcium with induced changes in plasma specific gravity, total protein, and albumin. Br Med J. 1973; 4: 640-643.

22 Payne RB, Little AJ, Williams RB, et al. Interpretation of serum calcium in patients with abnormal serum proteins. Br Med J. 1973; 4: 643-646.

23 Brauman J, Delvigne C, Deconinck I, et al. Factors affecting the determination of ionized calcium in blood. Scand J Clin Lab Invest Suppl. 1983; 165: 27-31.

24 Marshall RW, Hodgkinson A. Calculation of plasma ionised calcium from total calcium, proteins and pH: comparison with measured values. Clin Chim Acta. 1983; 127: 305-310.

25 Nikolakakis NI, De Francisco AM, Rodger RS, et al. Effect of storage on measurement of ionized calcium in serum of uremic patients. Clin Chem. 1985; 31: 287-289.

26 Evangelista A, Flachskampf F, Lancellotti P, et al. European Association of Echocardiography recommendations for standardization of performance, digital storage and reporting of echocardiographic studies. Eur J Echocardiogr. 2008; 9: 438-448.

27 Lancellotti P, Tribouilloy C, Hagendorff A, et al. European Association of Echocardiography recommendations for the assessment of valvular regurgitation. Part 1: aortic and pulmonary regurgitation (native valve disease). Eur J Echocardiogr. 2010; 11: 223-244.

28 Lancellotti P, Moura L, Pierard LA, et al. European Association of Echocardiography recommendations for the assessment of valvular regurgitation. Part 2: mitral and tricuspid regurgitation (native valve disease). Eur J Echocardiogr. 2010; 11: 307-332.

29 Zittermann A, Fuchs U, Kuhn J, et al. Parameters of mineral metabolism predict midterm clinical outcome in end-stage heart failure patients. Scand Cardiovasc J. 2011; 45: 342-348.

30 Cubbon RM, Thomas CH, Drozd M, et al. Calcium, phosphate and calcium phosphate product are markers of outcome in patients with chronic heart failure. J Nephrol. 2015; 28: 209-215.

31 Miura S, Yoshihisa A, Takiguchi M, et al. Association of hypocalcemia with mortality in hospitalized patients with heart failure and chronic kidney disease. J Card Fail. 2015; 21: 621-627.

32 Rozentryt P, Niedziela JT, Hudzik B, et al. Abnormal serum calcium levels are associated with clinical response to maximization of heart failure therapy. Pol Arch Med Wewn. 2015; 125: 54-64.

33 Rozentryt P, Nowak J, Niedziela J, et al. Serum phosphorus level is related to degree of clinical response to up-titration of heart failure pharmacotherapy. Int J Cardiol. 2014; 177: 248-254.

34 Myrda K, Rozentryt P, Niedziela JT, et al. Clinical and laboratory determinants of low serum level of 25-hydroxyvitamin D during escalation of pharmacotherapy in heart failure patients. Kardiochir Torakochirurgia Pol. 2015; 12: 216-227.

35 Yusoff R, Clayton N, Keevil B, et al. Utility of plasma N-terminal brain natriuretic peptide as a marker of functional capacity in patients with chronic severe mitral regurgitation. Am J Cardiol. 2006; 97: 1498-1501.

36 Petracca F, Affuso F, Di Conza P, et al. Usefulness of NT-proBNP in the assessment of patients with aortic or mitral regurgitation. J Cardiovasc Med (Hagerstown). 2009; 10: 928-932.

37 Potocki M, Mair J, Weber M, et al. Relation of N-terminal pro-B-type natriuretic peptide to symptoms, severity, and left ventricular remodeling in patients with organic mitral regurgitation. Am J Cardiol. 2009; 104: 559-564.

38 McGee EC, Jr. Should moderate or greater mitral regurgitation be repaired in all patients with LVEF <30%? Surgery, mitral regurgitation, and heart failure: the valves are all repairable but the patients are not. Circ Heart Fail. 2008; 1: 285-289.

39 Pilz S, Marz W, Wellnitz B, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. J Clin Endocrinol Metab. 2008; 93: 3927-3935.

40 Zittermann A, Schleithoff SS, Frisch S, et al. Circulating calcitriol concentrations and total mortality. Clin Chem. 2009; 55: 1163-1170.

41 Ameri P, Ronco D, Casu M, et al. High prevalence of vitamin D deficiency and its association with left ventricular dilation: an echocardiography study in elderly patients with chronic heart failure. Nutr Metab Cardiovasc Dis. 2010; 20: 633-640.

42 Schierbeck LL, Jensen TS, Bang U, et al. Parathyroid hormone and vitamin D-markers for cardiovascular and all cause mortality in heart failure. Eur J Heart Fail. 2011; 13: 626-632.

43 Liu L, Chen M, Hankins SR, et al. Serum 25-hydroxyvitamin D concentration and mortality from heart failure and cardiovascular disease, and premature mortality from all-cause in United States adults. Am J Cardiol. 2012; 110: 834-839.

44 Witte KK, Byrom R, Gierula J, et al. Effects of Vitamin D on Cardiac Function in Patients With Chronic HF: The VINDICATE Study. J Am Coll Cardiol. 2016; 67: 2593-2603.

45 Sugimoto T, Tanigawa T, Onishi K, et al. Serum intact parathyroid hormone levels predict hospitalisation for heart failure. Heart. 2009; 95: 395-398.

46 Sugimoto T, Dohi K, Onishi K, et al. Interrelationship between haemodynamic state and serum intact parathyroid hormone levels in patients with chronic heart failure. Heart. 2013; 99: 111-115.

47 Tagliafico AS, Ameri P, Bovio M, et al. Relationship between fatty degeneration of thigh muscles and vitamin D status in the elderly: a preliminary MRI study. AJR Am J Roentgenol. 2010; 194: 728-734.

48 Kim DH, Sabour S, Sagar UN, et al. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol. 2008; 102: 1540-1544.

49 Rywik TM, Janas J, Klisiewicz A, et al. Prognostic value of novel biomarkers compared with detailed biochemical evaluation in patients with heart failure. Pol Arch Med Wewn. 2015; 125: 434-442.

50 Laczmanski L, Lwow F, Mossakowska M, et al. Association between vitamin D concentration and levels of sex hormones in an elderly Polish population with different genotypes of VDR polymorphisms (rs10735810, rs1544410, rs7975232, rs731236). Gene. 2015; 559: 73-76.