Relationship Between Calcium-phosphorous Product, Native and Bioprosthetic Mitral Valve Calcifications

MIHAIL ENACHE[1,2], RALUCA OZANA CHISTOL[1,2]*, CRISTINA FURNICA[2]*, GRIGORE TINICA[1,2]

[1] Institute of Cardiovascular Diseases, 50 Carol I Blvd., 700503, Iasi, Romania

[2] Grigore T. Popa University of Medicine and Pharmacy, 16 Universitatii Str., 700115, Iasi, Romania

Calcification is a common cause of failure of natural and bioprosthetic valves (BPV). Prior research on patients with chronic kidney disease identified increased calcium-phosphorus (Ca-P) product as a risk factor for both arterial and valvular calcifications, an aspect not thoroughly investigated in general population. The aims of our study were to analyse the functional impact of native and bioprosthetic mitral valve (MV) calcification detected on cardiac computed tomography angiography (CCTA), to evaluate risk factors and to assess potential differences from a morphological and chemical point of view between BPV and native MV calcification. The authors performed a retrospective study on 270 patients who underwent CCTA for suspected coronary artery disease, 225 patients with no history of MV replacement and 45 patients with bioprosthetic MV. Mitral leaflet calcification (MLC) was registered in 21 (9.33%) and mitral annular calcification (MAC) in 27 (12%) of the 225 patients suspected for CVD. Echocardiography identified MV sclerosis in 20 cases (8.89%) and MV stenosis in 12 cases (5.33%) with MLC and/or MAC. In the BPV group, 13 patients (28.89%) presented visible BPV calcification associated to echocardiographic regurgitation in 3 (30.77%) cases and higher mean transvalvular gradients. Increased Ca-P product and diabetes mellitus proved to be risk factors for both native and BPV calcification and time since surgery only for BPV calcification. Native and BPV calcification are associated to valve dysfunction and share structural characteristics, thus indicating similar calcification mechanisms. Good glycaemic control in diabetic patients and careful administration of bisphosphonates, calcium and vitamin D supplements are mandatory especially in patients with BPV in order to prevent valve dysfunction due to calcification.

Keywords: mitral valve calcification, calcium-phosphorus product, hydroxyapatite, diabetes mellitus

Calcification is a common cause of failure of natural and bioprosthetic valves, severe degeneration and calcification of bioprosthetic mitral valve (MV) being a significant problem in cardiac surgery. Calcium deposits can prevent opening and closure movements, causing problems such as stenosis and regurgitation [1]. Several studies have suggested that mitral annular calcification (MAC) reflects the global atherosclerotic burden and is independently associated with a higher risk of cardiovascular diseases (CVD) and cardiovascular death [2, 3]. Prior research carried out on patients diagnosed with chronic kidney disease (CKD) identified increased calcium-phosphorus product (Ca-P product) above the solubility threshold as a risk factor for both arterial and valvular calcifications but this aspect has not been thoroughly investigated in general population [4].

The regulation of phosphocalcium metabolism is dependent on a double hormonal system composed of parathyroid hormone and, on the other hand, vitamin D (1,25-dihydroxycholecalciferol) synthesized in the skin and transformed into active substance in liver and kidney. These two systems ensure calcium homeostasis by regulating the digestive absorption, urinary excretion and bone mobilization (on demand) of calcium. They aim to elevate calcemia. The calcitonin secreted by the parafollicular C cells of the thyroid has an antagonic effect, hypocalcemic and hypophosphatemic. The metabolisms of phosphorus and calcium are closely related and regulated by the same hormones. Normally, 99% of calcium and phosphorus are found in teeth and skeleton in the form of phosphocalcic hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and 1% in the inner medium of which 0.9% in the cells. A misbalance of calcium intake, bisphosphonates administration, vitamin D, and involved hormones can lead to the formation of vascular and other extraosseous phosphocalcium deposits [5, 6].

The aims of our study were to analyse the functional impact of native and bioprosthetic mitral valve calcification detected on cardiac computed tomography angiography (CCTA), to evaluate risk factors and to assess potential differences from a morphological and chemical point of view between bioprosthetic and native mitral valve calcification.

Experimental part

Material and methods

The authors performed a retrospective study on 270 patients who underwent CCTA for suspected coronary artery disease between September 2012-January 2017, 225 patients with no history of MV replacement (group 1, randomly selected) and 45 patients with bioprosthetic MV (group 2). Patients with chronic kidney disease (CKD) were excluded in order to eliminate bias.

All patients benefited from the same evaluation including blood tests, electrocardiogram (ECG), cardiac echocardiography, and CCTA. The presence of MAC, mitral leaflet calcification (MLC) and bioprosthetic leaflet calcification (BLC) was correlated with clinical symptoms, lab results and echocardiographic results for assessing their functional impact. In the same time, a literature review was conducted in order to identify studies comparing the chemical structure of MAC, MLC and BLC. Informed consent for data usage was obtained from all patients prior to image analysis.

All CCTA examinations were performed using a 2nd generation 256-slices dual source multi-detector CT (MDCT) scanner (Siemens Somatom Definition Flash) with

* *email: ralucachistol@gmail.com; cristinafurnica@yahoo.com*

the following scan parameters: 120 kV tube voltage, tube current modulated by CareDose 4D algorithm, 128 × 0.6 mm collimation, gantry rotation time 280 ms. Two imaging protocols are used depending on heart rhythm and cardiac pathology: high pitch retrospective scanning in patients with high (≥71 beats per minute) or irregular heart rates and high pitch prospective scanning in patients with stable heart rate ≤70 beats per min. After patient installation, two puffs of nitro-glycerine spray (0.8 mg) were administered sublingually for coronary artery dilation. Image acquisition started with an unenhanced scan for calcium scoring evaluation followed by bolus tracking injection protocol for optimal contrast opacification. An average volume of 100 mL of contrast media and 50 mL of saline chaser were injected intravenously and the patient was scanned in a single breath-hold when the descending aorta opacification reached 150 *Hounsfield units* (HU). Optimal reconstructions at different R-R interval percentages were performed (thickness 0.75 mm) and submitted to the *Syngo.via* workstation (Siemens Medical Solutions, Germany) for image analysis. The presence of MAC, MLC and BLC was assessed on unenhanced images by extrapolating the *Agatston calcium score* used for CAD evaluation. *Agatston score* is a semi-automated tool that calculates a coronary artery calcification score based on quantification of calcium on an unenhanced cardiac CT. The calculation is based on the weighted score given to the highest attenuation value in HU multiplied by area of the calcification:

$$\text{Agatston score} = \text{Area} \times \text{X-Factor},$$

the X-Factor is a value between 1 and 4 based on the value range of the highest intensity pixel in the calcified plaque (130-199 = X-Factor 1, 200-299 = X-Factor 2, 300-399 = X-Factor 3, ≥400 = X-Factor 4).

Echocardiographic evaluation included 2D, Doppler and M-mode studies with qualitative and quantitative assessment.

Calcium–phosphorus product (Ca-P, product of the serum calcium and phosphorus concentrations) was evaluated in all cases.

Statistical analysis was carried out using IBM SPSS 23.0 for Mac. Continuous variables are expressed as mean values ± standard deviation and compared using *Student's t test*. Categorical variables are expressed as ranks or percentages and analysed using *chi-square test*.

Results and discussions

Baseline characteristics did not register significant statistical differences between the two study groups except for gender. In group 1 there were included 131 men (58.22%) and 94 women (41.78%) with a sex ratio of 1.39:1.00. In group 2, there were 24 women (53.33%) and 21 men (46.67%), with a sex ratio of 0.9 corresponding to the global incidence of MV disease, higher in women than men. Mean age was 63.5±11.7 years in group 1 compared to 66.8±9.1 years in group 2. Mean interval since bioprosthetic valve implantation in group 2 was 6.7±3.9 years.

In group 1, MLC was registered in 21 (9.33%) and MAC in 27 (12%) of the 225 patients suspected for CVD. MLC were mild/moderate in all cases but MAC proved severe and extensive in 3 patients (table 1). Echocardiography identified MV sclerosis in 20 cases (8.89%) and MV stenosis in 12 cases (5.33%). In 1 case with MV sclerosis, CCTA revealed no MLC or MAC but all cases with MV stenosis presented MLC, MAC or both. Calcification severity correlated with MV disease (table 1).

A high degree of MAC and MLC overlapping was noted (19 cases) with 2 patients presenting only MLC and 8 only MAC (fig. 1). Detection rate of MAC and MLC was the same for both genders (16 men – 12.21%, 13 women – 13.83%). Patients with MAC and MLC were older (68.9±8.7 years) compared to those without calcification (62.5±10.8 years) (p = 0.002). Two (0.97%) of the 205 patients without MV disease presented mild MLC, and 8 (3.9%) MAC.

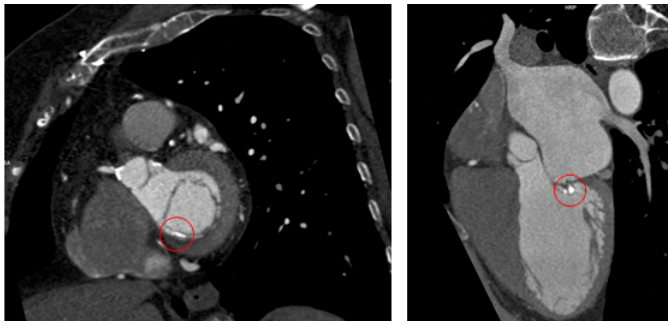

Fig. 1. Mitral annular calcification (red circle) in a patient with MV sclerosis (left) and mitral leaflet calcification (red circle) in a patient with MV stenosis (right)

Patients with MLC and MAC had higher Ca-P product (2.58±0.29 $mmol^2/L^2$) compared with patients not presenting calcification (2.39±0.35 $mmol^2/L^2$) (p = 0.0031). Diabetes mellitus, diagnosed in 9 patients with MLC/MAC (31%) and in 21 patients without calcification (10.71%) was also a factor associated with MLC and/or MAC (*chi square test* p = 0.0015).

In group 2, 13 patients (28.89%) presented visible BPV calcification (mean Agatston score 6.7). These patients were implanted earlier (8.8±3.8 years) compared to patients without BPV calcification (7.3±4.1 years) (p = 0,003). Three (30.77%) of the 13 patients with BPV calcification presented valve regurgitation (versus 1 patient without BPV calcification – 3.12%). Mean transvalvular gradient was increased in patients with BPV calcifications (7.22.1 mmHg) compared to patients with no BPV calcifications (4.81±1.9 mm Hg). From the baseline parameters, time since surgery (p = 0.0027), diabetes mellitus (chi square test p = 0.0001) and as Ca-P product (2.61±0.25 $mmol^2/L^2$ for patients with BPV calcification versus 2.43±0.29 $mmol^2/L^2$ in the absence of calcification) were associated with the presence of BPV calcification. Diabetes mellitus was diagnosed in 5 patients with BPV calcification (38.46%) and in 4 patients without calcification (12.5%).

Extracellular fluid is supersaturated with respect to the precipitation of hydroxyapatite ($Ca_5(PO_4)_3(OH)$). The occurrence of calcification requires energy for the change of phase of the solution to the solid crystal form, and the required energy can be reduced by the presence of nucleation sites, such as surface defects, cell debris, collagen fibers, mitochondria, lipids, and certain regulatory proteins, which serve as substrates for heterogeneous calcification in cardiac valves [7-9].

	MV stenosis	MV sclerosis without stenosis
MLC Agatston score	52.5	4.2
MAC Agatston score	247.7	53.6

Table 1
CALCIFICATION SEVERITY CORRELATED WITH MITRAL VALVE DISEASE

In case of BPV made from porcine and bovine pericardium, in addition to the factors already mentioned, calcification is also caused by glutaraldehyde ($C_5H_8O_2$) treatment before implantation, a standard procedure that prevents degradation, increases durability, and reduces the antigenicity of valves. Despite this, BPV still fail, especially in a mitral position (30% degeneration at 12-15 years), mainly due to degradation and calcification. Stabilization of tissues by glutaraldehyde promotes cell death, and it has been shown that its activity persists for many months after implantation, slowly releasing active glutaraldehyde monomers that destroy fibrocytes and macrophages that come into contact with the toxic material [10-16].

Calcification of native valves and BPV occurs after a period of years, unlike the calcification *in vitro* models, which starts immediately. This fact may indicate the presence of some *in vivo* factors that inhibit calcification of valves [14].

In general, valvular deposits of calcium phosphate are chemically and crystallographically similar to those found in bones, and it is believed that calcification of heart valves seems to be a process regulated similarly to bone formation, with nucleation of apatite crystals, growth, and possible degradation in association with an extracellular matrix that regulates tissue mineralization [3, 8].

In order to prevent such calcification, it is important to understand how it occurs and to discover its risk factors. XANES (X-ray absorption near-edge structure) analysis at the K edge of calcium absorption, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) studies proved the presence of identical spheroidal and crystalline deposits in regions most susceptible to mechanical stress both in native and BPV. These deposits presented Ca/P molar stoichiometric ratios similar to that of hydroxyapatite. Fitzpatrick et al., using micro energy analysis X-ray diffraction, observed that the calcification deposits formed in atherosclerotic human coronary arteries presented chemical composition identical to that of bone hydroxyapatite [14, 17]. Similar structural characteristics of calcified deposits in both native and BPV indicate possible comparable calcification mechanisms thus allowing a unidirectional development of anti-calcification treatments.

Recent studies have proved that osteogenic mechanisms are also involved as inflammatory cells, tissue osteopontin (OPN) and osteocalcin (OCN) are expressed by explanted degenerated BPV [9, 12]. High phosphate serum is able to stimulate OPN and OCN production. These observations triggered the idea of this research, that Ca-P product could be a predictor of valve calcification both in case of native and BPV. Computed tomography and echocardiography proved to accurately identify and localize native and BPV calcification in aortic or mitral position [18-20].

In our study group, in the absence of CKD, all patients presented with Ca-P product within normal range. However, subtle alterations have been identified in case of patients with native and BPV calcification. In general population, this product can be altered by diabetes mellitus, calcium, vitamin D and bisphosphonates administration, especially in post-menopausal osteoporotic women [21, 22]. In diabetic patients, the phosphates are mobilised towards the extracellular compartment and the bioavailability of vitamin D is affected due to its sequestration in the fat mass [23]. The results of our research plead for good glycaemic control in diabetic patients and careful administration of bisphosphonates, calcium and vitamin D supplements especially in patients with BPV as they could promote valve degeneration and formation of calcium deposits. The size and calcium impregnation of these deposits increase with valve disease severity from mild/moderate in case of leaflet sclerosis to severe in stenosis.

Conclusions

Native and bioprosthetic mitral valve calcification are associated to valve dysfunction and share structural characteristics, thus indicating similar calcification mechanisms. Increased calcium-phosphorus product and diabetes mellitus are risk factors for both native and bioprosthetic mitral valve calcification and time since surgery only for bioprosthetic valves. Good glycaemic control in diabetic patients and careful administration of bisphosphonates, calcium and vitamin D supplements are mandatory especially in patients with bioprosthetic valves in order to prevent valve dysfunction due to calcification.

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