Calcification of Bioprosthetic Heart Valves
Biochemical substrate and prevention

CRISTINA FURNICA[1], RALUCA OZANA CHISTOL[1,2]*, MARIA-MAGDALENA LEON CONSTANTIN[1], ANISIA-IULIANA ALEXA[1]*, GRIGORE TINICA[1,2]

[1]Grigore T. Popa University of Medicine and Pharmacy, 16 Universitatii Str., 700115, Iasi, Romania
[2] Institute for Cardiovascular Diseases, 50 Carol I Blvd., 700503, Iasi, Romania

Calcification of the leaflets of bioprosthetic heart valves (BHV) made from glutaraldehyde treated bovine or porcine pericardium or porcine aortic valves is related to the failure of these devices. The aim of this study is to evaluate the degeneration and calcification of explanted BHV and to analyze the underlying chemical substrate and risk factors. The authors performed a retrospective study on 47 cases with dysfunctional aortic BHV that were explanted and replaced between January 2000-November 2014. Hospital records, laboratory test results, cardiac computed tomography angiography (cardiac CTA) results, and operative data were reviewed and statistically analyzed. Mean valve survival time was of 11.5 ± 4.2 years. Coronary CTA revealed the presence of BHV calcifications in 38 out of the 47 cases (25 patients presented only punctate calcifications, 11 cases presented moderate cuspal calcifications and 2 cases presented gross calcifications associated to cuspal thickening and deformation). Glutaraldehyde fixation process of BHV may lead to degeneration and calcification of this type of valves, and in our opinion, the most promising preventive strategies have to include binding of calcification inhibitors to glutaraldehyde fixed tissue, removal or modification of calcifiable components, modification of glutaraldehyde fixation, and use of tissue cross linking agents other than glutaraldehyde.

Keywords: bioprosthetic heart valve, calcifications, glutaraldehyde, chemical substrate, biomaterials

Calcification plays a major role in the failure of bioprosthetic heart valves (BHV) and other tissue heart valve substitutes. The mechanism involves reaction of calcium-containing extracellular fluid with membrane-associated phosphorus to yield calcium phosphate mineral deposits [1]. Calcification is accelerated by young recipient age, valve factors such as glutaraldehyde ($CH_2(CH_2CHO)_2$) fixation, and increased mechanical stress. Deposition of mineral salts of calcium (especially hydroxyapatite – $Ca_{10}(PO_4)_6(OH)_2$) is normal in case of bones and teeth and restricted to specific anatomic sites. Since the biomaterials contained by medical devices outside of the musculoskeletal and dental systems are not intended to calcify, calcification of these materials is considered as pathological and related to tissue degeneration. Recent studies have suggested that pathologic calcification is regulated by inductive and inhibitory factors, similar to the physiologic mineralization of bone [2, 3].

The available heart valve prostheses on the market are mechanical and biological substitutes. However, both types of available heart valves have negative side effects because they are fabricated from foreign materials. Mechanical heart valves have proven to be very durable, but are susceptible to thrombosis and thromboembolism and necessitate long-term anticoagulation therapy [4]. On the other hand, BHVs require little or no anticoagulation; however, the underlying problem with them is a limited life because of structural changes such as leaflet wear and calcification leading to valve failure [5].

The focus of this study is on valvular calcification, as part of the vascular calcification, especially in the aortic position. Although new generations of prosthetic heart valves and a variety of anticalcification therapies have been developed over the years, prosthetic heart valve calcification is still poorly understood and remains the main obstacle for a life-long function of these valves, which makes it hard to develop treatment strategies and effective therapies. This study summarizes current concepts in the pathophysiology of tissue valve calcification and evaluates the degeneration and calcification of explanted BHV in order to analyze the underlying chemical substrate and risk factors.

Experimental part
Material and methods

The authors performed a retrospective study on 47 cases with dysfunctional bioprosthetic aortic valves that were explanted and replaced at the Institute of Cardiovascular Diseases from Iasi, Romania between January 2000-November 2014. Hospital records, laboratory test results, cardiac computed tomography angiography (cardiac CTA) results, and operative data were reviewed.

All patients were examined using a 2nd generation 256-slices dual source CT scanner (Siemens Somatom Definition Flash, Siemens Medical Solutions, Germany) with the following scan parameters: 100 or 120 kV tube voltage, tube current modulated by CareDose 4D algorithm (320 mAs reference), 128 x 0.6 mm collimation, gantry rotation time 280 ms, and high pitch retrospective cardiac synchronization scanning. Test bolus injection protocol was used in all cases for optimal contrast timing. A volume of 20 mL of contrast agent (Iomeron 400, Bracco, Milan, Italy) followed by 25 mL of saline chaser were injected via an 18G cannula placed in the right mediobasilic or mediocephalic vein at a flow rate of 6mL/sec. A series of dynamic, low-dose monitoring scans were performed (region of interest in the ascending aorta) beginning 10

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

seconds after initiation of contrast agent injection in order to determine the time interval between the start of the test bolus injection and the peak of aortic enhancement. After the aortic peak time evaluation (APT) 2 puffs of nitroglycerin (0.8 mg) were administered sublingually for coronary artery dilation, thus allowing the start of the main examination. An average volume of 85 mL of contrast media and 50 mL of saline chaser were injected and scanning in a single breath hold began after a delay based on the previously determined APT+5 seconds. Optimal reconstructions at different R-R interval percentages were performed (thickness 0.75 mm). Images were reconstructed at 30-80% of the R-R interval in 5% increments while the optimal reconstruction interval was chosen for image analysis on a Syngo.via workstation (Siemens Medical Solutions, Germany) by two independent radiologists, for more objective results. Renal function was evaluated the day of the examination in all cases. In patients treated with metformin derivate, the medication was suspended for 48 hours after the examination and reinitiated after a renal function control in order to avoid lactic acidosis.

Image analysis started with evaluation of coronary calcium score and aortic valve calcifications on the non-contrast acquisition. Images obtained after contrast injection (0.75 mm thick) were analyzed using the syngo.CT Coronary Analysis software application in order to characterize atherosclerotic plaques and valve functionality.

Results and discussions
The authors analyzed a total of 47 cases that needed replacement of a dysfunctional bioprosthetic aortic valve between 2000-2014. Of the 47 patients, 28 were men and the mean age when the first valve was implanted was 53 ± 15.2 years (27-66 years). Mean valve survival time was of 11.5 ± 4.2 years. Seven patients needed urgent valve replacement for endocarditis with valve destruction and severe regurgitation or important paravalvular leak. A cardiac CTA was performed in all cases for preoperative evaluation of the valve and coronary arteries. Coronary CTA revealed the presence of BHV calcifications in 38 out of the 47 cases (25 patients presented only punctate calcifications, 11 cases presented moderate cuspal calcifications and 2 cases presented gross calcifications associated to cuspal thickening and deformation). 29 of the patients with BHV calcifications also presented coronary artery disease (atherosclerotic plaques with various degrees of stenosis). Relevant laboratory data and patients history are displayed in table 1.

Overall surgical outcome
None of the patients had aneurysmal dilatation of the ascending aorta or registered severe postoperative complications related to the aortic bioprosthesic valve after the first implantation.

Calcification assessment by coronary calcium score
Severe calcifications were present in less than 1/10 of explanted valves, but more than half of these valves displayed punctate calcifications (fig. 1). 29 of the patients with calcifications of the prosthetic valve also presented coronary artery disease (mean Agatston score 189.7) thus suggesting that the pathogenesis of atherosclerosis resembles that of BHV degeneration. Calcifications and lipids, the principal features of atherosclerotic plaques, were also present in degenerated valves.
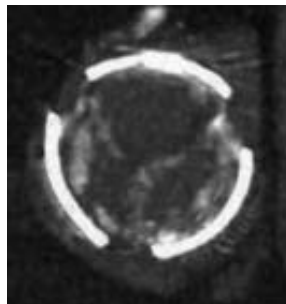

Fig. 1. Severe calcifications of a bioprosthetic aortic valve (non-contrast CT image)

There are different methods to treat valvular heart disease depending on severity and type of diagnosis. Heart valve replacement procedures, which have been used successfully since the 1960s, are safe and effective even though 10-year survival rate reviews still range from 37-58% [6].

Aortic valve anatomy
The aortic valve consists of the sinotubular junction, three aortic sinuses and three aortic leaflets.

The sinotubular junction (STJ), the region of the ascending aorta between the aortic sinuses and the superior aspect of the aortic root, presents a circular shape and supports the peripheral attachment of the aortic leaflets. The expanded portions of the aortic root around the leaflet attachments compose *the three aortic sinuses*. The aortic sinuses are named according to their arising arteries as *right-coronary sinus*, *left-coronary sinus* and *non-coronary sinus*. The curved shapes of the sinuses are thought to create sufficient gap between the aortic leaflets and the coronary arteries so that the aortic leaflets do not occlude the coronary inlets during systole [7]. This space is also supposed to improve the turbulent current behind the leaflets. According to this view, the aortic leaflets will be caught and closed by the blood flow at the end of systole. Finally, this specific shape for the sinuses can minimize the mechanical stress concentration between

Parameter	Value
Age	53 ± 15.2 years (27-66 years)
Sex	28 males, 19 females
Mean prosthesis survival time	11.5 ± 4.2 years
Causes of bioprosthetic valve dysfunction	leaflet tear – 29 cases, degeneration – 10 cases, endocarditis – 5 cases, paraprosthetic leak – 2 cases, perforation – 1 case.
Arterial hypertension	30 cases
Smoking after first valve implantation	11 cases
Diabetes mellitus	12 cases
Hypercholesterolemia (≥ 200 mg/dL)	28 cases (15 cases receiving statins)

Table 1
STUDY GROUP
CHARACTERISTICS

the aortic sinuses and the leaflets. Gundiah et al. demonstrated that the aortic sinuses and the ascending aorta have different material properties and showed that the aortic sinus is significantly stiffer than the ascending aorta, while both demonstrated anisotropic behaviors with the circumferential direction being stiffer than the longitudinal direction [8]. The leaflets are connected to the aortic wall by the *semilunar attachment line* and the *commissures*, which are formed by two portions of attachment lines of adjacent leaflets running side by side. During valve loading, adjacent leaflets come in contact with each other over the coaptation area and create a seal against backflow from the aorta into the left ventricle [9]. Measurements by Silver & William revealed that sinuses volume, sinotubular junction area and leaflets area increase with age and heart weight [10]. The aortic leaflets are pliable and thin in young people and become stiffer and thicker with age [9]. The leaflets are also known to be thinner in the leaflet belly and the coaptation area, and thicker at the leaflets attachment and free margin [7].

Ultrastructure of the aortic leaflets

The aortic leaflets are mostly (90%) water and connective tissue with unique mechanical properties. The main structural component of connective tissue consists of proteins collagen types I and III, elastin, GAG's (glycosaminoglycans - long chain sugars) and other small amount of cells. The internal collagen of leaflets forms a framework with three distinct layers: the fibrosa, spongiosa and ventricularis [11]. The layer facing the aorta is the fibrosa, which is arranged as a series of parallel bundles of collagen fibers into a stretch-resisting sheet of tissue. Fiber bundles are oriented in a circumferential direction, starting at one commissure, then spreading out into an isotropic mesh near the belly and finally combining again at the opposite commissure to provide the essential strength of the leaflets. Radial expansion of the leaflets allows them to mate together and seal off the aortic orifice [8]. The ventricularis facing the left ventricle consists of elastin sheets and collagen. This layer has more extensible properties than the fibrosa due to its higher concentration in elastin. The spongiosa fills the space between the fibrosa and ventricularis and is composed of collagen, elastin, proteoglycans and mucopolysaccharides. The spongiosa has a gelatinous, watery consistency due to its long, multichain proteins. The specific function of the spongiosa is not well understood; however it is believed that it is facilitates the localized movement and shearing between the fibrosa and the ventricularis during loading and unloading and thereby minimizes mechanical interaction between the two fibrous layers [9].

Treatment of valvular diseases

In the current study, the mean survival time of the original BHV was of 11.5 years and the main causes of valvular dysfunction were leaflet tear and degeneration. CT showed the presence of valvular calcifications in 38 of the 47 cases, a hallmark of tissue degeneration [12]. Most of the patients also presented arterial hypertension, hypercholesterolemia and coronary artery disease that indicate the presence of severe atherosclerosis and imply an increased stress on the aortic valve leading to degeneration.

The mechanical type of heart valve is durable but susceptible to blood clot formation requiring anticoagulation therapy. Anticoagulation therapy is necessary to reduce thromboembolic complications. Conversely, xenografts, or BHVs, are made of either porcine valves or bovine pericardium. These valves are more biocompatible and less thrombogenic than mechanical valves, yet they too undergo tissue degeneration and failure over time due to immune response to the graft. Therefore, xenograft valve durability ranges between 5 to 20 years, and can be even shorter in younger patients with more pronounced immune responses. This creates a need to re-operate sooner in these patients. Although the various replacement valves have their own advantages and disadvantages, they all share a common weakness [2].

Mechanism of valvular calcification

Calcification is a major contributor in tissue and BHV failure. Calcium and phosphorous are the most abundant elements in a human body. 99% of the total calcium content and 85% of the total phosphorous content in a human body reside in the skeleton (bone, teeth). The rest of these elements are distributed through intracellular and extracellular fluids and in soft tissue. Under controlled conditions, calcium is released from the bone into the bloodstream and transported from there into the body [12-14] Calcium concentration in the extracellular plasma in normal condition is 1 mg/mL (approximately 10^{-3} M) and since the calcium is pumped out by membranes of healthy cells, calcium concentration in the cytoplasm becomes 1,000 to 10,000 times lower (approximately 10^{-3} M). However, this normal mechanism for elimination of calcium from the cells does not happen in tissues that have been pretreated with glutaraldehyde [15, 16]. Phosphorous constitutes a large amount of the cell membranes and other intercellular structures (as phospholipids, especially phosphatidyl serine, and the phosphate backbone of the nucleic acids). They can bind with calcium and provide a nucleation site. Further calcification deposits accumulate in these initial nucleation sites and eventually combine and become larger. As a result of this mineralization, the tissue becomes stiff and therefore weak and causes malfunction in bioprosthesis performance [14].

Aortic calcification is a kind of vascular calcification through which calcium deposits build up on the aortic valve leaflets. The calcium deposits thicken and cause narrowing at the opening of the aortic valve. This impairs blood flow through the valve, causing chest pain or a heart attack. In severe cases, patients should undergo surgery and their aortic heart valve should be replaced with mechanical or BHV. Replacement valves, however, are subject to same calcification whether they are made up of tissue, or polymers. This is to make them immunologically inert and improve the tissue durability. It is believed that the glutaraldehyde fixation process leads to calcification of these types of valves. The glutaraldehyde pretreated cells become nonviable and produce the primary sites for calcification [15]. Through this fixation process, cross-linking of antigens happens, which is supposed to make the valves immunologically inert and improve tissue durability [16]. Unlike the old conception of vascular calcification as a passive process, it is currently considered to be an actively regulated one.

Recent studies have suggested a similarity between pathologic calcification and physiologic bone mineralization, which is regulated by inductive and inhibitory factors. Many aspects of the pathophysiology of calcification process have been elucidated through *in vitro* and *in vivo* pathological analysis on BHVs. Factors such as host metabolism, implant structure and mechanical stresses determine the mineralization in BHVs or other biomaterials. Calcification mineralization is further enhanced at the sites that are under intense mechanical

stresses, such as commissures in heart valves. In the cusps of BHVs, the mineralization process is dominantly initiated in connective tissue cells that are no longer viable due to glutaraldehyde pretreatment procedures. These cells have become devitalized but not removed from the structure of the valve. As stated above, dystrophic calcification of the cells happens due to reaction of calcium in extracellular fluid with membrane-associated phosphorus. This is likely because the glutaraldehyde pretreated cells have become nonviable and their calcium ion expulsion has been disrupted.

No medical therapy, as of yet, has been demonstrated to be effective in the prevention of the progression of BHV calcification, but several studies demonstrated the similarities between calcific aortic sclerosis in the BHV valve and atherosclerosis. Other authors stipulated that serum cholesterol levels are associated with increased BHV calcification and suggested that statin treatment could be effective in preventing both atherosclerosis and BHV degeneration [17-20]. Coronary calcifications are linked to BHV calcifications both in our study and the one performed by Farivar and Cohn [1]. Apart from post-implantation treatment, BHV calcification could also be prevented by an adequate pretreatment. Clark and col. proved that pretreatment with aluminum chloride (AlCl$_3$) (0.1 moles/L) and ethanol (80%, pH 7.4) inhibits calcification of both the glutaraldehyde-fixed porcine aortic BHV cusp and the aortic wall [2, 18]. Ethanol prevents mineralization of the cusps by removal of cholesterol and phospholipids and major alterations of collagen intrahelical structural relationships. AlCl$_3$ pre-treatment prevents aortic wall calcification by inhibition of elastin mineralization due to the following mechanisms: binding of Al to elastin resulting in a permanent protein-structural change conferring calcification resistance, inhibition of alkaline phosphatase activity, diminished upregulation of the extracellular matrix protein, tenascin C, and inhibition of matrix metalloproteinase- mediated elastolysis [2, 19, 20].

Compared to other metallic ion pretreatments, aluminum has been shown to be the most effective inhibitor of bioprosthetic aortic wall calcification and alkaline phosphatase activity of BHV tissue.

Conclusions

BHV calcification is a clinically important pathologic process limiting the anticipated durability and, hence, use of tissue-derived valves. In our opinion, the most promising preventive strategies have to include binding of calcification inhibitors to glutaraldehyde fixed tissue, removal or modification of calcifiable components, modification of glutaraldehyde fixation, and use of tissue cross linking

agents other than glutaraldehyde. Interesting approaches to preventing this problem through synergistic and simultaneous employment of multiple anticalcification therapies or novel tissue treatments are under investigation. Since some anticalcification approaches have been used in clinical valves for nearly a decade, documentation of favorable 15 to 20 year outcomes will require yet approximately another decade.

References

1.FARIVAR, R.S., COHN, L.H. J., Thorac. Cardiovasc. Surg., **126**, no. 4., 2003, p. 969.
2.CLARK, J.N., OGLE, M.F., ASHWORTH, P., BIANCO, R.W., LEVY, R.J., Ann. Thorac. Surg., **79**, no. 3, 2005, p. 897.
3.RUTKOVSKAIA, N.V., SAVOCTIANOVA, I., BARBARASH, O.L., Kardiologiia., **54**, no. 6, 2014, p. 76.
4.BLOOMFIELD, P., *Heart.*, **87**, 2002, p. 583.
5.GRUNKEMEIER, G.L., FURNARY, A.P., WU, Y., WANG, L., STARR, A. J., Thorac. Cardiovasc. Surg., **144**, no. 6, 2012, p. 1381.
6.BRE, L.P., MCCARTHY, R., WANG W., Curr Med Chem., **21**, no. 22, 2014, p. 2553.
7.ANDERSON, R.H., Heart., **84**, 2000, p. 670.
8.GUNDIAH, N., MATTHEWS, P.B., KARIMI, R. J., Heart. Valve Dis., **17**, no. 6, 2008, p. 606.
9.HO, S.Y., Eur. J. Echocardiogr., **10**, 2009, p. i3.
10.SILVER, M., WILLIAM, C.R., Am. J. Cardiol., **55**, 1965, p. 454.
11.WESKA, R.F., AIMOLI, C.G., NOGUEIRA, G.M., LEIRNER, A.A., MAIZATO, M.J., HIGA, O.Z., POLAKIEVICZ, B., PITOMBO, R.N., BEPPU M.M., Artif. Organs., **34**, no. 4, 2010, p. 311.
12.RUGGIERI, V.G., ANSELMI, A., WANG, Q., ESNEAULT, S., HAIGRON, P., VERHOYE, J.P., J. Heart Valve Dis., **22**, no. 2, 2013, p. 236.
13.SCHOEN, F.J., LEVY, R.J., Ann. Thorac. Surg., **79**, no. 3, 2005, p. 1072.
14.ACHARYA, G., ARMSTRONG, M., MCFALL, C., QUINN, R.W., HILBERT, S.L., CONVERSE, G.L., TOTH, P.B., SHERMAN, A.K., LEE, C.H., LOFLAND, G.K., HOPKINS, R.A., J. Heart. Valve Dis., **23**, no. 3, 2014, p. 259.
15.CONNOLLY, J.M., BAKAY, M.A., ALFERIEV, I.S., GORMAN, R.C., GORMAN, J.H., KRUTH, H.S., ASHWORTH, P.E., KUTTY, J.K., SCHOEN, F.J., BIANCO, R.W., LEVY, R.J., Ann. Thorac. Surg., **92**, no. 3, 2011, p. 858.
16.SALEEB, S.F., NEWBURGER, J.W., GEVA, T., BAIRD, C.W., GAUVREAU, K., PADERA R.F., DEL NIDO P.J., BORISUK, M.J., SANDERS S.P., MAYER, J.E., Circulation., **130**, no. 1, 2014, p. 51.
17.ANTONINI-CANTERIN, F., ZUPPIROLI, A., BALDESSIN, F., POPESCU B.A., NICOLOSI G.L., Cardiovasc. Ultrasound., **29**, no. 4, 2006, p. 26.
18.ILIE, A., ANDRONESCU, E., GHITULICA, C.D., CUCURUZ, A.T., Rev. Rom. Mater., **44**, no. 1, 2014, p. 66.
19.ANTONIAC, I.V., BURCEA, M., IONESCU, R.D., BALTA, F., Mat. Plast., **52**, no.1, 2015, p. 109.
20.LOGHIN, I., EARAR, K., NECHITA, A., SANDU, I.G., DOROBAT, C., MATEI, M.N., Rev.Chim. (Bucharest), **66**, no. 3, 2015, p. 387.