ISSN: 2573-9565

Research Article

Journal of Clinical Review & Case Reports

Biomineralization of Heart Valves

Maciej Pawlikowski

Department of Mineralogy, Petrography and Geochemistry, AGH University of Science and Technology, Poland

***Corresponding author**
Maciej Pawlikowski, Department of Mineralogy, Petrography and Geochemistry, Faculty of Geology, Geophysics and Environmental Protection, Bio-mineralogy Lab, AGH University of Science and Technology, Krakow, 30-059 Cracow, al. Mickiewicza 30, Poland, Tel: 126175232; E-mail: mpawlik@agh.edu.pl

Submitted: 13 Feb 2019; **Accepted**: 19 Feb 2019; **Published**: 01 Mar 2019

Abstract

Studies were conducted on biomineralization of mitral valves and aortic valves as well as heart valve prostheses. Mineralogical methods were used, including optical microscopy, scanning electron microscopy with analysis (EDS), as well as X-ray diffractometry and infrared spectrophotometry. It was observed that biomineralization is concentrated both inside the valves and on their surface. It develops in places of damage that are the centers of crystallization. Such damage may have different genesis: it may be genetic, caused by excessive heart work (overloads), by toxins associated with infections, or by external factors.

Substances that mineralize the valves are phosphates and crystalline apatite. Among organic compounds the dominant one is cholesterol. Phosphates and cholesterol usually co-exist in different proportions.

Keywords: Heart Valves, Biomineralization

Introduction

The basis for prevention and treatment of heart valves diseases, as well as for heart valve surgery, understands of the development of biomineralization processes.

Heart valve literature is diverse. A significant part of it is publications on dysfunction assessment methods and examination methods [1-12]. An important portion are publications discussing the techniques of surgical procedures, the relationship between coronary and aortic vessel mineralization and valve calcifications, as well as studies of valve prostheses, i.e. artificial heart valves. Results of studies on frozen heart valves used in transplants are particularly interesting [3,4,13-22].

In connection with this publication, the most interesting are publications regarding calcification of valves and various methods of studying this phenomenon [16,17,19,21-32]. However, there are no mineralogical works among these studies, even though the substances that mineralize the valves are suitable for examination using mineralogical techniques. Tests carried out with these techniques offer interesting results of significant importance for learning about both the origin of calcification and its forms [33].

Materials and research methods

Materials

Samples for this study were mitral and aortic valves as well as individual valve prostheses. They were postoperative materials obtained during heart valve implantations. After the surgery, this material is sent for utilization. It was offered for research instead, courtesy of prof. Roman Pfitzner from the John Paul II Hospital in Cracow, for which I offer him the most heartfelt thanks [34-38].

As a result, 14 samples of valves were chosen for the study, including 8 aortic valves, 6 mitral valves and 2 prostheses of the aortic valve. In this article, due to the reduction in the length of the publication, only particularly interesting research results are presented.

Methods

First, the samples were subjected to inspection using a binocular magnifier to select the most interesting samples from the material obtained and pass them on for further research. At this stage of the study, macroscopic photographs of the samples were taken.

The selected material was examined under a polarized transmitted light microscope to choose samples for SEM-EDS tests. A microscope manufactured by the Chinese company MOTIC with a photomicrography attachment was used.

The chosen material was tested using Jeol 540 scanning microscope and FEI Quanta 200 FEG. Samples were analyzed without sputter coating so as not to interfere with the results of chemical analyzes. The observed phenomena were documented with micrographs.

Infrared tests were carried out using BIO-RAD FTS6000 spectrometer and phase analyzes were conducted using Philips APD X'Pert PW 3020 X-ray diffractometer.

Results

Forms of mineralization

Mitral valves

Mineralization in the valve structure

Chemical analyzes carried out with the EDS method indicate that despite lack of visible mineral concentrations, valve leaflets may contain increased levels of calcium and phosphorus, and sometimes sulfur, nitrogen and others. As a consequence, a valve that looks normal but contains elemental substitutions in biological structures may show different behavior during work.

Mineralization is observed in the form of nodules and bumps on surface of the valve leaflets, but sometimes also in the tendinous cords (Photo 1A). In extreme cases with intense mineralization, it is possible to separate a large amount of phosphate particles (Photo 1B) from a single valve, using a suitable solvent. They are phosphate grains with different crystallinity and a variable degree of hydration, which will be discussed further. The mineralization of the internal structures of the valve is often combined with surface mineralization, visible on the valves.

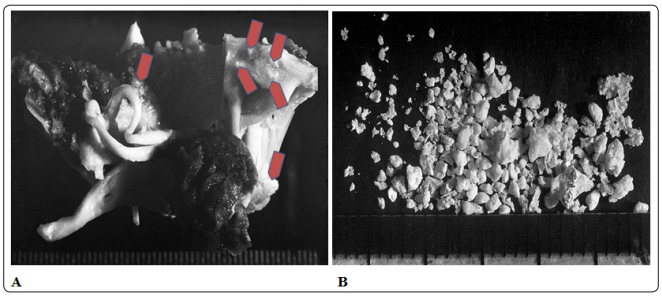

Photo 1: A - an example of mitral valve with marked spots (micro-bumps) where phosphate and/or cholesterol grains reside. B – Phosphate grains separated from a single, strongly mineralized (calcified) valve after chemical removal of organic substances of the valve (scale in mm)

Surface mineralization

It is often located in the areas of deformation and damage to valve leaflets. Biomineralization is favored by the micro-spacing between the collagen fibers that build the valve components, especially when the valves come from a bank of frozen valves (Photo 2A). In various spots on the valve surface, apart from regular phosphate or cholesterol crystals, cholesterol-phosphate aggregates with variable proportions of both components are observed (Photo 2B).

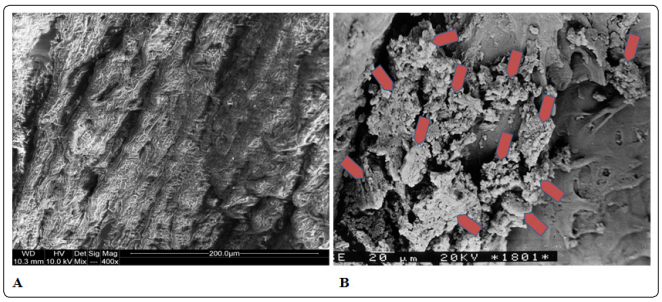

Photo 2: A - surface of a frozen valve (from the valve bank) with visible spaces between collagen strands. B – phosphate-cholesterol aggregates (arrows) crystallized on the surface of a valve affected by biomineralization near its ring (SEM)

Aortic valves mineralization

Mineralization on the surface of the valves and within their structure

Studies show that the mitral and aortic valve mineralization is of a similar nature. Hidden mineralization has been found there, which consists of the incorporation of elements (mostly calcium and phosphorus) into the atomic structures, mainly of collagen. It can develop inside the tissue structure of the elements of valves, as well as on their surfaces. It is related to the location of valve damage sites (more precisely - damages of biological structures of valve-building compounds - mainly collagen). These phenomena are clearly visible under the scanning microscope, especially at higher magnifications (Photo 3A, B).

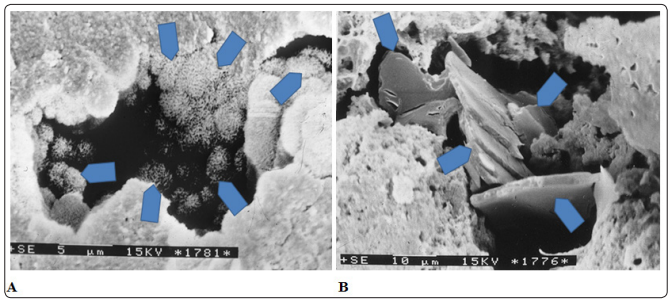

Photo 3: A – initial stage of the phosphate mineralization of aortic valve leaflet at the site of its damage. The arrows show micro-aggregates of phosphate crystals. B - Nicely developed cholesterol crystals crystallized at the site of aortic valve leaflet damage. Arrows show cholesterol plaque crystals SEM

Mineralization of valve prostheses

The phenomenon of biomineralization also affects valve prostheses-and not only biological, but mechanical ones, too. In biological valve prostheses, biomineralization affects the same elements as in the case of original valves. It seems, however, that (in biological valves) tissue areas associated with the preparation of valves are preferred. Such areas are, for example, the place of contact of leaflets (pig or other) with the collagen ring (Photo 4A). The resulting mineralization sometimes creates exceptionally beautiful apatite crystals (Photo 4B). Although they are tiny, their edges are sharper than a razor blade or a scalpel and the valves can mechanically damage (cut) the surrounding tissue during their activity (Photo 4B).

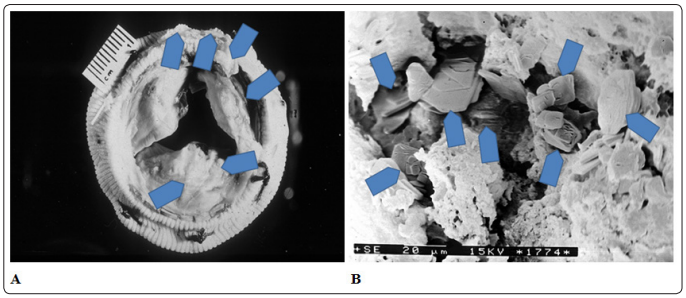

Photo 4: A - removed biological prosthesis affected by the phosphate-cholesterol biomineralization process (arrows). B – Apatite micro-crystals (arrows) crystallized in biological arterial valve prosthesis, near the place of connection between leaflets with the collagen ring SEM.

Valve-mineralizing substances

Studies of these substances require special equipment, which is

related to the method of mineralization of the valves.

Secret mineralization, i.e. substitution of elements into the biological structures of valve components, is possible to recognize and mark only by sensitive chemical methods (ASA, EDS).

Visible mineralization, present in the form of grains or even crystals of very small size, can be studied with infrared absorption spectrophotometry due to the tiny amount of research material required for the apparatus. In the case of slightly larger grains, phase analysis can be carried out using the X-ray diffractometry method.

Phosphates (inorganic mineralization)

It occurs both in the body of the valves and on their surface. It was found that the degree of crystallinity and hydration of phosphates in the valves is related to the age of patients.

Grains from younger patients' valves are less crystalline and more hydrated than those from older patients (Figure 1). It can be assumed that this situation is more related to how long the phosphate grains have been in the body than to the age of patients. In other words, the grains that formed in the valves age along with the patient. This leads to their dehydration and higher crystallinity.

However, this hypothesis requires verification in a larger number of tests.

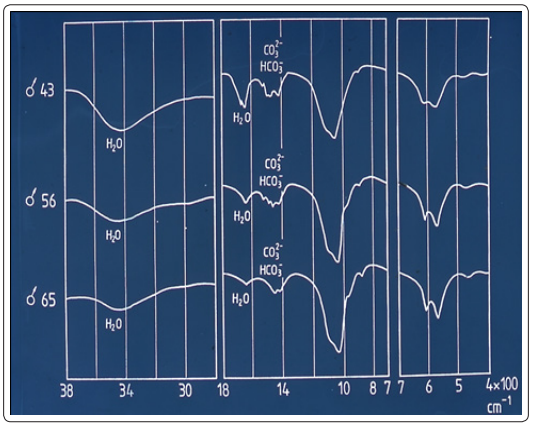

Figure 1: Spectra from infrared absorption spectrophotometry of mineral grains separated from valves of patients of different ages. In addition to the classic bands from PO_4 groups, spectra show acidic carbonate bands (1400-1600 cm^{-1}) and water bands (approximately 1650 cm^{-1}). Decrease of this band with the age of patients indicates dehydration of phosphates.

Grains that we managed to mechanically isolate from the valves were subjected to diffrotactometric phase analysis. The resulting diffractogram (Figure 2) shows that the main component of the "calcifications" is crystalline apatite.

The distances between net planes in the atomic structure of this apatite depend both on the number of substitutions of elements for Ca2+ and substitutions of CO_3^{2-} for PO_4^{3-} in the structure of $Ca_5(PO_4, CO_3)$. The apatite structure, including the size of its cell unit, is also influenced by the amount of OH^-, Cl^- and F^- anions in the apatite structure.

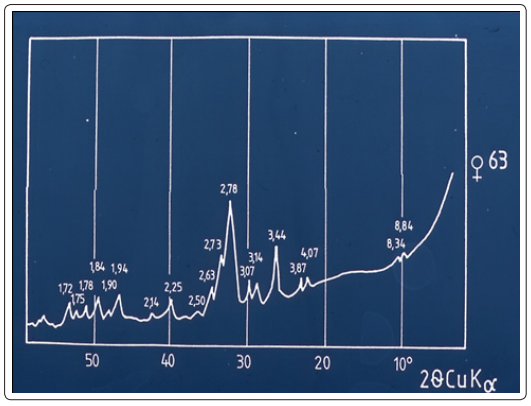

Figure 2: X-ray diffractogram of apatite crystals separated from the mitral valve of a 63-year-old female patient

Organic substances

Undoubtedly, cholesterol is the most common organic component that mineralizes elements of heart valves. In most cases, it has an ordered atomic structure, which results in a good X-ray diffraction (Figure 3). In addition to crystalline cholesterol, organic non-crystalline substances are also present in the organic concentrations that mineralize valve elements. Their presence is indicated by clear increases in the diffraction background in the range of 15-26 ° 2 Θ Cu K_α (Figure 3).

In individual samples, the proportion of cholesterol to organic substances with a disordered atomic structure was variable.

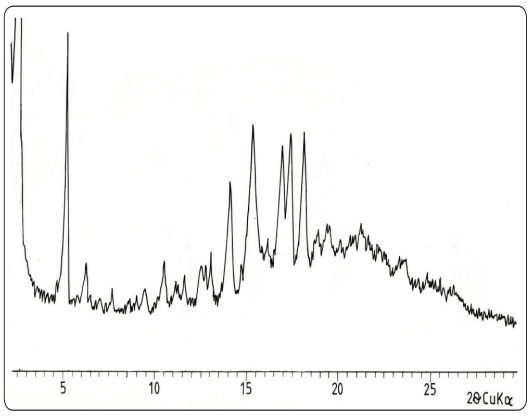

Figure 3: X-ray diffractogram of cholesterol grains separated from a valve (male, age 70). Increase of the background in the range of 15-26 ° 2 Θ Cu K_α proves the co-occurrence of non-crystalline organic substances with cholesterol

Types of mineralization

Summing up the study, it can be concluded that there are two types of substantial biomineralization in the valves (as well as in other organs): inorganic (mainly phosphate) and organic (mainly cholesterol) biomineralization. Usually both types of biomineralization coexist in different proportions. Sometimes one of these types of biomeralization is exclusive.

In terms of the form of occurrence, we have secret mineralization or visible mineralization. Obtained results indicate that in the first stage of mineralization of the valves, secret mineralization develops. It may eventually (though not necessarily) transform into visible

mineralization (grains, crystals, concrements). The schematic course of inorganic and organic biomineralization phenomena is shown in (Figure 4A, B).

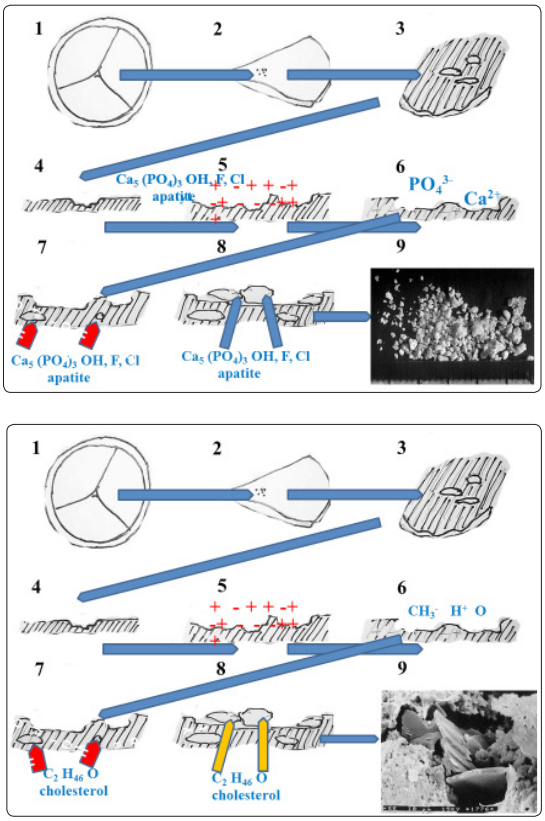

Figure 4: A – phosphate (inorganic) valve biomineralization
1 - aortic valve image with normal appearance, 2 - valve leaflet with traces of surface damage, 3 - enlarged area of valve leaflet damage, 4 - cross-section of the valve leaflet with a defect on its surface, 5 - schematic view of electrical charges in the defect (presence of charges in damaged collagen atomic structures), 6 - charged ions (CH_3^- , H^+ - cholesterol components) in blood flow by the valve damage and are attached to the defective biological structures. Secret mineralization is formed, 7 - development of visible mineralization (phosphate grains are formed in the body of the valve leaflet), 8 - phosphate crystallization in the body of the valve and the site of its surface damage, 9 - microscopic image of phosphate grains separated from the studied mitral valve.

B – Cholesterol (organic) valve biomineralization

1 - aortic valve image with normal appearance, 2 – valve leaflet with traces of surface damage, 3 – enlarged area of valve leaflet damage, 4 – cross-section of the valve leaflet with a defect on its surface, 5 - schematic view of electrical charges in the defect (presence of charges in damaged collagen atomic structures), 6 - charged ions in blood flow by the valve damage and are attached to the defective biological structures. Secret mineralization is formed, 7 – development of visible mineralization (cholesterol grains and crystals are formed in the body of the valve leaflet), 8 – further crystallization of cholesterol and other organic compounds in the body of the valve and the site of its surface damage, 9 – microscopic image of cholesterol crystals crystallized in the damaged aortic valve leaflet.

Usually both types of valve biomineralization (organic and inorganic) occur simultaneously. They are in various stages of development (secret and visible mineralization). Factors causing biomineralization

Both crystallization centers and substances that can crystallize in these centers are required for biomineralization of valves to develop. The presence of the crystallization centers themselves or the substances potentially mineralizing the valves themselves is insufficient and does not cause the development of the so-called calcifications.

Crystallization centers

Genetic factors

Those include different kinds of structural defects in the substances building the valves. All defected sites that are charged are places of biomineralization. These factors determine patients' susceptibility to biomineralization of the same places in subsequent generations.

Mechanical damage to biological structures (physical exertion)

This type of damage to biological structures is the result of excessive exertion and overload of the heart with intense activity. It can be the result of physical work, but also intense sport activities. It leads to destruction of atomic structures (breaking the bonds between atoms in biological structure, e.g. in collagen).

External factors (infections, environment, diet, air pollution, stimulants)

Many external factors can favor the development of crystallization centers. One of the most significant are all types of infections.

Bacteria, viruses and other organisms that attack the body produce various kinds of toxins as part of their life processes (during the infection). They are chemically active compounds that affect tissues, including the cardiovascular system, especially heart valves. Blood flowing through them is under high pressure. The blood (including the toxins) hits the valve leaflets with significant force. It causes damage to the valves (atomic structures of collagen). The size of resulting damage depends on the type of infection, i.e. on the type and aggressiveness of the toxins.

Formation of centers of crystallization is similarly affected by environmental pollution, i.e. toxins and solid particles (penetrating the body via inhalation or along with drinks and foods).

Mineralizing substances

Supplementation of calcium and phosphorus

These are undoubtedly two main elements that cause development of calcifications (inorganic biomineralization). Their source is for example osteoporosis, which causes transfer of calcium and phosphorus from bones, usually developing with age. Another, and perhaps primary source of these elements is diet high in calcium (milk, cheese, other dairy), as well as large doses of calcium administered - unnecessarily – to older people, allegedly to prevent osteoporosis.

Supplementing calcium and phosphorus in older age does not cause mineralization of bones. For osteogenesis, we need collagen matrix built from specific type of collagen with adequate crystallization centers. In these centers, the alkaline phosphatase produced by osteoblast mitochondria may crystallize into carbonate hydroxyapatite.

If these conditions are not present, supplemented calcium and phosphorus go to crystallize to crystallization centers in places other than bones, e.g. in valves. It results in acceleration of calcification of many organs, arteries, and heart valves.

It can therefore be concluded that both the high-dairy diet and the supplementation of large amounts of CA and P are unfavorable in older age, to say the least.

Cholesterol

Autogenous cholesterol is a derivative of bile acids produced by the liver. Hence too high cholesterol measure in blood is associated with one of the bile acids.

Rarely do cholesterol crystals float in the blood. However, blood carries ingredients from which cholesterol can crystallize. They get into the blood from the "biliary system" and specifically from the bile that is "added" to the digestive matter that travels through the digestive system. Here, those ingredients are absorbed from the intestines into the cardiovascular system and distributed with blood throughout the body. This is how they end up in the centers located in the valves (and arteries) where the cholesterol crystallizes. This is particularly the case with the "imperfect" liver function and overproduction of the bile acid.

The role of „external" cholesterol in these processes is not clear. It is not supplied to the body in the ionic form, but as „ready-made" cholesterol. It must be therefore transferred into its ionic form to be able to crystallize in crystallization centers.

Understanding the role of allogenic cholesterol in biomineralization of valves (and arteries) required research and clarification of numerous details.

Usually all of the aforementioned factors overlap, which makes the clinically observed image of biomineralization, appears complicated and unclear.

References

1. Agatston AS, Janowitz WR., Hildner FJ, Zusmer NR, Viamonte M Jr, et al. (1990) Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 15: 827-832.
2. Boughner DR, Thornton M, Dunmore-Buyze J, Holdsworth DW (2000) The radiographic quantitation of aortic valve calcification: implications for assessing bioprosthetic valve calcification in vitro. Physiol Meas 21: 409-416.
3. Budoff MJ, Lane KL, Bakhsheshi H, Mao S, Grassmann BO, et al. (2000) Rates of progression of coronary calcium by electron beam tomography. Am J Cardiol 86: 8-11.
4. Callister TQ, Raggi P, Cooil B, Lippolis NJ, Russo DJ (1998) Effect of HMG-CoA reductase inhibitors on coronary artery disease as assessed by electron-beam computed tomography. N Engl J Med 339: 1972-1978.
5. Gohlke-Barwolf C (2000) Anticoagulation in valvar heart disease: new aspects and management during non-cardiac surgery. Heart 86: 715-721.
6. Kizer JR, Gefter WB, de Lemos AS, Scoll BJ, Wolfe ML, et al. (2001) Electron beam computed tomography for the quantification of aortic valve calcification. J Heart Valve Dis 10: 361-366.
7. Mako WJ, Vesely I (1997) In vivo and in vitro models of calcification in porcine aortic valve cusps. J Heart Valve Disease 6: 316-323.
8. Mohler ER (2000) Are atherosclerotic processes involved in aortic-valve calcification? Lancet 356: 524-525.
9. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD (1994) Characterisation of the early lesion of "degenerative" valvular aortic stenosis: histological and immunohistochemical studies. Circulation 90: 844-853.
10. Otto C.M., Burwash IG, Legget ME, Munt BI, Fujioka M, et al. (1997) Prospective study of asymptomatic valvular aortic stenosis: clinical, echocardiographic, and exercise predictors of outcome. Circulation 95: 2262-2270.
11. Pibarot P, Dumesnil J (2000) Hemodynamic and clinical impact of prosthesis-patient mismatch in the aortic valve position and its prevention. J Am Coll Cardiol 36: 1131-1141.
12. Gnann JW, Dismukes WE (2000) Prosthetic valve endocarditis. Heart 85: 590-593.
13. Herzog CA, Ma JZ, Collins AJ (2002) Long-term survival of dialysis patients in the United States with prosthetic heart valves. Should ACC/AHA practice guidelines on valve selection be modified? Circulation 105: 1336-1341.
14. Kleczyński P, Dziewierz A, Daniec M, Bagieński M, Rzeszutko Ł, et al. (2017) Impact of post-dilatation on the reduction of paravalvular leak and mortality after transcatheter aortic valve implantation. Kardiol Pol 75: 742-748.
15. Lindblom D, Lindblom U, Qvist J, Lundström H (2001) Long-term relative survival rates after heart valve replacement. J Am Coll Cardiol 15: 566-573.
16. Harbaoui B, Montoy M, Charles P, Boussel L, Liebgott H, et al. (2016) Aorta calcification burden: towards an integrative predictor of cardiac outcome after transcatheter aortic valve implantation. Atherosclerosis 246: 161-168.
17. Kapila R (1986) Hart calcific cerebral emboli and aortic stenosis: detection by computed tomography. Stroke 17: 619-621.
18. Ryś M, Hryniewiecki T, Michałowska I, Stokłosa P, Różewicz-Juraszek M, et al. (2018) Quantitative estimation of aortic valve calcification in multislice computed tomography in predicting the development of paravalvular leaks following transcatheter aortic valve replacemen. Postepy Kardiol Interwencyjnej 14: 85-89.
19. Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, et al. (1998) Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol 31: 126-133.
20. Bloomfield P (2002) Choice of heart valve prosthesis. Heart 87: 583-589.
21. Iivanainen AM, Lindroos M, Tilvis R, Heikkilä J, Kupari M (1996) Calcific degeneration of the aortic valve in old age: is the development of flow obstruction predictable? J Intern Med 239: 269-273.
22. Peter M, Hoffmann A, Parker C, Lüscher T, Burckhardt D (1993) Progression of aortic stenosis: role of age and concomitant coronary artery disease. Chest 103: 1715-1719.
23. Bertazzo S, Gentleman E (2017) Aortic valve calcification: a bone of contention. Eur Heart J 38: 1189-1193.
24. Haensig M, Lehmkuhl L, Rastan AJ, Kempfert J, Mukherjee C, et al. (2012) Aortic valve calcium scoring is a predictor of significant paravalvular aortic insufficiency in transapical-aortic

valve implantation. Eur J Cardiothorac Surg 41: 1234-1240.
25. Khetarpal V, Mahajan N, Madhavan R, Batra S, Mopala P, et al. (2009) Calcific aortic valve and spontaneous embolic stroke: a review of literature. J Neurol Sci 287: 32-35.
26. Khalique OK, Hahn R, Gada H, Nazif TM, Vahl TP, et al. (2014) Quantity and location of aortic valve complex calcification predicts severity and location of paravalvular regurgitation and frequency of post-dilation after balloon-expandable transcatheter aortic valve replacement. JACC Cardiovasc Interv 7: 885-894.
27. Koos R, Mahnken AH, Dohmen G, Brehmer K, Günther RW, et al. (2011) Association of aortic valve calcification severity with the degree of aortic regurgitation after transcatheter aortic valve implantation. Int J Cardiol 150: 142-145.
28. Lindroos M, Kupari M, Valvanne J, Strandberg T, Heikkilä J, et al. (1994) Factors associated with calcific aortic valve degeneration in the elderly. Eur Heart J 15: 865-870.
29. Nagueh S, Sun H, Kopelen HA, Middleton KJ, Khoury DS (2001) Hemodynamic determinants of the mitral annulus diastolic velocities by tissue Doppler. J Am Coll Cardio 37: 278-285.
30. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS (1999) Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. N Engl J Med 341: 142-147.
31. Rumberger JA, Simons DB, Fitzpatrick LA, Sheedy PF, Schwartz RS (1995) Coronary artery calcium area by electron-beam computed tomography and coronary atherosclerotic plaque area: a histopathologic correlative study. Circulation 92: 2157-2162.
32. Stewart BF, Siscovick D, Lind BK, Gardin JM, Gottdiener JS, et al. (1997) Clinical factors associated with calcific aortic valve disease. J Am Coll Cardiol 29: 630-634.
33. Takamiya Y, Miura S, Sako H, Shirai K, Morishige N, et al. (2007) Pseudoaneurysm of the mitral-aortic intervalvular fibrosa following infective endocarditis in a patient with acute heart failure: a case report. J Cardiol 49: 353-356.
34. Ewe SH, Ng AC, Schuijf JD, van der Kley F, Colli A, et al. (2011) Location and severity of aortic valve calcium and implications for aortic regurgitation after transcatheter aortic valve implantation. Am J Cardiol 108: 1470-1477.
35. Enriquez-Sarano M (2002) Timing of mitral valve surgery. Heart 87: 79-85.
36. Massel D, Little SH (2001) Risks and benefits of adding anti-platelet therapy to warfarin among patients with prosthetic heart valves: a meta-analysis. J Am Coll Cardiol 37: 569-578.
37. R Pfitzner, Barecka D, Pawlikowski M, Kopytek M, Rudnicka-Sosin L, et al. (2018) Influence of cryopreservation on structural, chemical and immunoenzymatic properties of the aortic valve allografts. Transplantation Proceedings 50: 2195-2198.
38. Pawlikowski M, Pfitzner R (1999) Mineralization of heart, (Mineralization of heart and big blood vessels). Wyd IGSMiE PAN Kraków 1999: 142.