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Bone and cartilage metaplasia in calcific aortic stenosis

Koštana i hrskavičava metaplazija u kalcifikantnoj aortnoj stenozi

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Abstract

Background/Aim. Calcification is a frequent change in aortic cusps and the most frequent cause for the occurrence of aortic valve stenosis. Recent studies have shown that the process of aortic calcification is often active and closely related to the formation of bone tissue in calcific aortic stenosis. The aim was to analyze the demographic characteristics of patients with calcific aortic stenosis, the most common symptoms, comorbidities and risk factors, incidence of bone and cartilage metaplasia and its possible association with the present comorbidities, as well as its clinical importance. **Methods.** We retrospectively analyzed the medical records of 115 patients referred to the University Hospital from January 2013 to December 2015. **Results.** Calcific aortic stenosis occurred more frequently in males. The average age of patients was 67.3 years. The majority of patients were non-smokers, overweight. The most common clinical symptoms were fatigue, shortness of breath and chest pain. Eighteen (15.6%) patients had no symptoms. Seventeen (14.8%) patients had cartilaginous and osseous metaplasia. Gender, age, smoking and body mass index (BMI) had the same distribution among patients with and without metaplasia. Metaplasia was equally prevalent among patients with moderate, severe and critical aortic stenosis. **Conclusion.** Age, sex, smoking, BMI and blood pressure values are not risk factors neither for osseous nor for cartilaginous metaplasia.

Key words:
aortic value stenosis; aortic valve calcification; metaplasia; risk factors.

Apstrakt

Uvod/Cilj. Kalcifikacija je česta promena aortnog kuspisa i najčešći uzrok nastanka stenoze aortne valvule. Prema novijim istraživanjima, ona predstavlja aktivan proces, usko povezan sa stvaranjem koštanog tkiva u kalcifikantno obolelim kuspisima. Cilj istraživanja je bio analizirati demografske karakteristike bolesnika sa kalcifikantnom aortnom stenozom, najčešće simptome bolesti, prisutne komorbiditete i faktore rizika, kao i učestalost javljanja koštane i hrskavičave metaplazije i njenu udruženost sa komorbiditetima, ali i njen klinički značaj. **Metode.** Retrospektivno je analizirana medicinska dokumentacija 115 bolesnika kojima je u periodu od januara 2013. do decembra 2015. godine na Univerzitetskoj klinici postavljena dijagnoza kalcifikantne aortne stenoze. **Rezultati.** Kalcifikantna aortna stenoza češće se javljala kod muškog pola. Prosečna starost bolesnika bila je 67,3 godine. Najveći broj bolesnika bili su nepušači, prekomerne telesne mase. Najčešći klinički simptomi bolesti bili su zamaranje, gušenje i bolovi u grudima. Osamnaest (15,6%) bolesnika nije imalo simptome. Kod sedamnaest (14,8%) bolesnika patohistološki su nađene koštana i hrskavičava metaplazija. Pol, starost, pušenje, indeks telesne mase (BMI) imali su istu raspodelu među bolesnicima sa i bez patohistološki potvrđene metaplazije. Metaplazija je bila podjednako zastupljena kod bolesnika sa umerenom, teškom i kritičnom aortnom stenozom. **Zaključak.** Godine, pol, pušenje, BMI i vrednosti krvnog pritiska nisu faktori rizika ni za koštanu, ni za hrskavičavu metaplaziju.

Ključne reči:
zalistak aortni, stenoza; zalistak aortni, kalcifikacija; metaplazija; faktori rizika.

Introduction

As the life expectancy is getting longer, there is an increasing number of patients with calcific aortic stenosis. It is estimated that the number of patients is even greater than the known statistical estimates, since the patients only report in the late stage of the disease, when the symptoms are pronounced, and the prognosis is worse. Knowing the possible risks and mechanisms for the occurrence of the disease would allow early diagnosis and treatment of these

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patients, possibly non-surgical. The question arises whether the development of bone and cartilage tissue is a common process or occurs in the predisposed patients.

The aim of our work was to analyze the demographic characteristics of patients with calcific aortic stenosis, the most common symptoms of the disease, the presence of comorbidities and risk factors, as well as to examine the frequency of bone and cartilage metaplasia in calcific aortic valve stenosis and its possible association with the present comorbidities. We also aimed to discover a clinical importance of metaplasia in calcific aortic stenosis.

Methods

The study included 115 patients with the replacement of the aortic valve during the period from January 2013 to December 2015 at the University Hospital due to the diagnosed calcific aortic stenosis. Furthermore, in some of the patients, revascularization coronary artery bypass grafting (CABG), thrombanderterectomy (TEA), replacement of the ascending aorta, mitral and tricuspid valve anuloplasty, patch plastics of the interstitial septum or atrial septal defect (ASD) closure were performed in addition to aortic valve replacement (AVR). The material for histopathological (HP) analysis was processed and analyzed in the Pathology Center, and demographic and clinical-morphological data included in the research were: age, sex, existing comorbidities, risk factors, disease symptoms, aortic valve area (AVA) obtained by Doppler and measured in cm^2, and histological type of metaplasia in aortic valve.

The only criteria for inclusion in the study were surgery: aortic valve replacement or myocardial revascularization with aortic valve replacement. Data for all patients were from the history of the disease and HP referrals. The exclusion criterion was a possible lack of data from the medical records.

Treatment of tissue samples for histopathological analysis

Aortic valve tissue taken during the operation was processed for a standard HP analysis, which involved fixing in 10% neutral formalin, paraffin molding, and microtome cutting to tissue sections of 4 micron thickness, followed by staining with hematoxylin-eosin (HE) method.

Statistical data processing

The data obtained by the research were entered into a separately created database of Microsoft Excel packages. The data were displayed as frequency distribution. The relationship between variables and determining the statistical significance of the results were calculated in the SPSS program using *t*-test, χ^2-test, Mann–Whitney *U* test, Kruskal–Wallis *H* test, correlation and regression analysis. The results are shown in tables and/or graphically.

Results

The study covered 115 patients with diagnosed calcific aortic stenosis.

Age and gender distribution

Patients were aged 24 to 83 years. The average age of the examined patients was 67.3 years (Figure 1). Out of 115 patients, 71 (61.7%) were male, while 44 (38.3%) were female.

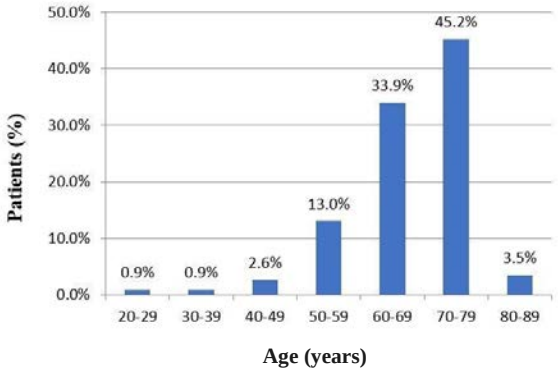

Fig. 1 – Age distribution among patients.

Other risk factor distribution

Of the 115 patients, 38 (33%) were smokers or former smokers, while 77 (67%) of patients stated that they never smoked. The highest value of systolic blood pressure among the patients was 190 mm Hg and diastolic 120 mmHg. The lowest systolic blood pressure was 90 mmHg and diastolic 60 mmHg. The average blood pressure among patients was 140/83 mmHg. We also analyzed body mass index (BMI) among the affected patients (Table 1).

Table 1

Histopathological findings among patients with metaplasia

Pathological findings	Patients		
	n	yes (%)	no (%)
Inflammation	6	35.3	64.7
Cartilaginous metaplasia	8	47.1	52.9
Red bone marrow	8	47.1	52.9
Yellow bone marrow	12	70.6	29.4
Osseous metaplasia	13	76.5	23.5
Neovascularization	14	82.4	17.6
Fibrosis	17	100.0	0.0
Myxomatous degeneration	17	100.0	0.0

Symptoms

Of the 115 patients, 18 (15.6%) did not have symptoms. Among symptomatic patients, 64 (55.7%) patients reported fatigue, 51 (44.3%) patients hard breathing, 37 (32.2%) chest pain, 14 (12.2%) dizziness or faint, 8 (7%) arrhythmia and 7 (6.1%) swelling of the legs, while 6 (5.2%) patients reported a history of the loss of consciousness.

Aortic valve area

Patients were classified in four groups following the American Heart Association (AHA) guidlines for the severity of aortic stenosis. None of them was in the group of mild aortic stenosis (AVA 1.5–2 cm^2). Sixty two (54%) patients were classified as those with severe aortic stenosis (AVA 0.6–1.0 cm^2), 38 (33%) as critical (AVA < 0.6 cm^2) and 15 (13%) as moderate aortic stenosis (AVA 1.0–1.5 cm^2). We used χ^2-test and Kruskal–Wallis *H* test to identify a possible association between the age and AVA, but no statistically significant association was found. The χ^2-test and Mann–Whitney *U* test showed that there was no statistically significant association between smoking and the severity of aortic stenosis. The ANOVA test showed that there was no statistically significant association between BMI and AVA, and the correlation analysis showed very low correlation (0.087) between these two variables. Using multinominal and linear regression analysis, the predictability of AVA values based on BMI and age was very low, namely 3.7–4.4% of variance was explained by the model. The Mann–Whitney *U* test showed that there was no statistically significant association between the presence of metaplasia and the severity of aortic stenosis.

Presence of metaplasia

Of all patients with calcific aortic stenosis, in 17 (14.8%) patients, the presence of bone and/or cartilage tissue in aortic cusps was found (Table 2, Figures 2, 3 and 4).

Table 2

Body mass index (BMI) of patients with calcific aortic stenosis

BMI (kg/m^2)	Patients, n (%)
< 18.5 (malnutrition)	0 (0)
18.5–24.9 (normal body mass)	32 (27.8)
25.0–29.9 (overweight)	52 (45.2)
≥ 30.0 (obesity)	31 (27)

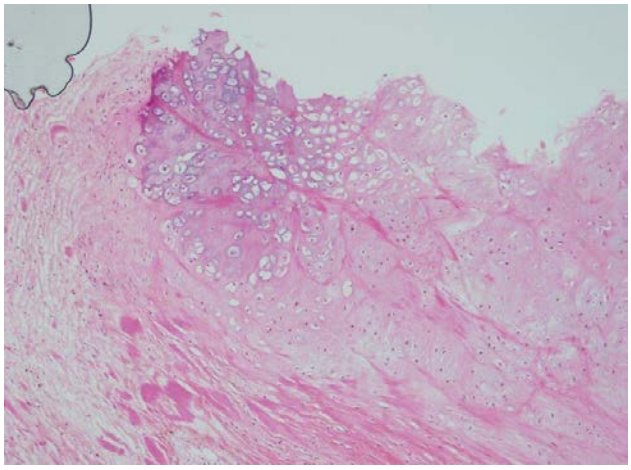

Fig. 2 – Hyaline cartilage in the aortic valve tissue [hematoxylin-eosin (HE), ×100].

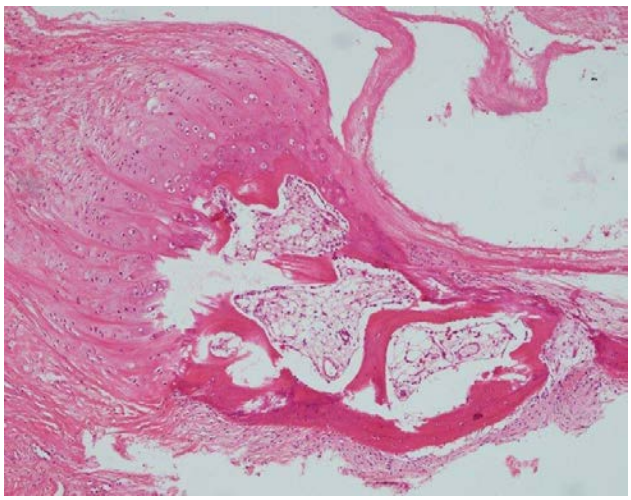

Fig. 3 – Endochondral ossification in the aortic valve [hematoxylin-eosin (HE), ×100].

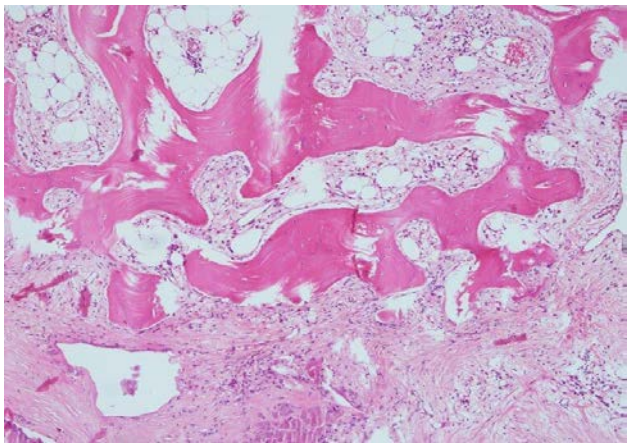

Fig. 4 – Yellow and red bone marrow in the aortic valve [hematoxylin-eosin (HE),×100].

Of the 17 patients with metaplasia, 8 (47.1%) of the patients belonged to the age group from 60 to 69 years, 6 (35.3%) of the age group from 70 to 79 years, while 3 (17.6%) patients were in the age group of 50 to 59 years. The average age of these patients was 66.8 years. Eleven (64.7%) patients were male, while 6 (35.3%) were female. Seven (41.2%) patients were overweight, while 6 of them (35.3%) were normally fed. Nine (52.9%) patients were non-smokers, while 8 (47.1%) were smokers.

The χ^2-test and *t*-test showed that there was no statistically significant association between the age of patients, sex, BMI and smoking, and the occurrence of metaplasia in calcified degenerate aortic cusps. Logistic regression analysis showed no statistically significant model when BMI and age were used as predictors, and only 0.8%–1.3% of variance of the occurrence of metaplasia could be explained by this model.

Treatment

Aortic valve replacement (AVR) was performed in all 115 patients. Of the 115 patients, 28 (24.3%) were revascularized – coronary artery bypass surgery (single,

double, triple or quadruple), in 6 (5.2%) patients the replacement of the ascending aorta had been done, in 4 (3.5%) thrombandarterectomy, in 3 (2.6%) mitral and tricuspid valve annuloplasty, and in one (0.9%) patient patch plastics of interstitial septum and atrial septal defect (ASD) suture.

Discussion

Calcification is a frequent change in aortic cusps and the most common cause of aortic valve stenosis. Possible initiators of this pathological process are abnormal haemodynamic forces in hypertension and increased stretching of cusps that initiate the remodeling process and inflammation, further leading to calcification and valvular ossification [1, 2].

Research on aortic swine valves showed that endothelial cells from the aortic side express different molecules from those on the ventricular side [5]. This suggests the possibility that endothelial cells of the aortic side participate in the calcification and oscillation process, especially when taking into account that these changes are almost always on the aortic side. The importance of endothelial cells in pathogenesis of valvular disease is also shown in studies on cloned endothelial cells of maturated sheep that showed the possibility of endothelial mesenchymal transformation in *in vitro* conditions. It seems that this process, otherwise decisive in the occurrence of specific cusp structure in embryonic period, can be re-initiated in adults, possibly creating osteoprogenitor cells [6].

Recent research defines five phenotypes that best present a family of valvular interstitial cells, since each subgroup has different roles in physiological and pathological conditions. These are embryonic progenitor endothelium/mesenchymal cells, resting interstitial cells (qVICs), activated interstitial cells (aVICs), progenitor (pVICs) and osteoblast interstitial cells (obVICs). They have the ability to cross one into another [7]. Resting interstitial cells allow the maintenance of the valvular structure and function. They express certain transmembrane proteins that are supposed to serve for their mutual communication.

In case of damage to the valve, certain cytokines that recruit bone marrow or blood progenitors are released, and these cells reach the site of damage by identifying specific ligands. Studies in mice show that hematopoietic stem cells inserted into the heart valve of the recipient differ in the direction of cells that are morphologically similar to the native interstitial cells of the recipient [8].

Mechanical stress and damage lead to the transition of resting into activated valvular interstitial cells. Activated cells exhibit the increased expression of α-smooth muscle actin (α-SMA) as well as the increased contractility, all for the purpose of repairing the damage to the valve. The disorder of this complex process leads to fibrosis, calcification and angiogenesis leading to the clinical manifestation of a valvular disease.

Valvular interstitial cells can create cartilage or mature bone. Calcificated nodules do not occur spontaneously in the culture of valvular interstitial cells. The calcification process has been proven to be dependent on alkaline phosphatase activity [9]. The presence of hydroxyapatite, osteopontin, bone sialoprotein, bone morphognetic protein-2 (BMP-2) and osteocalcin has been detected in calcified valves, suggesting that calcification is not a passive degenerative process, but an active process that implies the existence of an osteoblast cellular phenotype [4].

There are some similarities between the process of calcification and atherosclerosis. It has been proven that the patients with aortic sclerosis have a 40% higher risk of myocardial infarction and a 50% greater chance of sudden cardiac death. A possible explanation is that aortic sclerosis is an indicator of the developing process of atherosclerosis in the body, namely, atherosclerosis and aortic sclerosis are the two manifestations of one and the same disease. According to literature data, age, male sex, smoking, hypertension and hyperlipidemia are risk factors for both aortic sclerosis and atherosclerosis – which supports this theory [10, 11]. Our results are in line with the literature, given that calcific aortic stenosis in our patients was more common among men, most often in the seventh decade of life. The highest value of systolic blood pressure among our patients was 190 mm Hg and diastolic 120 mm Hg. The average blood pressure was 140/83 mm Hg. Blood pressure was measured at the moment of hospital admission. Among our patients, as opposed to literature, there were more non-smokers. This has to be interpreted bearing in mind the fact that the most people in Serbia are non-smokers, and not in direction of excluding smoking as a risk factor.

Low density lipoprotein (LDL), angiotensin converting enzyme (ACE) and its product-angiotensin II18 were found in the interstititium of calcified cusps, and even in the macrophages themselves [12]. Although there is a possibility that part of the ACE is produced in the cusps itself, most of it is still extracellular and occurs in the presence of apolipoprotein B, suggesting the possibility that ACE in the lesion has been linked to LDL particles [12]. The results of our research have shown that most of the affected patients are overweight. Since obesity, as a part of the metabolic syndrome, is associated with the increased production of LDL particles and increased production of inflammatory cytokines, our results support the assumptions that these events are likely to be a part of the pathomechanism that leads to calcific aortic stenosis [13–15]. However, in our study BMI had the same distribution among patients with moderate, severe and critical aortic stenosis. Also, high blood glucose level enhances valve interstitial cell (VIC) matrix calcium deposition [15].

An interesting fact is that diabetes mellitus reduces the occurrence of ossification in calcified altered valves [16].

Recent studies have shown a high association of warfarin therapy and the occurrence of aortic valvular calcification [17, 18]. Warfarin leads to calcification influencing the synthesis and function of the matrix Gla-protein, which is otherwise an inhibitor of the calcification process [16, 18].

Taking everything mentioned together, it might be that the haemodynamic and mechanical forces along with

oxidized lipids and exogenous substances (*eg*, bacterial lipopolysaccharides) transform calm valve interstitial cells (VICs) into activated VICs [19]. Activated VICs may be subjected to osteogenic transdifferentiation. Mechanical forces affecting cusps lead to the activation of the atrioventricular (AV) endothelium resulting in the increased expression of vascular cell adhesive molecule (VCAM), intercellular adhesive molecule (ICAM), bone morphogenetic protein 4 (BMP-4, proinflammatory osteogenic morphogen), transforming growth factor beta (TGF-beta) as well remodeling an extracellular matrix leading to an increased stiffness of cusps [20, 21]. The increased stiffness of cusps increases the effect of mechanical force on the cusps. The activation of the Toll-like receptor on VICs, possibly under the action of the liberated double-stranded RNA from injured cells, increases the bone morphogenic protein-2 (BMP-2) expression on VICs. The increased expression of BMP-2 on VICs leads to an increase in Runx2 protein levels, the factor of transcription of osteoblast gene expression, which is necessary for the formation of an improved bone and helps regulate the differentiation of chondrocyte and osteoblasts [22, 23]. BMP-2 also induces Sox9, the second factor of transcription that stimulates chondrogenesis [19]. VCAM and ICAM expression stimulate the infiltration of cusps with inflammatory cells [24, 25]. Chronic inflammation stimulates angiogenesis that is essential for bone formation and the release of TNF-alpha from activated leukocytes [26]. VIC-exposed TNF-alpha increase the expression of BMP-2 [27]. TGF-beta mediates extracellular matrix (EMC) remodeling and stimulates the production of reactive oxygen radicals [28]. Oxidative stress that is particularly high in calcification areas can lead to the transformation of activated VICs into osteoblasts, possibly via Wnt3a signaling [29, 30]. The occurrence of metaplasia in cusps can also have a genetic basis. Notch1 mutation results in the greater expression of BMP-2 by VICs [31].

According to literature, aortic stenosis is most often clinically manifested with the symptoms of choking, chest pain, dyspnea, or syncope [32, 33]. According to our results, the triassic symptoms in the affected people include fatigue, choking and chest pains, while fainting as a clinical symptom predominated in 14 (12.2%) patients, and only 6 (5.2%) patients were reduced to loss.

In 17 (14.8%) of our patients, the presence of bone and/or cartilage metaplasia was pathohistologically shown. In a study of Torre et al. [34] metaplasia appeared in 11.5% of the patients with tricuspid aortic valve. This study also showed more frequent reporting of metaplasia in males, which is in line with our results.

In our study, in patients with pathohistologically proven metaplasia, the presence of yellow bone marrow was more common, which corresponds to the results of other studies on this topic [35].

Metaplasia was equally prevalent among patients with moderate, severe and critical aortic valve stenosis, which may point out that metaplasia has no influence on the severity of aortic valve stenosis. It is still a question whether metaplasia is just a pathological finding or it has some influence on the clinical course of the disease. However, a greater sample is required to discover it.

To this date, no medical therapy has been proven to prevent or to stop the progression of aortic valve stenosis. Although there have been attempts to find another, the only therapeutic approach for now is aortic valve replacement [36, 37]. In order to develop the new treatment strategies, we have to discover the complex pathophysiological pathway of this disease.

Conclusion

Age, sex, smoking, body mass index and blood pressure values show the same distribution among patients with and without histopathologically proven metaplasia, which may point out that these are not risk factors for metaplasia among the patients with calcific aortic stenosis. Metaplasia shows no impact on the severity of the disease. Howewer, further studies are required to show whether there are specific risk factors that lead to metaplasia and whether it is connected with the late stage and worse outcome of the disease.

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