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Effects of calcific and inflammatory mechanisms on different aortic cusps in etiopathogenesis of degenerative aortic stenosis

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ABSTRACT

Objectives: This study aimed to evaluate pathological differences in aortic valve cusps from patients undergoing aortic valve replacement due to aortic stenosis.

Patients and methods: In this single-blind observational study, aortic valves from 20 patients (10 males, 10 females; mean age: 69±6.36 years; range, 62-84 years) were divided into left coronary, right coronary, and noncoronary cusps between January 2013 and December 2014. The cusps were stabilized in 10% formalin and sent to the pathology lab in a blinded manner. Sections 5 µm in thickness were prepared, stained with hematoxylin-eosin and Van Gieson, and analyzed for inflammation, fibrosis, and calcification using light microscopy. Comparisons were made between right, left, and noncoronary cusps.

Results: Inflammation comparisons between right, left, and noncoronary cusps showed no statistically significant differences (p=0.199, p=0.789, and p=0.379, respectively). For fibrosis, left and right coronary cusps and right and noncoronary cusps were not significantly different (p=0.079 and p=0.880); however, the comparison between left coronary and noncoronary cusps was significant (p=0.046). Regarding calcification, left and right coronary cusps and right coronary and noncoronary cusps showed no significant difference (p=0.285 and p=0.180), while the comparison between left coronary and noncoronary cusps was statistically significant (p=0.011).

Conclusion: While pathological differences between aortic valve cusps are rarely emphasized in the literature, this study identified significant fibrosis and calcification differences in left coronary and noncoronary cusps. These findings could contribute to a better understanding of calcific aortic stenosis and potentially guide new treatments.

Keywords: Aortic stenosis, calcification, fibrosis, inflammation.

Aortic stenosis is a major indication for valve replacement surgeries in Türkiye, with degenerative calcific aortic stenosis more common in older patients.[1] While acute rheumatic fever remains the primary cause of aortic stenosis in younger populations, degenerative aortic stenosis in older adults results from idiopathic calcification and atherosclerosis, often affecting individual valve cusps without fusion.[2,3] This form of the disease is now recognized as part of a systemic atherosclerotic process, with lipid deposits, inflammation, and calcification as primary pathological features.[4-7] Calcification, a hallmark of degenerative aortic stenosis, is typically localized to the aortic side of the valve cusps and plays a central role in disease progression.[5,8]

The progression of aortic stenosis shares similarities with atherosclerosis, with endothelial damage due to mechanical stress playing a key role. The distribution of lesions on the valve is influenced by factors such as tangential tension, which varies across the cusps, and mechanical stress at attachment sites on the aortic root.[9] This damage triggers localized inflammation and calcification, processes regulated by inflammatory cytokines, oxidative stress, and lipoprotein metabolism.[4-6]

Advanced imaging techniques, such as computed tomography (CT), are critical for quantifying

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calcification and evaluating the severity of aortic stenosis.[8,10] These techniques have been shown to correlate with disease progression, particularly in patients with elevated lipoprotein (a) levels.[6,11-13]

Despite advances in imaging and understanding of disease mechanisms, the precise pathophysiology remains unclear, and medical therapies to slow progression are still unavailable.[5,6] Therefore, identifying reliable indicators of disease progression and the need for surgical intervention is crucial. This study aimed to evaluate the pathological effects of degenerative aortic stenosis on different valve cusps using samples from aortic valve replacement surgeries performed in our clinic.

PATIENTS AND METHODS

The single-blind observational study was conducted with 20 patients (10 males, 10 females; mean age: 69±6.36; range, 62-84 years) who underwent aortic valve replacement due to degenerative calcific aortic stenosis at the Cardiovascular Surgery Clinic of the Celal Bayar University between January 2013 and December 2014. Patients with the history of rheumatic fever, congenital aortic stenosis, and bicuspid valves were excluded. The study protocol was approved by the Celal Bayar University Faculty of Medicine Ethics Committee (date: 10.12.2012, no: 20,478,486,246). Written informed consent was obtained from all patients. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Aortic valve surgery was performed in standard fashion with single stage canulation following median sternotomy. The removed valve was divided into left coronary, right coronary, and noncoronary cusps. It was then fixed in 10% buffered formalin solution. When the total number of valves reached 20 and the number of cusps reached 60, the cusps were numbered from 1 to 60 in a mixed manner and sent to the pathology laboratory. While the surgical clinic knew the number that identified each cusp, the pathology clinic remained blinded. The cups that were observed to be excessively calcified in manual examination of the valves were kept in decalcification solution for 12 h and remaining for 1 h and were subjected to routine tissue follow-up in a fully automatic tissue tracking device without any further action. At the end of this process, the valves were placed on one surface and embedded in paraffin in a way to provide the widest possible examination through the embedding device. Sections of 5 μm in thickness were taken from the tissue blocks obtained in this way by means of a standard rotary microtome, and these sections were stained with hematoxylin-eosin and Van Gieson after deparaffinization and rehydration.

Sections stained with hematoxylin-eosin were examined under a standard light microscope by a blinded, expert pathologist and evaluated for calcification, fibrosis, and inflammatory infiltration. A score of 1 was assigned if areas of calcification and fibrosis were less than 25% of the cusp surface, 2 if between 25% and 50%, and 3 if more than 50%.[1] Sections stained with Van Gieson were also used to determine the fibrosis score, and cell-free type 1 collagen areas stained with bright red were considered fibrosis areas (Figure 1).

In order to determine the level of inflammatory infiltration, absence of inflammatory cells (lymphocytes or plasma cells) was considered Grade 0, presence of rare inflammatory cells scattered on the section surface or a single group of no more than 20 inflammatory cells was identified as Grade 1, the presence of more than one group of more than 20 inflammatory cells on the surface was classified as Grade 2, and the presence of more than 20 groups or a group of more than 100 cells was considered Grade 3 (Figure 2).

In addition, CD3 (clone SP7, 1/150 dilution, incubation time 30 min; Thermo Fisher Scientific, Waltham, MA, USA), CD20 (clone L26, 1/200 dilution, incubation time 30 min; BioCare, Concord, CA, USA), CD4 (clone 4B12, prediluted, incubation time 60 min; DAKO, Denmark ApS, Glostrup, Danmark), CD8 (clone SP16, 1/100 dilution, incubation time 60 min; Thermo Fisher Scientific, Waltham, MA, USA), and CD138 (clone MI15, prediluted, incubation time 40 min; Thermo Fisher Scientific, Waltham, MA, USA) were added for six cusps with grade II and III inflammation. All immunohistochemical studies were performed with the Ventana fully automatic immunostaining device (Roche Diagnostics, Rotkreuz, Switzerland) and standard kits for this machine.

Statistical analysis

Data were analyzed using IBM SPSS version 19 software (IBM Corp., Armonk, NY, USA).

Table 1
Cross-tables of calcification degrees, fibrosis grades, and inflammation degrees by cusp types

	Calcification degree			Fibrosis grades			Inflammation degree			
	1	2	3	1	2	3	0	1	2	3
Right-coronary cusp										
Frequency	6	2	12	2	8	10	5	13	2	0
%	30.0	10.0	60.0	10.0	40.0	50.0	25.0	65.0	10.0	0.0
Left-coronary cusp										
Frequency	8	4	8	5	10	5	1	17	2	0
%	40.0	20.0	40.0	25.0	50.0	25.0	5.0	85.0	10.0	0.0
Non-coronary cusp										
Frequency	1	4	15	1	9	10	5	12	2	1
%	5.0	20.0	75.0	5.0	45.0	50.0	25.0	60.0	10.0	5.0
Total										
Frequency	15	10	35	8	27	25	11	42	6	1
%	25.0	16.7	58.3	13.3	45.0	41.7	18.3	70.0	10.0	1.7

Student's t-test was used to determine mean values and standard deviations, and the Mann-Whitney test was used for nonparametric tests. The distribution of the data according to the cusp type was analyzed through cross tables. A p-value <0.05 was considered statistically significant.

RESULTS

Right coronary and left coronary cusps were compared based on calcification, fibrosis and inflammation. The results are given in Table 1. The z-value for the degree of calcification was –1.069 (p=0.285), the z-value for the degree of fibrosis was –1.759 (p=0.079), and the z-value for the degree of inflammation was –1.285 (p=0.199). There was no difference between the two valve types in terms of these features.

Right coronary and noncoronary cups were also compared (Table 2). While the z-value for the degree of calcification was 1.341 (p=0.180), the z-value for the fibrosis degree was –0.151 (p=0.880), and the z-value for the degree of inflammation was –0.267 (p=0.789). It was determined that there was no difference between the two valve types in terms of these features.

Finally, left coronary and noncoronary cusps were compared (Table 2). The z-value for the degree of calcification was –2.555 (p=0.011), the z-value for the degree of fibrosis was –1.995 (p=0.046), and the z-value for the degree of inflammation was –0.880 (p=0.379). The degree of calcification and fibrosis yielded statistically significant results for the comparison of left coronary and noncoronary cusps.

DISCUSSION

Calcific aortic stenosis constitutes the leading class of valve diseases in the Western world. In the past 10 years, the number of aortic valve replacement surgeries performed in some countries has doubled according to the literature. Considering the increasing elderly population, it is predicted that cases of aortic stenosis will double again in the next 20 years.[14]

Although surgical aortic valve replacement is common, transcatheter aortic valve implantation is an alternative treatment method in patients with severe aortic stenosis, particularly those at high risk for surgical aortic valve replacement.[15] As the number of transcatheter aortic valve implantation procedures increases, understanding the pathology of aortic disease becomes more important. Calcification degrees should be assessed before procedures, and specific measures should be taken.[16-18]

This progressive disease is characterized by inflammation, fibrosis, and calcification, with the latter often serving as a primary marker of disease severity.[5,8] Advanced imaging modalities such as CT allow for accurate assessment of calcification,

Table 2
Comparison of right coronary, left coronary, and noncoronary cusps

	Right-coronary/left-coronary valves			Right-coronary/non-coronary cusps			Left-coronary and non-coronary cusps		
	Calcification degree	Fibrosis degree	Inflammation degree	Calcification degree	Fibrosis degree	Inflammation degree	Calcification degree	Fibrosis degree	Inflammation degree
Mann-Whitney U test	164	140	164	159	195	191.5	116	132.5	174.5
According to Wilcoxon test									
W	374	350	374	369	405	401.5	326	342.5	384.5
Z	–1.069	–1.759	–1.285	–1.341	–0.151	–0.267	–2.555	–1.995	–0.88
Asymp. sig. (2-tailed)	0.285	0.079	0.199	0.18	0.88	0.789	0.011	0.046	0.379
Exact sig. [2*(1-tailed Sig.)]	0.341	0.108	0.341	0.277	0.904	0.820	0.023	0.068	0.495

Figure 1. Van Gieson-stained sections were used to determine the fibrosis score, and cell-free type 1 collagen areas stained with bright red were considered as fibrosis areas (Van Gieson ×200).

Figure 2. Grade 3 inflamation (H&E, ×200).

which is crucial for evaluating disease severity and prognosis.[8,10] The role of lipoprotein (a) is particularly noteworthy, as elevated levels have been shown to correlate with faster calcification progression and worse outcomes.[6] Recent evidence suggests that lipoprotein (a) mediates calcification via oxidative stress and inflammatory pathways, underscoring its importance as a therapeutic target.[6] Furthermore, inflammatory regulation of extracellular matrix remodeling and immune cell infiltration have also been implicated in disease progression.[2,5]

Left ventricular afterload increases due to the progressive narrowing of the aortic valve. In the face of the increasing pressure load, myocytes initiate a

hypertrophic process. While this process initially reduces the stress on the wall, it is later insufficient. In patients with aortic stenosis, the emergence of symptoms related to the progression of the disease, the observance of adverse effects, and the need for surgery depend on the narrowing of the valve and the resulting left ventricular hypertrophy.[19,20]

In calcific aortic stenosis, the valves become progressively thickened, fibrotic, and calcified. As a result, valve stiffness, decreased movement, and narrowing of the valve opening are observed in contrast to the fusion that develops in rheumatic valve diseases. Historically, calcific aortic stenosis was thought to be caused by wear and age-related degeneration after prolonged use. However, recent evidence shows that aortic stenosis occurs as a result of active inflammation involving biochemical, humoral, and genetic factors.

Although the differences were not statistically significant in our study, we believe that it is mainly due to the limited number of patients. Radiological examinations are effective in determining the calcification levels of the aortic cusps; however, there is still no definite answer about the underlying process. As we expand our knowledge on the etiopathogenesis of the disease, the management of treatment will also advance. Although pathological differences between aortic cusps rarely take part in current literature, we believe that it will shed light on new developments in medical and surgical treatment of calcific aortic stenosis.

This study was limited by its small sample size. Larger studies incorporating advanced imaging and molecular biomarkers, as recommended in recent literature, are needed to validate these findings and inform clinical practice.[8,10]

In conclusion, these findings emphasizes the role of lipoprotein metabolism, advanced imaging techniques, and inf lammation in disease progression. Understanding these mechanisms could guide new medical and surgical therapies aimed at improving patient outcomes.

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