Different Calcification Stage in Each Cusp of a Calcified Tricuspid Aortic Valve

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Calcific aortic stenosis (AS) is a slow and progressive disease characterized by thickening, calcification and stiffening of the heart valves. Although calcified deposits in the heart valves have been investigated, there has been little evidence for differing calcification growth stages in each respective calcified cusp of an aortic valve. A 76-year-old woman was admitted to hospital because of cardiogenic shock and pulmonary edema. Transthoracic echocardiogram indicated calcareous deposits in the heart valves (Figure 1A). On color Doppler echocardiography,

Figure 1. Preoperative (A) transthoracic and (B) Doppler echocardiograms of the heart; and (C) aortic view of the intact tricuspid aortic valve excised from a female aortic stenosis patient, showing the positions of Raman spectroscopy traces 1–5 on each cusp. AVA, aortic valve area; LA, left atrium; LC, left coronary; LV, left ventricle; LVOT, left ventricular outflow tract; NC, non-coronary; RC, right coronary; RV, right ventricle; SV, stroke volume.
aortic valve area was 0.61 cm², peak pressure gradient was 65 mmHg, mean pressure gradient was 35 mmHg, and left ventricular ejection fraction was 46%, indicating severe AS (Figure 1B). N-terminal pro-B-type natriuretic peptide was 7,164 pg/mL, and no significant lesion was seen on carotid Doppler. The patient was diagnosed with severe AS, and heart valve replacement surgery was performed.

After aortic valve replacement surgery, the tricuspid aortic valve (TAV) was observed and isolated. Calcified nodules and thickening in each valve were observed from the aortic side of the intact TAV (Figure 1C). In addition, a partial commissural fusion was also found.

Simultaneous differing calcification growth stages were noted in each respective cusp of this excised TAV on Raman spectroscopy (Figure 2). Raman spectra at 5 random positions on each cusp of the aortic valve (traces 1–5) were obtained directly using a portable Raman spectrometer. The low-intensity band at 1,003 cm⁻¹ due to phenylalanine of proteins implies that proteins were not abundant in all the cusps (Figure 2). Traces 1,2 in the left coronary cusp (LCC; Figure 2A) were similar, in which lipid-related spectral bands manifested mostly as saturated (1,063 and 1,445 cm⁻¹) and unsaturated (1,256 and 1,658 cm⁻¹) chemical bonds.³ The band at 1,658 cm⁻¹ could be assigned to the amide I mode of protein, but it was instead assigned to the stretching vibration of the cis C=C double bond of lipids.³ This indicates that the LCC was composed of large amounts of lipid-rich components. From traces 3–5 in the LCC (Figure 2A), a new unique band at 969 cm⁻¹ appeared, and the band intensity at 945 and 969 (970) cm⁻¹ was gradually enhanced. Both bands were attributed to β-tricalcium phosphate.⁴ Interestingly, the Raman spectral shift occurred from 1,658 cm⁻¹ (cis-configuration of unsaturated lipids) to 1,662 cm⁻¹, and then to 1,664 cm⁻¹ (trans-configuration of unsaturated lipids).³ This is the first evidence of conversion of unsaturated lipids from cis to trans in the LCC.

Three similar Raman spectra (traces 1–3) were observed in the right coronary cusp (RCC; Figure 2B), similar to trace 5 (Figure 2A). Both bands at 945 and 970 cm⁻¹ due to β-tricalcium phosphate were also observed. A new band at 959 cm⁻¹ assigned to hydroxyapatite (HA) was observed in trace 4, but two characteristic bands at 959 and 1,069 cm⁻¹ clearly appeared in trace 5 (Figure 2B). The band at 1,069 cm⁻¹ was assigned to B-type carbonated HA.⁴ The progressive calcification from β-tricalcium phosphate (as a precursor) to HA and then to B-type carbonated HA in the RCC is clearly seen (Figure 2B). The Raman spectra of the non-coronary cusp (NCC) were divided into two groups: 945- and 970-cm⁻¹ bands (traces 1,2) and 959- and 1,070-cm⁻¹ bands (traces 3–5; Figure 2C), suggesting that the calcification process in the NCC predominantly involved the formation of B-type carbonated HA (major) and β-tricalcium phosphate (minor).

In the present TAV the stage of calcification progressed in the order of LCC, RCC, NCC, with the LCC having the lowest stage of calcification, and the NCC, the highest. Different proportions of mineral components such as β-tricalcium phosphate, HA and B-type carbonated HA were detected in each respective cusp. The NCC had the highest stage of calcification, which might be due to the fact that the NCC has lower shear stress than the LCC or RCC, meaning that the calcification process is often seen first and most severely on the NCC.⁵,⁶ The advanced calcification in the present AS patient might be possibly due to the thickening of collagen tissue, destruction of the normal layered architecture, foci of calcification and even ossification, and/or infiltration of chronic inflammatory cells, resulting in a worsening of AS and heart failure.⁷ To our knowledge, this is the first study to show a simultaneous difference in calcification growth in each respective cusp of a TAV in a patient with calcific AS. In addition, cis- and/or trans-unsaturated lipids and their conversion products were also identified in each cusp, in different ratios depending on the stage of calcification.
Aortic Valve Cusps: Differing Calcification Stages

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Disclosures
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Supplementary Files

Supplementary File 1
Supplementary Methods
Please find supplementary file(s);
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