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Subclinical Endocarditis Might be a Hidden Trigger of Early Prosthetic Valve Calcification: A Histological Study

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ABSTRACT

Objective: Despite various improvements in valve prosthetics, early valve deterioration still occurs, leading to prosthetic failure. Studying the early phase of this deterioration is quite difficult, as the prosthesis to be examined is almost always explanted only after extensive deterioration. The objective of this research is to study the pathology of early valve deterioration in an early stage in order to reveal the possible trigger of the process.

Methods: Three cusps of the same type of bovine pericardium valve prosthesis underwent comparative examination. Two cusps (cusps 1 and 2) were retrieved from a valve prosthesis explanted three months post-implantation, and the third cusp was from a non-implanted valve prosthesis and used as a reference cusp (ref. cusp). The examination included macroscopic examination, Non-linear Optical Microscopy using a multiphoton microscope, and histological examination with staining, using Hematoxylin & Eosin, Movat Pentachrome stain, Von-Kossa stain, and Alizirin-Red stain. Parallel sections were decalcified using Osteosoft® solution prior to Von-Kossa and Alizirin-Red staining to exclude false positive results.

Results: Macroscopically, cusp 1 showed early deterioration, and cusp 2 showed endocarditic vegetations. Histologically, cusp 1 showed calcifications in acellular deposits on the surface of the cusp, with pathological signs of subacute/healed endocarditis and intact cusp tissue. The examination did not show calcifications of the cellular remnants within the valve tissue. Cusp 2 showed florid endocarditis, with microscopic destruction of the valve tissue.

Conclusion: Early prosthetic valve deterioration can exist as early as three months post-implantation. Subacute or subclinical endocarditis can be the cause for early valve calcification and deterioration.

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INTRODUCTION

The primary problem that arises with bioprostheses, whether porcine or pericardial, is structural valve deterioration in the long term, mainly due to dystrophic calcifications [Pettenazzo 2008]. Several improvements have been introduced, such as stent-flexibility, low-pressure glutaraldehyde fixation, strut height reduction, new methods of tissue preservation, and calcium retardant agents. All of these improvements are targeted towards delivering a better valve design and tissue treatment, therefore decreasing the possibility of developing a calcification nucleus with subsequent

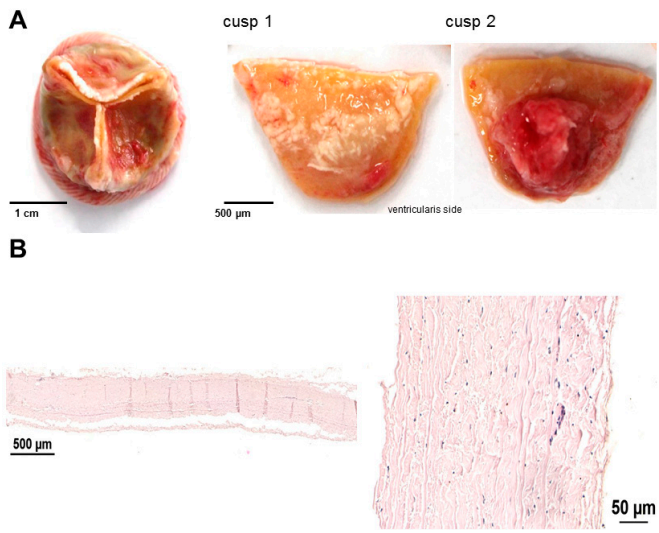

Figure 1. Macroscopic and histological morphology of Trifecta cusps. (A)Trifecta prosthesis explanted three months after implantation with different states of pathological alteration. Cusp 1 exhibits a white covering, cusp 2 an endocarditic vegetation on the ventricular side. (B)HE staining of a section of an original Trifecta cusp (cusp ref.) shows the collagen structure of the unimplanted fixed prosthesis cusp including nuclei.

valve deterioration in the long run [Pettenazzo 2008]. The methods are effective because the principal underlying pathologic process leading to valve deterioration is cuspal calcification [Schoen 2005]. Despite the fact that calcific deposits are usually localized to cuspal tissue (intrinsic calcification), it has been suggested in the literature that calcific deposits extrinsic to the cusps may also develop, as the calcification of minor thrombi on the surface of the prosthetic valve has been described [Schoen 2005; Ishihara 1981; Ferrans 1980]. Endocarditic vegetations have been suggested to play a role as nuclei for calcifications [Schoen 2005; Ferrans 1978].

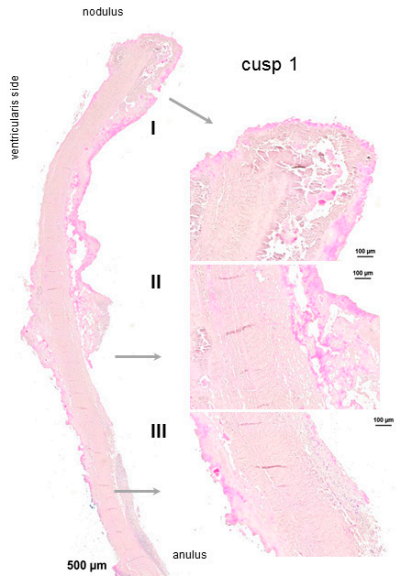

Figure 2. Histological sections of cusp 1 with mostly acellular fibrinogen covering in a subacute endocarditis.

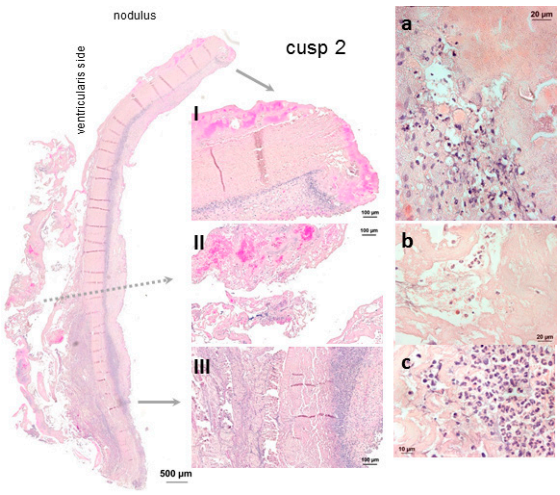

Figure 3. Active endocarditic covering of cusp 2 of the explanted prosthesis demonstrated at HE stained histological sections. Acellular fibrinogen covering is solely visible at the nodulus of the cusp (I), whereas cellular infiltrations of numerous immune cells are frequent in all other regions of the cusp.

Early valve deterioration has been repeatedly described, sometimes developing as early as three days after implantation [Ishihara 1981; Hetzer 1978]. With the development of new generations of biological valves with better design and treatment, as well as the avoidance of implantation into young patients, the number of cases of early degeneration has fallen markedly. Yet, even with markedly improved biological prostheses, early valve deterioration has not been abolished.

Although an extrinsic factor might be the trigger, there are no sufficient data to support the hypothesis that a subclinical endocarditis might be the hidden cause of early deterioration of modern biological valves. Herein, we report further evidence for the possibility of subclinical endocarditis as an etiological factor for early valve deterioration.

METHODS

In this study we present the morphologic observations of three separate Trifecta® bovine pericardium aortic valve prosthesis cusps (St. Jude Medical, Inc.; St. Paul, MN, USA). The first and second cusps (cusps 1 and 2) were obtained from a prosthesis that was explanted three months after implantation because of florid endocarditis. As a reference (cusp ref.),

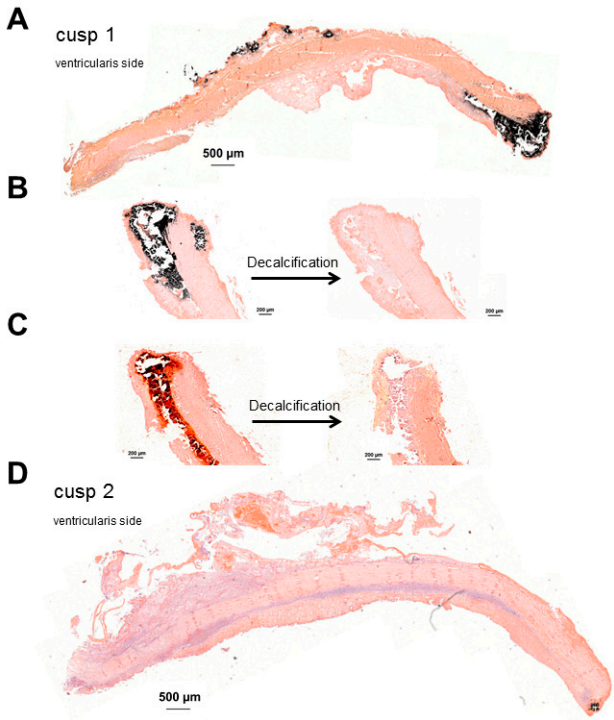

Figure 4. Cusp calcification status. (A)Von Kossa staining of cusp 1 reveals calcification sites at the nodulus of the cusp, mostly on the aortic side, and smaller spots of calcification at the ventricular side. (B)Decalcification of cusp 1 tissue sections by Osteosoft® treatment abrogates the von Kossa positive signal. (C)Alizarin positive staining of calcified cusp tissue is abolished after Osteosoft® treatment of cusp 1 tissue sections. (D)Von Kossa staining of cusp 2 shows no calcification at the site of the acute endocarditis. The staining shows only a calcified spot at the nodulus in a non-infiltrated area.

one cusp was obtained from a non-implanted valve as delivered from the manufacturer.

Sample Preparation

All cusps were macroscopically examined, dissected, and fixed in 4% formaldehyde. They were examined by Nonlinear Optical Microscopy (NLOM), and embedded in paraffin. After gross examination, histological sections were stained using Hematoxylin & Eosin (HE), Movat Pentachrome stain (Morphisto GmbH, Frankfurt, Germany), von-Kossa stain, and Alizarin-red (followed by an additional HE stain) according to their respective standardized staining protocols and manufacturers instruction [Böck 1989]. In the case of positive von-Kossa and Alizarin Red staining, the parallel sections were incubated overnight in a humidified atmosphere directly on specimen slides using 500 µl decalcifying solution Osteosoft® (Merck, Darmstadt, Germany), followed by von-Kossa and Alizarin red staining procedures. This allowed for the exclusion of false positive staining, and the support definition of calcified regions.

Nonlinear Optical Microscopy

A section of each cusp (about 2 mm thick) was subjected to nonlinear optical microscopy imaging. The fixed cusp sections were washed briefly in phosphate buffered saline (PBS) and placed on microscope glass slides, and then a cover slip was placed on top of the surface subjected to imaging. Both of the external surfaces of the fibrosa and the ventricularis side, as well as the cut surface, were imaged.

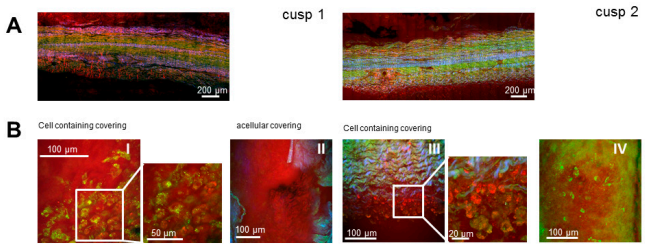

Figure 5. Trifecta collagen structure. Cross-sectional (A) and surface (B) analysis of the middle parts of cusp 1 and cusp 2. Nonlinear Optical Microscopy was adopted to visualize collagen (blue; SHG), elastin and cellular infiltrations (green; TPEF), and lipids (red; CARS). Cusp 2 shows cellular infiltration, in contrast with cusp 1.

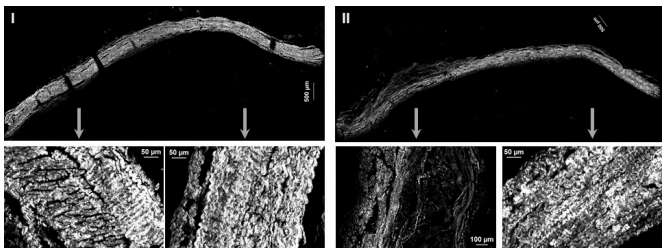

Figure 6. Isolated SHG signal of both cusps illustrates the intact collagen structures of cusp 1, and the destroyed, loosened, and sparse distribution of the collagen fibers in the annulus region of cusp 2.

The multiphoton microscope is a multimodal system with near-infrared ultrashort pulsed lasers, which offers the possibility of label-free imaging based on intrinsic tissue properties. It has been described in detail elsewhere [Uckermann 2015; Büttner 2014; Galli 2014]. In brief, the optical microscope is an upright Axio Examiner Z.1 coupled to a laser scanning module LSM 7 (Carl Zeiss AG, Jena, Germany). Endogenous two-photon excited fluorescence (TPEF) was used to address endogenous fluorophores of the tissue, like elastin and cellular structures, and second harmonic generation (SHG) was used to probe highly ordered structures lacking inversion symmetry, like collagen.

The excitation for TPEF and SHG is provided by a picosecond fiber laser (Femto Fiber pro NIR from Toptica Photonics AG, Munich, Germany) emitting at 781 nm. In order to visualize CH-bond rich molecules like lipids, the coherent anti-Stokes Raman scattering (CARS) was used, using a second picosecond fiber laser source (Femto Fiber pro TNIR from Toptica Photonics AG, Munich, Germany). The CARS, TPEF and SHG were simultaneously excited and acquired to build multimodal images (red: CARS; green: TPEF; blue: SHG).

Image Processing

Images were processed using the ZEN program (Carl Zeiss AG, Jena, Germany) to build orthogonal and maximum intensity projections from the acquired stacks of images. Further image analysis was performed using the ImageJ/Fiji image analysis platform (Laboratory for optical and computational instrumentation, University of Wisconsin-Madison, WI, USA).

RESULTS

Macroscopically, cusp 1 showed marked color changes on the belly and the free margin of the cusp, suggesting possible calcification. The cusp did not show evidence of cusp damage, tear, perforation, or endocarditis (Figure 1a). The total mobility of the cusp was not severely affected. Cusp 2 showed endocarditis vegetation on the ventricular side, with mild color changes, suggesting possible calcification (Figure 1a). There were no tears or perforations on this cusp, either.

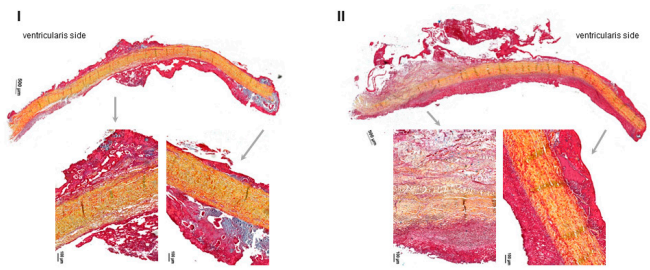

Figure 7. The yellow color of the Movat Pentachrom stain visualizes the unaffected collagen structure of cusp 1, in contrast to the infiltrated, loosened collagen fibrils at the annulus of cusp 2 with the active endocarditic covering.

Histologically, the explanted aortic valve prosthesis exhibits different states of pathological alteration (Figure 1). Potentially calcified deposits were apparent on cusp 1, which were evident on the belly of the ventricular side of the cusp and the free margin.

The histological analysis of the reference cusp stained with HE shows a regular collagen matrix with mesenchymal cells and cell nuclei (Figure 1b). Histological analysis of cusp 1 after HE stain revealed deposits of partly fresh, partly organized fibrin on both surfaces, but mostly at the aortic surface (Figure 2 I-II). At the base of the aortic side, there was an infiltration by mononuclear inflammatory cells and some granulocytes, consistent with a subacute endocarditis (Figure 2-III). In the nodular region, and partially at the middle part of the ventricular side of the cusp, there were reddish-brown acellular precipitates on the surface of the cusp. These were partially positive in the von-Kossa and Alizarin Red stains, denoting calcification (black and red, respectively, Figure 4a-c). After the treatment of parallel sections with Osteosoft® for decalcification, no positive von Kossa (Figure 4b) or Alizarin-Red (Figure 4c) staining signals were detected, proving that these precipitates had been calcified, and excluding a false positive result. In these calcified areas, no inflammatory cells could be identified (Figure 2-I).

Both surfaces of cusp 2 were covered heavily by mostly organized fibrin (Figure 3a, upper right corner; Figure 3b). More than 75% of the aortic layer, and at least the basal half of the ventricular layer, was covered by inflammatory cells. This mainly consisted of granulocytes, but also contained mononuclear cells (lymphocytes and macrophages), consistent with acute endocarditis (Figures 3, 3a, and 3c). Distal to the base of this cusp, parts of the fibrosa layer at the ventricular side were destroyed by the inflammatory cells (Figure 3-III). Similar to cusp 1, small calcium-containing precipitates were present at the nodular region of cusp 2 (Figures 3-I and 4d). This nodular calcified region of cusp 2 was free from inflammatory cells, in contrast with the other parts of this cusp. Figure 3a depicts the transition from the acellular to the cellular part of the cusp.

Cellular infiltrations in the fibrin covering were detected by multiphoton microscopy (Figures 5 and 6). The infiltrations were found solely at the annular region of cusp 1, and in the actively endocarditic covering on cusp 2 (Figures 5b-I and 5b-III). Examination of the cusp surfaces showed no further cellular infiltration of the surface in cusp 1, and confirmed the cellular infiltration of the cusp 2 surface (Figures 5b-II and 5b-IV). The SHG demonstrated that the collagen structure of cusp 2 was fanned out, destroyed, and loosened near the annulus (Figure 6-II). This was confirmed by the loss of collagen (yellow) staining intensity near the annulus in the Movat Pentachrome stain (Figure 7-II). In contrast, in cusp 1, collagen fibers were mostly intact over the whole tissue section (Figures 6-I and 7-I).

DISCUSSION

Prosthetic valve replacement still represents the treatment of choice for severe cardiac valve disease (1). Despite numerous improvements that have been accomplished in terms of design and tissue pretreatment, early valve degeneration is still a problem that leads to the need for premature replacement of the failing prosthesis.

Despite advancements in prosthetic valve design and pretreatment, some early valve deterioration can still be attributed to intrinsic factors. Sénage and colleagues published data reporting early valve deterioration in Mitroflow® Aortic valve prosthesis, attributing the deterioration to the absence of anticalcification treatment in the prostheses used in his study cohort [Sénage 2014]. Patient-prosthesis mismatch has been also reported as a cofactor [Sénage 2014; Flameng 2010]. However, prosthesis deterioration due to intrinsic factors usually occurs within years in the new generations of valve prostheses [Sénage 2014; Flameng 2010]. For early valve deterioration within months or weeks of implantation, extrinsic factors may be playing the bigger role.

Endocarditic vegetations have been reported as a site of calcification in earlier reports. The leading work of Ferrans et al, which studied prosthetic valve deterioration, showed calcification of vegetations in explanted valves [Ferrans 1980]. Cremer et al reported a case of prosthesis deterioration with severe stenosis and thrombotic depositions. However, the culture of the valve in their case grew Propionibacterium acnes, denoting that a subclinical endocarditis might have been present [Cremer 2015].

In our study, the examined cusp showed calcifications that were evident at the time of explantation. As the valve was explanted three months post-implantation, an extrinsic factor comes into focus as a possible cause of the early valve calcification. The result of our study showed that, compared to the reference cusp, the calcifications were on the surface of the cusp and not in the cusp structure itself, further denoting the possibility of an extrinsic factor leading to the calcification. The results of the histological examination of the cusps, together with the histological cellular findings, showed calcification of the deposits, which was consistent with a subacute/healed endocarditis. The explantation of a valve due to florid endocarditis later on enabled us to study early valve deterioration in an early stage, an event that is quite rare except for in animal models.

STUDY LIMITATIONS

This study was based on a single observation due to the rarity of the finding. Although the affected cusp showed only a subacute endocarditis, the other cusp from the explanted valve showed florid endocarditis, which adds the risk of possible bicuspid cellular infiltration affecting the observation. No electron microscopy or spectroscopic method was performed.

CONCLUSION

Early prosthetic valve deterioration and calcification can exist as early as three months post-explantation, even in the new generations of prosthetic valves. Subacute or subclinical endocarditis can be the cause for such early valve calcification and deterioration.

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