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Cellular and Neuronal Aspects in Aortic Stenosis

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1. Introduction

For quite some time the physiological approach to cardiac valve function held the belief that these are purely passive structures merely moving in response to changes in transvalvular pressure generated by atrial and ventricular contractions [1, 2, 3]. Today, this "passive hypothesis" is being challenged as numerous studies have shown that mammalian cardiac valves are capable of independent contraction during various stages of the cardiac cycle [1, 4, 5]. In order to understand this concept and the pathophysiological implications for valvular disorders one would need to understand the cellular components and appreciate the neural network innervating the diverse cellular milieu of the cardiac valve as we understand it today.

The diverse cellular population of the aortic valve, together with its neuronal network will be discussed in order to clarify the cellular and neuronal aspects of the complex and still incompletely understood process of aortic stenosis.

2. Macro structure of the aortic valve

As a unit, the aortic valve is composed of four parts: the aortic annulus, the aortic valvular cusps, the sinuses of Valsalva and the sinotubular junction [3]. The three semi-lunar cusps are attached at their base to a crown-shaped annulus [3]. The interaction between the cusps and the root is extremely important to ensure adequate coronary blood flow. Proper closure of the cusps in diastole helps to preserve the shape of the aortic root and this ensures the creation of vortices of blood flow in the sinuses of Valsalva which is a major determinant of coronary blood flow [3].

The aortic cusps are composed of three layers: the fibrosa, the spongiosa and the ventricularis [3]. The fibrosa lines the aortic side and is rich in collagen fibers, whereas the ventricularis lines the ventricular side and is rich in elastin fibers [3, 6]. The spongiosa lies between these two layers and is composed primarily of proteoglycans [3, 6].

3. Cellular components of the aortic cusps

Both sides of the aortic valve cusps, the fibrosa and ventricularis, are lined by endothelial cells [1, 2, 3]. These valvular endothelial cells are able to respond to stress placed on the cusps,

particularly shear stress and can produce several vasoactive mediators, such as nitric oxide, endothelins and prostaglandins [1, 7, 8], thus translating mechanical stimuli into biological responses, referred to as mechanotransduction [3]. The actual release of these various vasoactive mediators can be effected by neurotransmitters such as acetylcholine and substance P, released by nerve endings innervating the valvular cusps [7, 8]. These substances released by the endothelium can in turn stimulate the local nerve terminals and elicit various reflex responses [1, 9]. The role of this "endothelium-cusp-nerve interaction" in health and disease is an interesting and yet insufficiently explained arena in the healthy and diseased aortic valve.

Below the layer of endothelial cells lining the outer and inner layer of the aortic cusps (the fibrosa and ventricularis), the extracellular matrix is found, composed of elastin, collagen and proteoglycans [10]. In addition to the elastin, collagen and proteoglycans in this extracellular matrix, a population of valve interstitial cells can be found [2, 3]. These consist of fibroblasts, smooth muscle cells and myofibroblasts [2, 3, 11]. The proportion of these different cells comprising the interstitial cell component will depend on the condition and state of health of the valve [2]. These cells possess important secretory and proliferative properties to repair and maintain the extracellular matrix [2, 3]. They also have contractile properties and express the same structural genes as cardiac muscle, such as cardiac troponin T, I and C, cardiac myosin light chain and beta myosin heavy chain [2, 12]. Immunohistological studies have shown that almost 60% of aortic valve interstitial cells can express alpha-smooth muscle actin [2, 13].

The most recently identified cell type in the aortic valve is a population of resident stem cells which lie within the cusps [3, 14]. These cells are hematopoietic in origin with later mobilization towards the valve cusps [15].

The human aortic valve is an avascular structure, but is innervated by a network of afferent and efferent nerves [3].

4. The aortic neural network

The neural innervation of the aortic valve arise from two sources, the ventricular endocardial plexus [1] and the aortic adventitial wall [16]. These nerves are found in the entire leaflet, except at the coapting edge [1]. Compared to the two coronary leaflets, the noncoronary leaflet displays an attenuated density of innervation and the density of innervation declines in all the leaflets with advancing age [1]. This decline in valvular neural innervation with age seems to be limited to the aortic valve [1]. It seems that each cardiac valve is independently innervated with no nerve fibers extending between valves [2] . The thin aortic nerves display a circumferential pattern within each leaflet [2, 17]. Morphologically and neurochemically these valvular nerve endings appear similar to the nerve endings found in the epicardium and endocardium of the human heart [1, 18]. Aortic nerve terminals has been found to express immunoreactivity for tyrosine hydroxylase, neuropeptide Y, acetylcholinesterase, substance P and vasoactive intestinal peptide (VIP) [1, 2, 19] . These various neurotransmitters also have neuromodulatory effects, regulating the activity of several populations of neurons in the valve [2].

Thus, it is clear that there exists a rich cellular milieu inside the cusps of the aortic valve, with a rich supply of nerve endings, each one releasing neurotransmitters with diverse

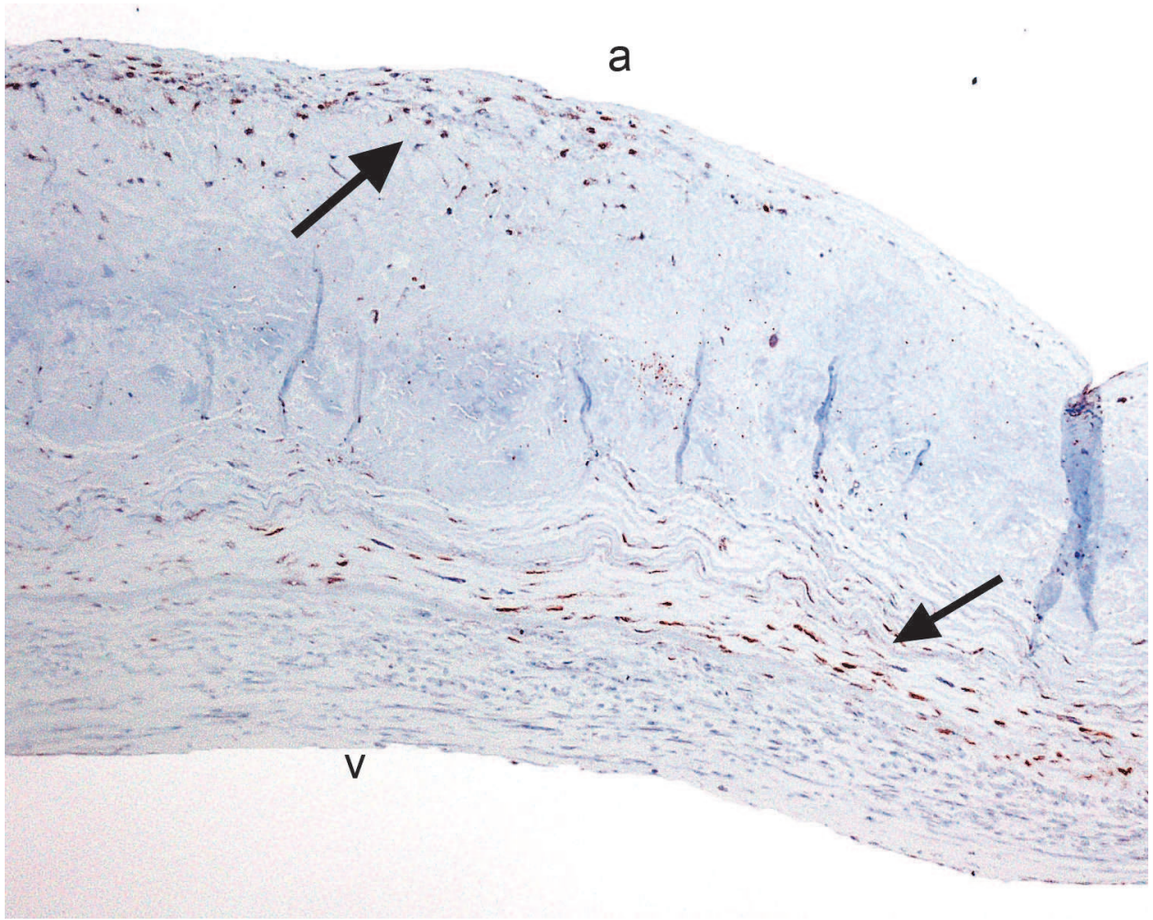

Fig. 1. S-100 staining of a stenotic valve shows the presence of positive spindle shaped cells suggestive of Schwann cells (arrows). (original magnification X 100).

physiological effects, effecting a complex array of contractile, secretory and homeostatic actions [2]. (Fig. 1). The aortic valve can thus be seen as a structure that is regulated by a local neural network very similar to the gastrointestinal tract, bronchial tree and blood vessels [2].

5. Diverse cellular changes in aortic stenosis

A cell not seen in the normal aortic valve is the osteoblast. However, during the inflammatory process leading to ultimate aortic stenosis, aortic valve myofibroblasts may differentiate into osteoblasts and thus contribute towards valve calcification [20]. (Fig 2).
Normal aortic valves are avascular and the oxygen demand of the cellular milieu is supplied by diffusion [21]. However, in the stenotic aortic valve a vascularization process is part of the pathogenesis of aortic stenosis [22]. (Fig 3).
The presence of myofibroblasts is associated with expression of alpha smooth muscle actin and osteoblastic markers such as osteocalcin and bone sialoprotein [23]. This contributes to fibrosis of the leaflets and the formation of calcified nodules [23]. Distinction between smooth muscle cells and myofibroblasts is difficult as both cell types express alpha-smooth muscle actin. It is possible to distinguish between these two groups of cells with h-caldesmon

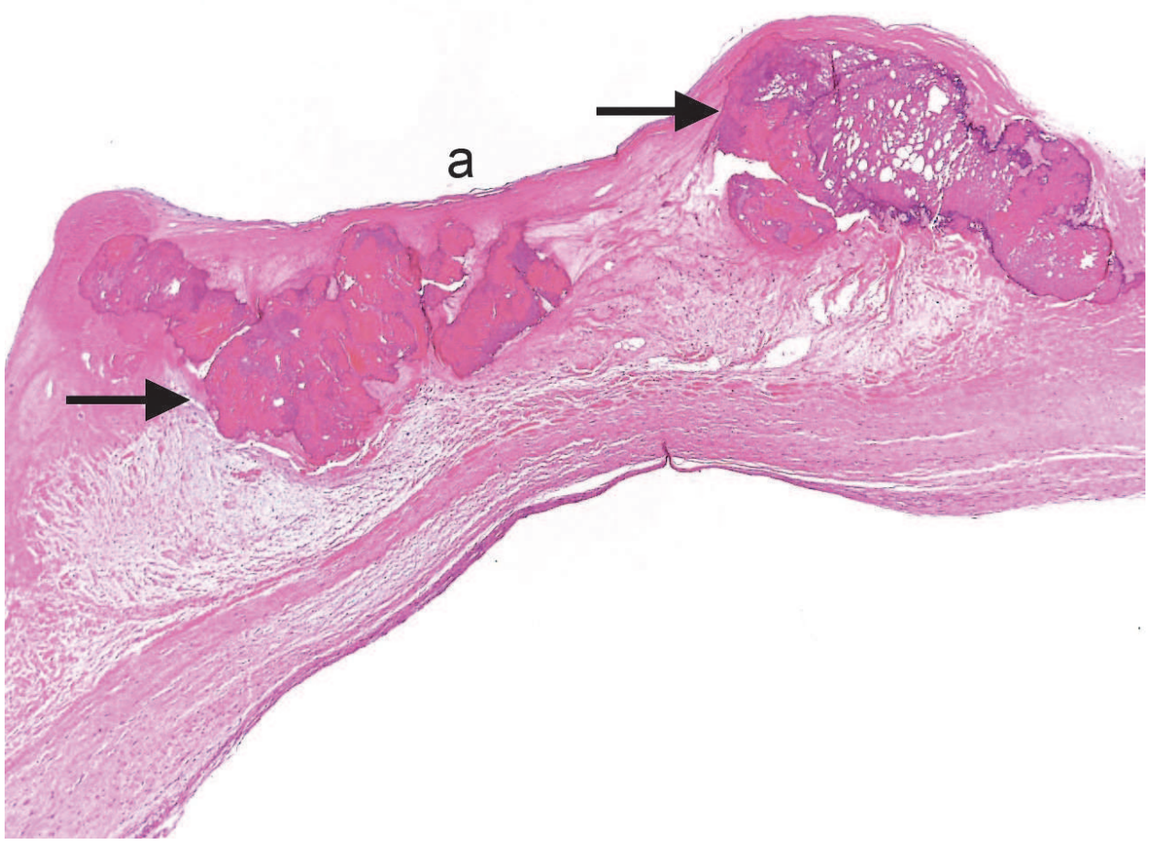

Fig. 2. Low power photomicrograph of a stenotic aortic valve shows the presence of calcifications (arrows) on the aortic side (a) of the valve (original magnification X40).

immunohistochemical evaluation. H-caldesmon is specific for smooth muscle cells and do not react with myofibroblasts [24]. (Figs 4 & 5).

6. Possible future therapeutic implications of cellular and neuronal aspects of aortic stenosis

From the preceding discussion it is clear that there are numerous possibilities for the development of new therapeutic agents in order to arrest the progression of the complex process of aortic stenosis. In the early stages the increase in smooth muscle cells and myofibroblasts may be inhibited by yet to be discovered inhibitors of differentiation.
The process of angiogenesis in the valve leaflet may be blocked, as well as the differentiation of valvular myofibroblasts into osteoblasts with subsequent calcification.
It is also possible that the still incompletely understood effects of neuronal stimulation of the valvular interstitial cells may play a causal role in the initial process of aortic stenosis and that these neurons may lend themselves susceptible to future inhibition by therapeutic agents.
Lastly, the possible therapeutic role of native stem cells inside the valvular cusps are endless and only the future will tell.
In conclusion, it can be said that the possible future therapy of the process of aortic stenosis may become a purely medical one, with surgery limited to cases discovered in the end stage of this complex process.

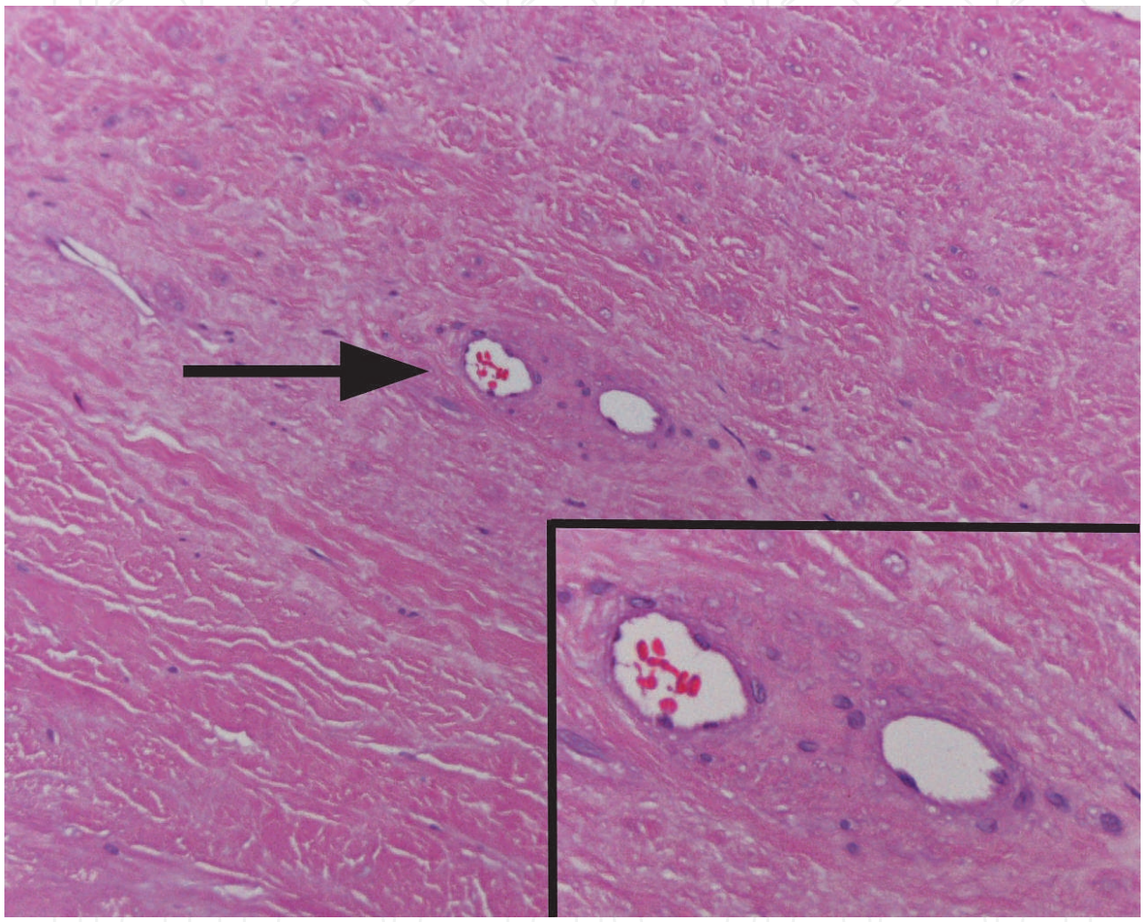

Fig. 3. Small blood vessels (arrow) in a stenotic aortic valve. (original magnification X 100). Inset: Higher magnification of the blood vessels. (original magnification X 200).

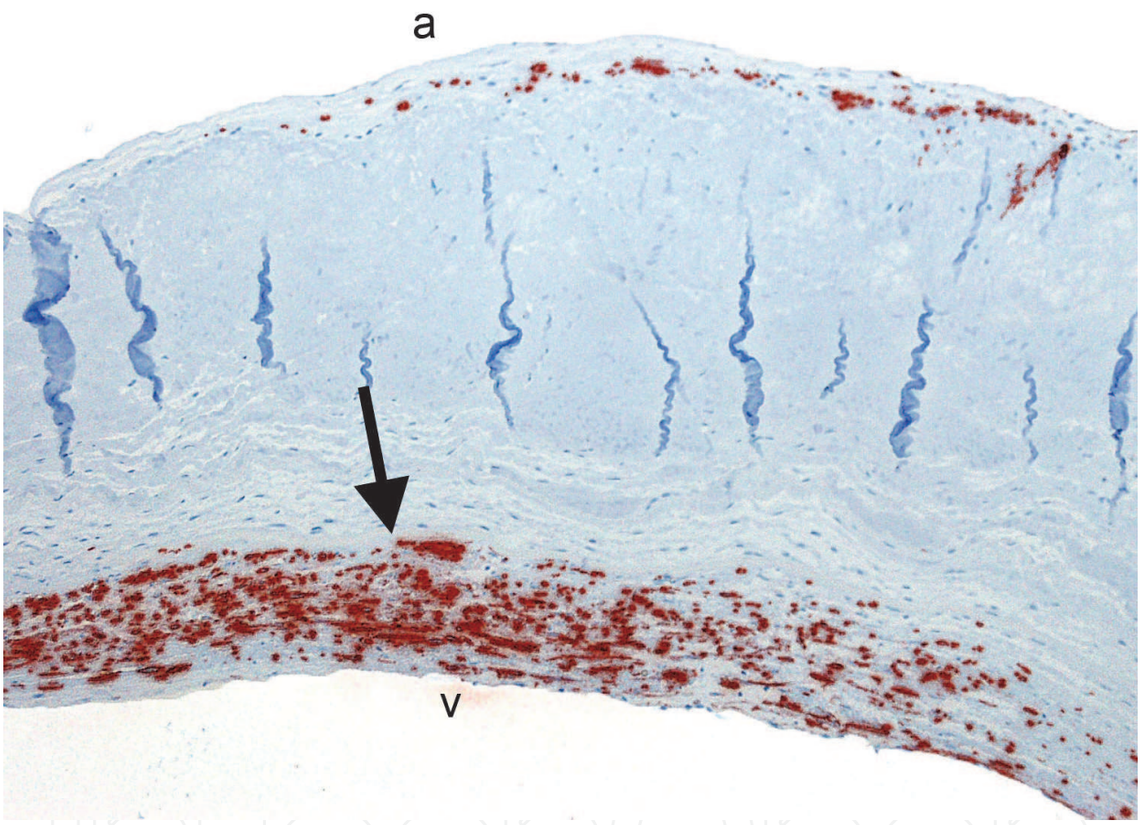

Fig. 4. Immunohistochemical staining of a decalcified stenotic aortic valve with smooth muscle actin (SMA) shows the presence of numerous positive cells (smooth muscle cells and myofibroblasts) (arrow) on the ventricular side (v) of the valve. Fewer positive cells are present on the aortic side (a). (original magnification X 100).

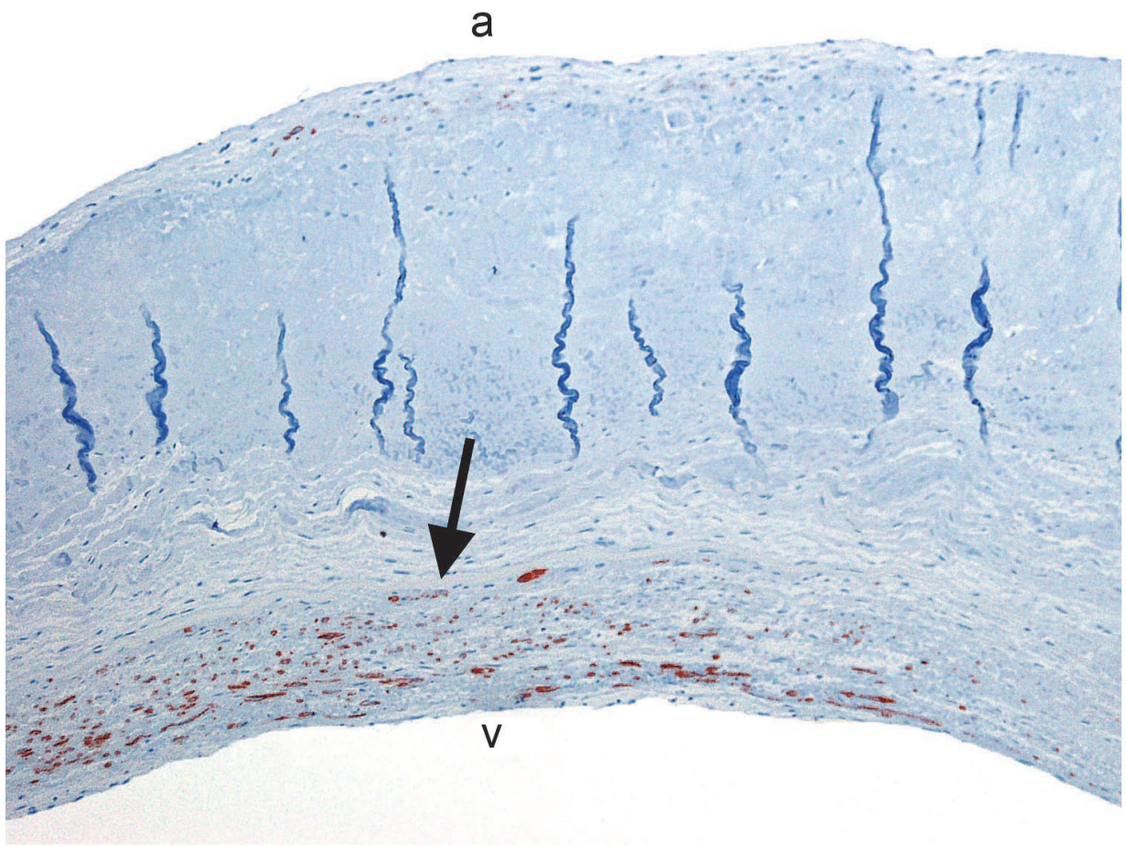

Fig. 5. H-caldesmon immunohistochemical stain (specific for smooth muscle cells) of the same area as in Fig. 4 demonstrates the portion of SMA positive cells that are smooth muscle cells (arrow). The SMA positive cells that did not stain with h-caldesmon are myofibroblastic in nature. (original magnification X 100).

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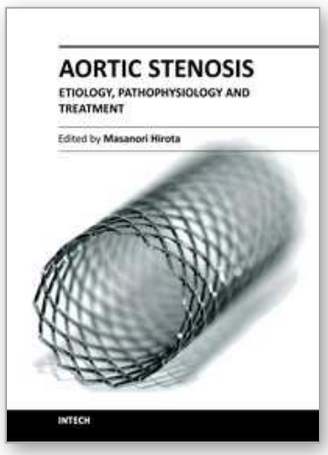

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Currently, aortic stenosis (AS) is the most prevalent valvular disease in developed countries. Pathological and molecular mechanisms of AS have been investigated in many aspects. And new therapeutic devices such as transcatheter aortic valve implantation have been developed as a less invasive treatment for high-risk patients. Due to advanced prevalent age of AS, further discovery and technology are required to treat elderly patients for longer life expectancy. This book is an effort to present an up-to-date account of existing knowledge, involving recent development in this field. Various opinion leaders described details of established knowledge or newly recognized advances associated with diagnosis, treatment and mechanism. Thus, this book will enable close intercommunication to another field and collaboration technology for new devices. We hope that it will be an important source, not only for clinicians, but also for general practitioners, contributing to development of better therapeutic adjuncts in the future.

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