# Histological and molecular characterization of human aortic stenosis: a matter of sex

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**Introduction:** Aortic stenosis (AS) is the most common heart valve disease and it is strongly prevalent with elderly. AS is a progressive, degenerative disease associated with fibrosis and calcification of the valve leaflets. Surgical valve replacement is the only treatment available. Molecular, cellular and interstitial events activate multifactorial and complex cues with a significant contribution by valve interstitial cells (VICs). Despite male sex is a risk factor for developing AS, there is scant information on sex-specific differences in aortic valve (AV) biology or pathology.

**Purpose:** The aim of our study was to analyse sex-specific differences in aortic valves from AS patients.

**Methods:** 185 patients with severe AS undergoing surgical valve replacement were recruited. 149 AVs (66 women; 83 men) were used for ex vivo analyses. Human VICs were isolated from 36 AVs (12 women; 24 men) for in vitro experiments. AVs structure were evaluated by haematoxylin-eosin, Movat, Alizarin Red, Congo red and Alcian blue/Sirius Red staining and immunohistochemistry. Western blot, ELISA and zymography were used for molecular biology studies.

**Results:** AVs from men presented increased inflammatory infiltrates (CD68 and CD45 positive cells) as compared to women. Complementarily, AVs from men exhibited higher levels of the inflammatory molecules interleukin (IL)-6 and IL-1b and RANTES. In line with these results, oxidative stress markers (eNOS, myeloperoxidase, malondialdehyde and nitrotyrosine) were upregulated in male AVs. Concerning, fibrosis, increased levels of collagen type I, fibronectin and syndecan-1 were found in AVs from men. Extracellular matrix (ECM) remodelling was characterized by reduced metalloproteinase-1 expression and increased tissue inhibitor of metalloproteinase-2 expression in male AVs. Importantly, calcification and osteogenic markers (bone morphogenetic protein-9, periostin, osteocalcin and Sox-9) was greatly enhanced in men AVs as compared to women. These findings were confirmed in isolated VICs. At baseline, male VICs presented higher myofibroblast-like phenotype than female VICs. In line with our ex vivo results, male VICs exhibited increased inflammatory, oxidative stress, fibrotic and osteogenic differentiation markers.

**Conclusions:** Our results suggest that the mechanisms driving the AV pathogenesis could be different in men and women patients with the same AS severity. Male AVs and isolated VICs presented more inflammation, oxidative stress, fibrosis and ECM remodelling including extensive calcification as compared to female. A better knowledge of the pathophysiological pathways in AVs and VICs will allow developing sex-specific options for AS treatment.