

Distribution of selected elements in surgically excised human stenotic aortic valves

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Aortic valve degeneration is an age-related process leading to valvular sclerosis and stenosis. In course of the disease, dystrophic calcification is a common phenomenon.

Recent years brought strong evidence supporting the concept of active mechanisms participating in the pathophysiology of valvular calcification. [1]. It is postulated that chondroblastic and/or osteoblastic transformation of cells localized in valvular stroma can lead to calcification and heterotopic bone formation [2,3]. Among degenerating aortic valves, congenitally bicuspid valves constitute a separate group. This malformation prone to severe pathological features, including early massive valve calcification, frequent perforation and ulceration. Synchrotron radiation microprobe technique have been successively applied by us to characterize elemental composition of atheromas in animal model [4].

The aim of the current project is to determine concentration of selected elements (Ca, Cl, Cu, Fe, K, P, S, Se, Sr, Zn) in surgically excised human stenotic aortic valves (tricuspid and congenitally bicuspid), insufficient aortic valves as well as normal aortic valves. The elemental distributions are comparing with images of consecutive sections stained histologically and immunohistochemically, allowing precise localization of the elements in morphologically defined areas.

Five aortic valves were included in the preliminary study. One normal valve (male, 46 years old), two insufficient valves without signs of calcification (females, age: 64 and 66), and two calcified stenotic valves (males, age: 66 and 68) from which one was tricuspid (TAV) and one bicuspid (BAV). Tissue serial sections (10 μm -thick) were cut frozen on cryostat, mounted on 3 μm -thick Mylar foil and subjected to measurements applying synchrotron radiation. Additional sections were processed to histological (HE) and immunohistochemical stainings: CD68 for macrophages, alpha smooth muscle actin (SMA) for myofibroblastic cells and tenascin C (TnC) a matrix glycoprotein expressed during tissue regeneration and remodeling. Double immunostaining was also done employing in situ zymography, a technique that enables localization of matrix-degrading metalloproteinases (MMPs) activity in histological sections (Fig. 1).

All micro-XRF measurements were carried out at beamline L of the storage ring DORIS III. The primary photon energy was set to 17.5 keV by a multilayer double monochromator. A polycapillary half-lens was used for beam focusing, hence the final beam size on the sample was approximately 15 μm in diameter. Emitted elemental spectra was recorded with Vortex SDD detector. Two-dimensional maps were acquired from microscopically selected areas of valves (resolution 15 μm , time of acquisition 3 s from each point). Precise point spectra were recorded from morphologically defined areas (resolution 15 μm , time of acquisition 300 s). The results were normalized to beam current, thickness of sample and time, and expressed in arbitrary units (mean \pm SD).

The distributions of such elements like potassium, chloride corresponded well with histological structure of the section and they were found in cellular components of the lesion.

Active remodeling areas rich in fibroblasts, myofibroblasts and macrophages expressing TnC and showing MMPs activity was found in both stenotic valves (tricuspid and bicuspid). In these areas higher (then in not affected part of valves) level of calcium,

phosphorus, sulfur, iron and strontium were found. Extremely higher concentration of calcium, however, were observed in mineral deposits. Similar distribution was also characteristic for phosphorus, although its concentration in calcifications was not as high as it was in the case of calcium. Differences in content of iron between tricuspid and bicuspid aortic valves were found, with much higher level in TAV.

Differences in elemental composition of not affected part of valves were also found. The highest calcium concentration was observed in BAV, while its concentration in stenotic TAV was only slightly higher than in normal non-stenotic valve and lower than in insufficient valves, which show macroscopically slight fibrotic changes without calcification. Observed differences between TAV and BAV in calcium and iron content and distribution can reflect differences in their etiopathology.

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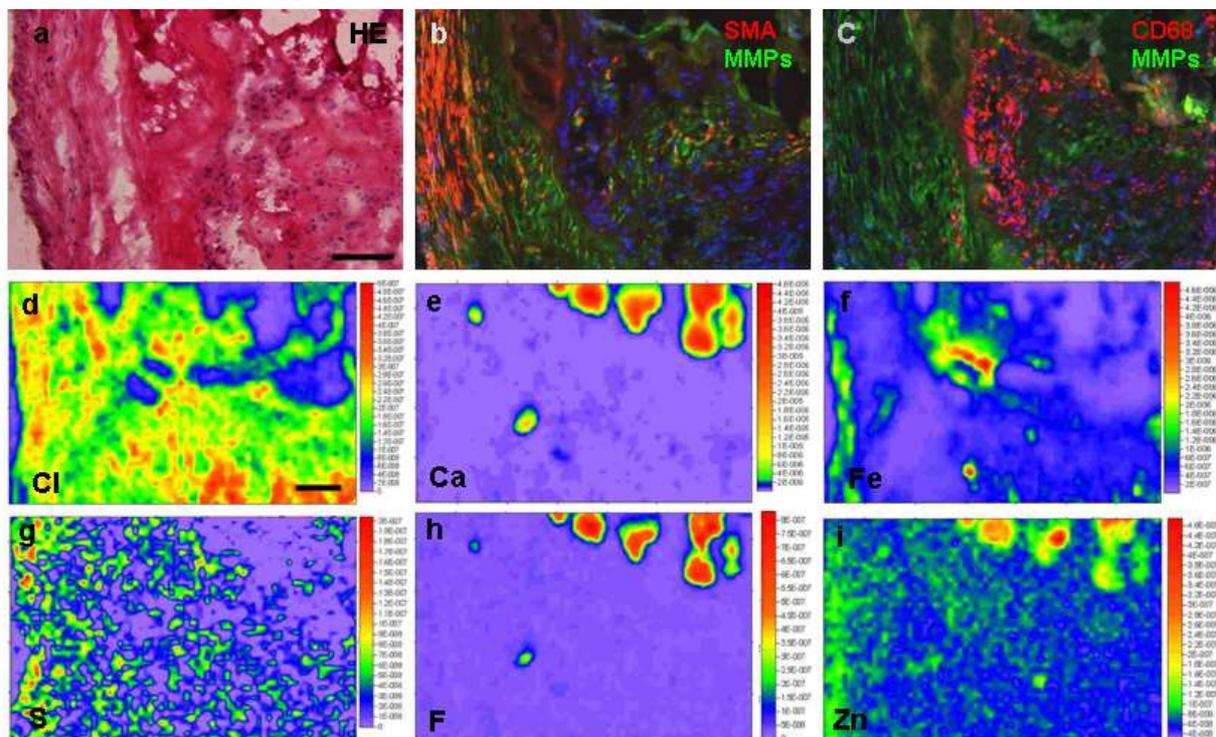


Figure 1: Consecutive sections of aortic active remodelling area of stenotic aortic valve (TAV) stained with HE (a), and double immunostained for smooth muscle actin (b) or CD68 (c) and MMPs. Cell nuclei stained with DAPI (blue). Distributions of selected elements in adjacent section (d-i). Scale bar = 100 μm

References

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