Distribution of iron in pathologically altered and normal human aortic valves studied by microscopy combined with SR-μXRF

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Aortic valve degeneration is an age related process which has become the most common type of valvular heart disease in the Western world, causing significant morbidity and mortality. It often includes ectopic mineralization of the valves, formerly regarded as passive deposition of minerals in pathologically altered areas of a tissue. Recent years brought strong evidence supporting the concept of active mechanisms participating in the pathophysiology of valvular calcification [1]. The exact role of iron in progression of aortic valve degeneration is unclear. The potential mechanisms of iron involvement leading to vessel and valve damage include modification of LDL, induction of lipid peroxidation by macrophages and endothelial cell activation [2,3].

The aim of the present study was to investigate degenerative processes in human aortic valves with the use of complementary histological/histochemical and physical methods. Current analyses are concentrated on the comparison of distribution of iron, calcifications and lipid deposits in human aortic valves.

Two-dimensional maps and line scans of SR-μXRF and μFTIR recordings, acquired from microscopically selected areas of valves, were compared with histological/histochemical stainings on consecutive sections to prove topographical co-localization of the examined elements and compounds. Ten aortic valves (normal, insufficient and stenotic) were included in the study. 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 double immunohistochemical stainings: CD68 (macrophages), tenascin C (TnC) and metalloproteinases (MMPs) activity (in situ zymography). 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 microsopically selected areas of valves (resolution 15 μm, time of acquisition 3 s from each point). The line scans (1D scans) were acquired horizontally or vertically, with the step of 30 μm for 100 s/point. The results were normalized to beam current, thickness of sample and time, and expressed in arbitrary units.

SR-μXRF analysis revealed high concentration of calcium and phosphorus in focal (nodular) deposits present exclusively in stenotic valves (Fig.1). Nodular calcifications were surrounded by a tissue rich in macrophages. TnC immunoreactivity appeared in leaflet areas showing intensified remodeling and in areas of nodular calcification. SR-μXRF analysis revealed in these areas focal accumulations of iron (Fig.1). SR-μXRF 2D maps and linear scans showed that in areas of nodular calcifications calcium- and iron-rich areas were in close apposition, what is in line with the observation of Debernardi et al. [4], who reported calcium and iron co-localization in aortic valve microcalcifications of the apolipoprotein E-deficient (APOE-/-) mice. Close co-localization of calcium and iron was observed in fibrosa (upper layer) of insufficient valves without nodular calcifications, as well as in noncalcified areas of stenotic valves, while in ventricularis (lower layer) of the valves generally much lower calcium content was accompanied by high level of iron (Figs. 1,2) In both calcified stenotic and noncalcified insufficient valves, as well as in normal

valves, areas of higher concentration of calcium, not showing nodular form, were found in fibrosa layer, which also showed slight accumulation of lipids (Fig. 2).
Some data on the role of iron in valve degeneration come from investigations on vascular atherosclerosis. Since there are many similarities between both pathologies, the proposed sources of iron found in atherosclerotic plaques: extravasated erythrocytes entering the tissues directly from the bloodstream and/or from leaking neovessels [5] can also be valid in aortic valve degeneration.

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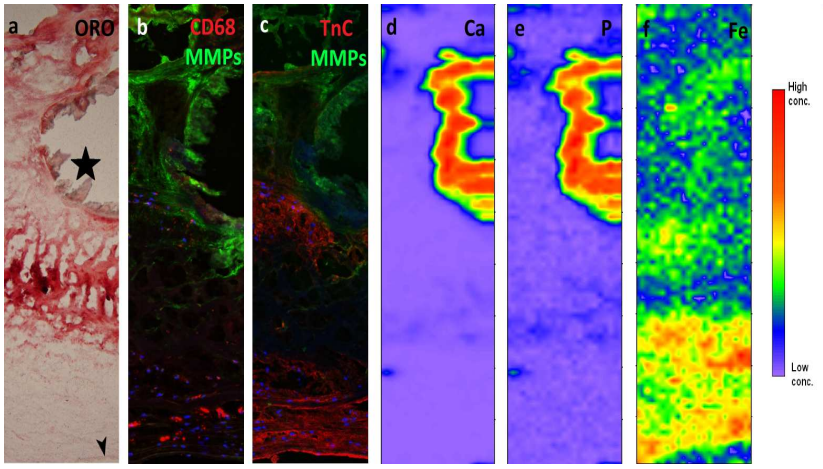

Figure 1: Consecutive sections of stenotic aortic valve imaged by histochemistry, immunofluorescence and SR-µXRF. (a) section stained for lipids (red), arrow: lower surface of valve, asterisk: nodular calcification; (b,c) double staining for MMPs activity (green) and CD68 (red) indicating macrophages (b) or tenascin C (c). Cell nuclei stained with DAPI (blue). (d-f) distributions of Ca,P and Fe by SR-µXRF in section shown in (a). Orig. magn. X 200.

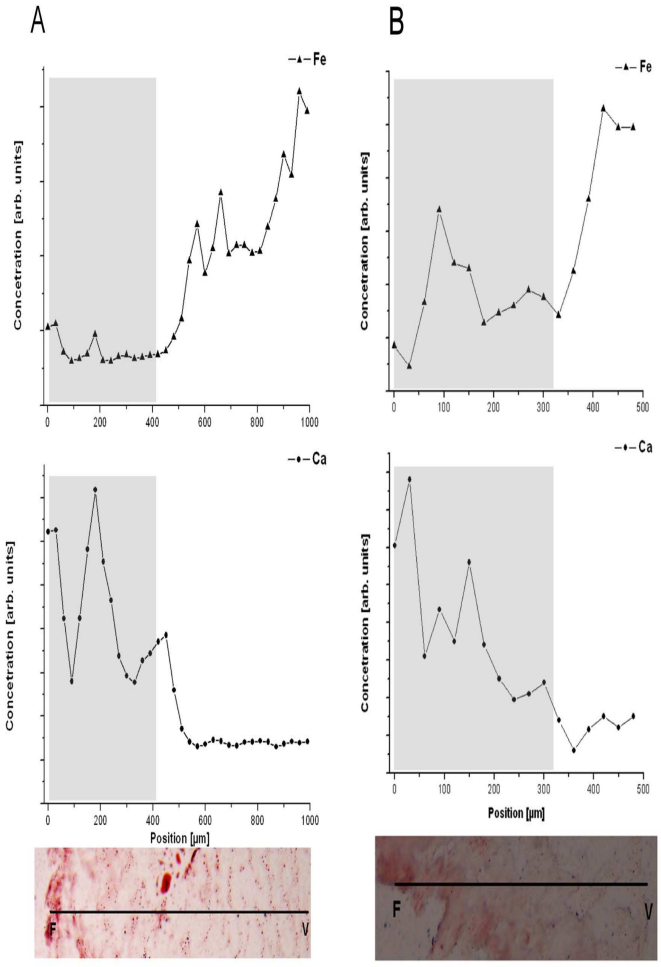

Figure 2: Distribution profiles of calcium and iron across the cusp of insufficient aortic valve (A) and normal aortic valve (B). Microscopic image of ORO stained section (basal segments) shows lipid distribution (red) in SR-µXRF scanned area. The scanning line is marked and corresponds with position (x axis) on the line scans (upper plots). F – fibrosa (upper layer); V – ventricularis (lower layer). The shadowed part of the plots corresponds to fibrosa. Orig. magn. X 200.

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