Degenerative Alterations in RVOT Samples – What to learn from Histopathological Analysis?

Introduction:
Re-obstruction of the RVOT is a common phenomenon in CHD-patients that lacks broader histopathological analysis and understanding. To examine the histological correlation of degenerative alterations, explanted specimen were processed and investigated.

Methods:
Human (homologous and autologous), bovine (pericardium and jugular vein conduits) and other explants (synthetic, equine or porcine) of 42 patients (male: n=27, 64.3%; female: n=15, 35.7%), who underwent re-operation of the RVOT at a median age of 12.5 years [1.9-57.0 years] (median 8.8 years [1.3-49.2 years] after primary surgery), were analysed. Alizarin red S staining was used to analyse calcification degree. Nuclear fast red dye and Image J analysis enabled determination of nuclei per mm². Immunohistochemistry, in detail expression of Tissue Inhibitor of Metalloproteinase 1 (TIMP 1) and Matrix Metalloproteinase 9 (MMP 9) was performed, then examined microscopically.

Macroscopic degeneration:
- Calcification was a major cause for early re-operation
- Severe calcification was encountered in a range of different types of explants
- Human explants showed a lower degree of calcification than bovine tissue and other materials

Calcification in different types of explants – Alizarin red S staining:
- Dark shades represent higher calcified samples in the different examined groups
- All in all, 18 of 42 samples were extremely calcified

Count of nuclei per mm²:
- 10x Nuclear fast red staining displaying nuclei in a bovine explant of a nine-year-old. Nuclei were counted using ImageJ software.

Immunohistochemistry TIMP1 and MMP9 staining:
- Degenerative changes were present in most of the analysed samples
- Severe calcification was encountered in a range of different types of explants leading to early re-operation
- Human explants were on average less calcified than bovine and other materials
- Count of nuclei per mm² was lower in human and bovine samples
- Balance of MMP 9 and TIMP 1 seems to be shifted towards MMP 9

Future Questions and Goals of Research:
- What is the chemical composition of calcification encountered?
- What role do inflammatory cells play in re-obstruction on a histological level?
- How can neo-endothelialisation be characterised?
- How are degenerative alterations linked to clinical data?

Conclusion:
- Calcification was a major cause for early re-operation
- Severe calcification was encountered in a range of different types of explants
- Human explants showed a lower degree of calcification than bovine tissue and other materials

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