The Myocardium In Aortic Stenosis Re-Visited: More Complex Than Just Myocytes And Interstitial Diffuse Fibrosis – Implications For Cardiovascular Magnetic Resonance

Running Title: Tissue characterization in aortic stenosis by histology and cardiac MRI

Patrizia Camelliti, DPhil[1], Janos Kriston-Vizi, PhD[2], Rocío Eiros, MD[3], George D. Thornton, MBBS[4], Konstantinos Savvatis, MD, PhD[4,5], Michael A. Ashworth, MD[6], Begoña Lopez, PhD[3], Arantxa González, PhD[3], James C. Moon, MD[4,5], Thomas A Treibel, MD, PhD[4,5]

1. School of Biosciences and Medicine, University of Surrey, Guildford, UK.
2. Laboratory for Molecular Cell Biology, UCL, UK.
3. Program of Cardiovascular Diseases, CIMA Universidad de Navarra and IdiSNA, Pamplona, Spain. CIBERCV, Instituto de Salud Carlos III, Madrid, Spain.
4. Barts Heart Centre, St Bartholomew's Hospital, London, UK.
5. Institute for Cardiovascular Sciences, University College London, London, UK.
6. Department of Histopathology, Great Ormond Street Hospital for Children National Health Service Trust, London, UK

Correspondence Address:
Dr Thomas Treibel
Barts Heart Centre
St Bartholomew's Hospital
2nd Floor, King George V Block
London EC1A 7BE, United Kingdom
Tel: +44 203 765 6336
Email: Thomas.Treibel.12@ucl.ac.uk

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Non-invasive imaging of myocardial function has limitations as a read-out of myocardial biology and therefore as the basis for cardiomyopathy treatments. Measuring scar by cardiovascular magnetic resonance (CMR) adds value but does not inform about non-scarred myocardium, the key therapeutic target in cardiology. The extracellular volume fraction (ECV%) is an intuitive measure of tissue remodeling and is elevated in edema, amyloid and fibrosis. ECV% and the derived intracellular volume fraction (ICV%) can be adjusted by the myocardial volume to derive total extracellular and intracellular volumes (1). But this two-compartment-approach is reductionist. Leaving aside the intravascular compartment, the ICV% cannot distinguish between different myocardial cell types including myocytes, fibroblasts, endothelial and inflammatory cells. Both compartments are woven, patterned and architected in 3D to generate the myocardium. Histological approaches are similarly reductionist; although correlating with ECV%, 2D-histology has inherent measurement limitations, let alone distinguishing non-myocytes.

Confocal microscopy can measure multiple cell-types with specific dye-tagged antibodies in 3D and build up a multi-parametric measurement of myocytes, non-myocytes and ECM (2). In this study, we combined macroscopic myocardial structure by CMR, whole biopsy collagen volume fraction (CVF%) by 2D light microcopy, and tissue composition by high-resolution confocal microscopy. The study was approved by the ethical committee of UK National Research Ethics Service (07/H0715/101). The study conformed to the principles of the Helsinki Declaration, and all subjects gave written informed consent.

Nine patients with severe aortic stenosis (AS) scheduled for surgical aortic valve replacement (54% male, median age 65 [58-80], indexed aortic valve area 0.4±0.04cm^2) underwent CMR for left ventricular (LV) structure, function and ECV% quantification (3). Two intraoperative myocardial biopsies were obtained for each patient by Trucut needle biopsy from the basal LV septum. One biopsy was fixed in formaldehyde, paraffin

embedded and stained with picrosirus red for CVF% quantification (~5mm^2/sample). The second biopsy was embedded in optimum cutting-temperature compound (Tissue-Tek) and snap frozen (3). Myocytes were labeled with a rabbit anti-dystrophin antibody, non-myocytes with a mouse monoclonal anti-vimentin antibody, and ECM and cell membranes with wheat germ agglutinin-TRITC (2). Sections were imaged with confocal laser-scanning microscopy (4) and analyzed using custom ImageJ plug-ins (~6.5mm^3/sample).

When quantified (Figure 1), in-vivo CMR median ECV% was 28.3% (24.8-32.3%) with ICV% therefore 71.7%. Whole-biopsy 2D-analysis revealed a median CVF% of 11.6% (4.2-25.8%). By confocal microscopy, the equivalent ECM fraction was 16.9% (10.0-23.7%), and the cell volume fraction was split into myocyte volume fraction of 70.8% (59.6-78.4%) and non-myocyte volumes fractions of 13.3% (9.3-16.7%). There was limited correlation (Spearman's) of confocal parameters with only total confocal cellular volume correlating with LGE (rho=0.57, *p=0.048*) and left atrial area (rho=0.74, *p=0.047*); they did not correlate with CVF% (p=0.4) or CMR parameters of LV size (EDV/ESV), LV ejection fraction or ECV%. CVF% correlated with NT-proBNP (rho=0.85, *p=0.003*) and high-sensitivity troponin T (rho =0.78, *p=0.03*), but not ECV%.

To understand myocardial biology, high-dimensional, layered information on structure, function, tissue characterization and biomarkers is needed. CMR captures hypertrophy by quantifying overall mass and then dichotomizing the myocardium into cellular and extracellular compartments. Histology (2D or confocal) identifies different tissues better than CMR, but within a very limited sampling area (confocal<0.007mL / CVF%=0.01mL / CMR>100mL) and only reports ratios of tissue/cell subtypes and their relative expansion. Hence, the lack of association between in-CMR and histology highlights that they ultimately offer complementary insights at vastly different dimensions. Whilst the approaches have limitations related to sampling (scalability/size/location), process

(vasodilatation/intra-myocardial an blood pool not captured) and staining, this represents an advance in visualization and combined insight. For the clinical and especially the imaging cardiologist, our data also serves as a reminder of the importance of non-myocytes (especially endothelial cells and fibroblasts), which despite of being much smaller than cardiomyocytes are more numerous (5), and occupy 10-15% of the left ventricular myocardium.

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Figure Legend

Figure 1: Cardiac MRI and Confocal microscopy images of the left ventricular myocardium
Cardiac MRI images: cine still of the mid-ventricular short-axis showing left ventricular hypertrophy and pericardial effusion suggesting decompensation (A); matching ECV map showing patchy ECV% elevation (green) amid normal (blue) myocardium (B). Whole-biopsy stained by picrosirus red (right). Confocal microscopy images (D-G): Myocytes labeled with dystrophin (D; green), non-myocytes with vimentin (E; blue); extracellular matrix with wheat-germ agglutinin (F; red), finally the merged image (G).