

Whole mount heart histology: a new gold standard for myocardial damage validation in experimental cardiac MRI studies?

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Introduction: Triphenyltetrazolium chloride (TTC) stain is commonly used for the validation of myocardial damage in experimental cardiac MRI studies using various animal species such as mice, dogs and pigs [1-3]. However, subtle myocardial damage, border zone or infarct heterogeneity associated with myocardial infarction (MI) is difficult to recognize on TTC stains. Although targeted microscopic histology staining is usually sufficient to demonstrate such clinically significant damage, the associated small piece of targeted tissue on the slide makes direct comparison with the stacks of images obtained with MRI difficult. In this study, we investigated the feasibility of whole-mount heart histology that preserved the 3D morphology with a digital display at the microscopic level as a new alternative in the validation of myocardial damage in a porcine model of MI in experimental cardiac late-enhancement (LE) MRI studies.

Materials and Methods: In eleven Yorkshire pigs (25-50 kg) myocardial infarction was created by a 90-minute percutaneous balloon occlusion of the left anterior descending coronary artery (LAD, n=6) or of the mid- to distal left circumflex coronary artery (LCX, n=5). After reperfusion and full recovery from anesthesia, animals were allowed to survive for three to six weeks. MR studies were conducted on a GE 1.5T Signa Excite system. Pre-contrast MRI included a steady-state free precession (SSFP) LV function study. LE- MRI was performed 10-20 minutes after double-dose bolus injection of Gd-DTPA. Both IR-FGRE and multi-contrast late-enhancement (MCLE) acquisitions in short-axis oblique slices (SAO, slice thickness of 5 mm without gaps) and/or two- or four-chamber views were obtained. For IR-FGRE, the TI varied from 200 to 300 ms, depending on the null point of healthy myocardium. For MCLE, a segmented SSFP readout is used following an inversion pulse, providing 20 cardiac-phase-resolved images at varying effective TIs [4]. The in-plane resolution was 1mm x 1mm and through-plane resolution was 5 mm for both IR-FGRE and MCLE. Upon the completion of MRI examinations all animals were sacrificed and the heart was removed. The heart was inserted in a box and filled with an inert quick-setting polymer (Histomer, Histotech). Then 4-5 mm thick whole-mount SAO heart slices based on LE-MRI images with 4-5 mm thickness were cut and kept in a 10% neutral-buffered formalin solution for extended fixation, followed by a long paraffin processing to allow extended paraffin infiltration [5]. Sections with 4-micron thickness were stained using haematoxylin and eosin (H&E) and/or Masson trichrome methods; these slides were then digitally scanned at a resolution of two to ten microns for further analysis (Figure.1).

Results: Macroscopic examination of the excised heart and gross whole-mount histology confirmed the presence of LV-MI in all animals, right ventricular (RV) involvement in 6 LAD-MI animals and papillary muscle (PM) involvement in 5 LCX-MI animals. The gross appearance of chronic MI was gray-white scarring in LV, RV and PM with increased collagen deposition in histology. Gross whole-mount histopathology slides were among the best for showing the scar tissue and the heterogeneity within the infarct (Figure.1). Subtle myocardial findings included a long rim of thinned healthy myocardium located in the endo- or epi-myocardial layers and bundles of finger-like cardiac myofibers extending into the scar tissue. Remodeled vessels can also be visualized in the infarction. Although these subtle histological findings cannot be identified easily from visual inspection of original LE-MRI images using either technique due to the limited spatial resolution, gray-zone mapping based on MCLE imaging can be used to show intermediate contrast between viable myocardium and scar tissue which corresponds well to the border zone consisting of bundles of finger-like myocardial fibers extending into the scar tissue in microscopic histology. This provides a means to directly correlate gray-zone measures with infarct heterogeneity. 3D reconstruction of whole-mount heart histology slides is planned. A detailed 3D display of heart structure at macro- and microscopic resolution level may provide a new gold standard in experimental cardiac MRI studies.

Conclusions: Whole-mount heart histology is feasible in the validation of myocardial damage in experimental cardiac MRI studies. MCLE with higher resolution may be needed to further explore the spatial structure of infarct heterogeneity.

References: 1. Ojha N, et al. *AJP* 2008; 294: H2435-H2443. 2. Saeed M, et al. *Radiology* 2008; 249: 560-571. 3. Yang Y, et al. *JMRI* 2007; 26: 1486-1492. 4. Detsky JS, et al. *MRM* 2007; 58:365. 5. Clarke GM. *Histopathology* 2007; 50:232-242.

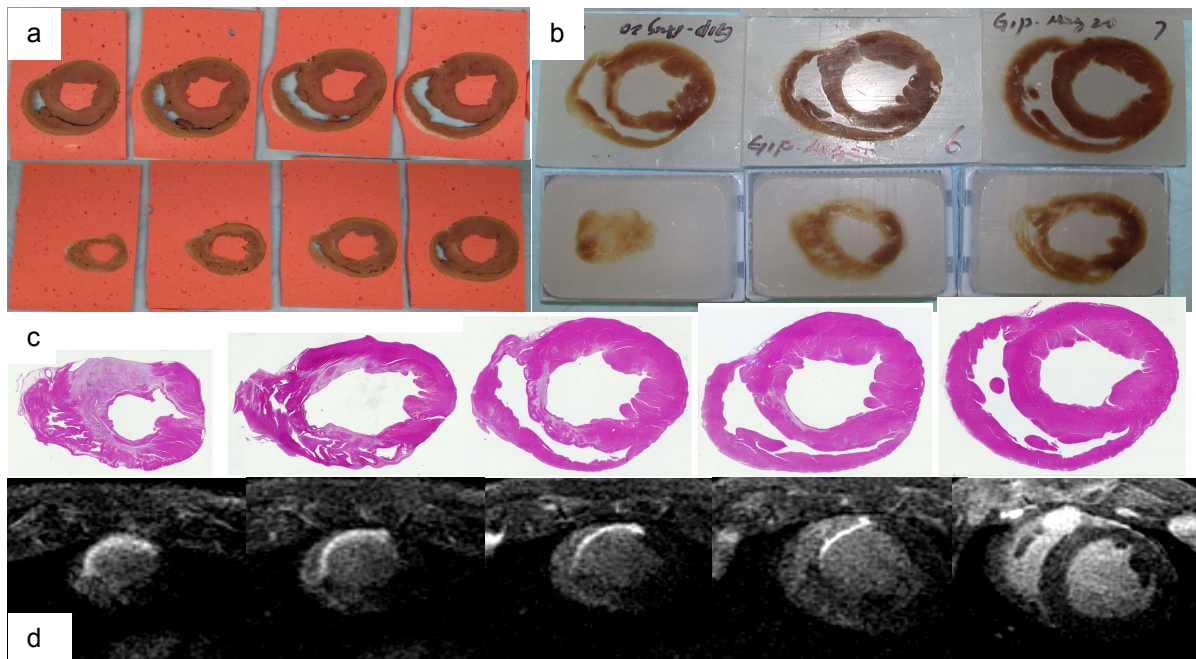


Figure.1 (a) Whole-mount heart histology in a LAD-MI pig. Fig.1a. Heart blocks cut with a Histomer setting. (b) Paraffin-embedded heart blocks. (c) Whole-mount heart slides with an H&E stain. (d) MCLE MRI images corresponding to histology sections in (c).