

## Myocardial T<sub>1</sub> Mapping in Swine with Non-ischemic Heart Failure with Comparison to Changes in Specific Collagen Types

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**Introduction:** Dilated cardiomyopathy is the most common type of non-ischemic heart failure, with persistent tachycardia being a common cause [1-2]. The degree of myocardial fibrosis is thought to play a critical role in determining the risk of sudden death, but the changes in the collagen types relating to fibrosis are not fully understood. Cardiac T<sub>1</sub> mapping MRI can enable signal quantification on a standard scale [3-5]. The purpose of this study was to investigate a free-breathing pulse sequence to quantify myocardial T<sub>1</sub> changes in tachycardia-induced heart failure swine model and to assess changes in collagen.

**Methods:** After obtaining IACUC approval, Yorkshire swine (N=9) were implanted with pacemakers and paced for 3 to 5 weeks at 200 beat per minute. Each animal was scanned in a 1.5 T MRI scanner at baseline (non-paced pacemaker in situ) and then at heart failure (N=6) (confirmed by echocardiography).

For the T<sub>1</sub> Mapping, we used a modified look-locker with saturation recovery SSFP sequence [6] (TE/TR 1.9/4.3ms, 45° FA, 256x160, 3 NEX, 20 VPS, 8mm slice, with 1-2 min free breathing) with three Look-Locker imaging blocks (2, 2, and 6 heartbeats respectively). The sequences starts with an initial TI of 50 ms, then adds TI increments of 40 ms if the heart rate is 100 bpm (RR interval is 600ms), thus acquiring ten TIs of 50, 90, 130, 160, 190, 220, 250, 280, and 310ms (50, 50+40, 50+2x40, 50+RR, 50+40+RR, 50+40x2 +RR, 50+40x2 +2xRR, 50+40x2 +3xRR, 50+40x2 +4xRR, 50+40x2 +5xRR). The images are then post-processed by home developed software.

Tissue was collected from the septum and the left ventricular free wall and placed in 10% formalin to fix. Tissue was embedded in paraffin using standard methods. For paraffin immunochemistry (IHC-P), tissue slides were incubated with primary antibodies (Collagen I Antibody, (Abcam, Cambridge, MA), 1:100; Collagen III Antibody, (Abcam, Cambridge, MA), 1:100; Collagen VI Antibody, (Abcam, Cambridge, MA) 1:100), for 48 hours at 4 degrees Celsius. The tissue was washed in PBS and incubated with secondary antibodies (Alex Fluor Goat Anti Mouse IgG and/or Alex Fluor Goat Anti Rabbit IgG, (Invitrogen, Carlsbad, CA), 1:100) for 2 hours at room temperature, and finally stained with DAPI (DAPI Nucleic Acid Stain, (Invitrogen, Carlsbad, CA) 1:1000) for 2 minutes. The images were then photographed using a Nikon Eclipse 80i microscope with digital DXM 12000c camera and analyzed using Nikon NIS Elements SW, vs. 3.1 (Nikon Instruments Inc., Melville, New York). Descriptive statistics performed using SPSS Statistical Software, v 16, (SPSS Inc., Chicago, IL).

**Results:** Baseline ejection fractions were 45 ± 4%; and for the heart failure group, 14 ± 7%. The mean T<sub>1</sub> value for the pre-contrast T<sub>1</sub> mapping sequence was 960 ± 96 ms at baseline; and for the heart failure group, 726 ± 94 ms, (Paired t-test, N= 6, p = .020). The 5 min post contrast T<sub>1</sub> value for the baseline pigs was 546 ± 180 ms; and 300 ± 171 ms, for the heart failure group (p = .005). Sample data for the immunochemistry is currently insufficient to make statistical inference; however, visual observation suggests increases in all collagen in the heart failure animals (Figures 2 and 3).

Figure 1

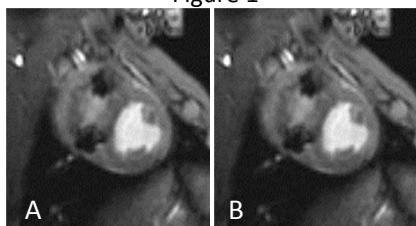


Figure 2

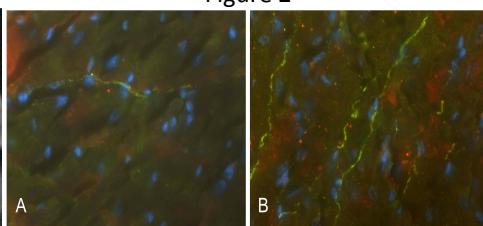
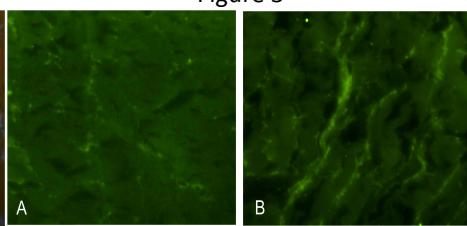


Figure 3



**Figure 1.** 2D Cine SSFP End Systolic Images at baseline (A), and heart failure (B).

**Figure 2.** IHC-P of fibrillar collagens I and III, stained with Primary Col-I Antibody, Col-III Antibody, Abcam, 1:100, and Secondary Alex Fluor Goat Anti Mouse IgG and Alex Fluor Goat Anti Rabbit IgG, Invitrogen, 1:100. 60 X Power. A = control, B = failure myocardium.

**Figure 3.** IHC-P of collagen VI, stained with Primary Col-VI Antibody, Abcam, 1:100, and Secondary Alex Fluor Goat Anti Mouse IgG Invitrogen, 1:100, 60 X Power. A is control, B is failure myocardium.

**Discussion:** This study demonstrates that T<sub>1</sub> mapping may be a promising technique to quantify myocardial changes in heart failure even when the subject cannot hold their breath. Many patients are too ill to hold their breath for cardiac scanning, or they may be sedated and therefore unable to follow breath holding commands. The immunochemistry results are too preliminary to make statistical conclusions, but visually there appears to be a change in the amount of Type I, III and VI collagen in the myocardium between the control and heart failure swine. More research needs to be done to further establish the relationship between T<sub>1</sub> mapping values and collagen changes.

References: 1. Grimm W, Alter P, Maisch B. Herz 2004;29(3):348-352. 2. Peters KG, Kienzle MG. Am J Med 1988;85(2):242-244. 3. Messroghli DR, Greiser A, Frohlich M, et al. JMRI 2007;26:1081-1086. 4. Messroghli DR, Walters K, Plein S, et al. Magn Reson Med. 2007 Jul;58(1):34-40. 5. Iles L, et al. J Am Coll Cardiol 2008;52:1574-1580. 4. Song T, et al, ISMRM Annual Scientific Meeting 2009, pp483.