Multiexponential T2 Analyses in a Murine Model of Hepatic Fibrosis at 11.7T MRI

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Purpose: The purpose of this study was to characterize the multiexponential T_2 relaxation of liver using a murine model of hepatic fibrosis in a highly controlled setting using *ex vivo* imaging at 11.7T MRI.

Methods:
Imaging experiments were performed using 11.7T MRI. 17 male C57BL/6 mice were divided into a control group (n=2) fed normal diet and an experimental group (n=15) fed a diet containing 3, 5-dicarbethoxy-1, 4-dihydrocollidine (DDC) to induce hepatic fibrosis. The experimental diet was continued for a total duration of 16 weeks and mice were sacrificed intermittently throughout this period for imaging. During imaging, formalin fixed *ex vivo* liver specimens were placed in phosphate buffered saline (PBS) and temperature was controlled at 23.5°C. A multi-echo (echoes=48, TE_1=6.4ms, echo spacing=6.4ms, TR=4000ms) spin-echo sequence was utilized for constructing parametric T_2 maps. The following geometric parameters were utilized: voxel dimensions = 150x150x700µm3 (reconstructed pixel size = 75x75µm) and matrix size = 100x100.

Two methods of multiexponential T_2 analysis were utilized: a constrained regularization method (CONTIN) [1] and a regularized non-negative least squares method (AnalyzeNNLS) [2]. Using the CONTIN algorithm, the geometric mean T_2 values of the individual peaks, the full width half maximum (FWHM) values for the peaks and the area fractions of the peaks were recorded. Using the AnalyzeNNLS algorithm, geometric mean T_2 values of the individual peaks were recorded as well as their area fractions. Peaks with area fractions less than 1% were excluded from analysis. Representative regions of interest (ROIs) on the directly acquired images were used as input to the multiexponential T_2 analysis algorithms.

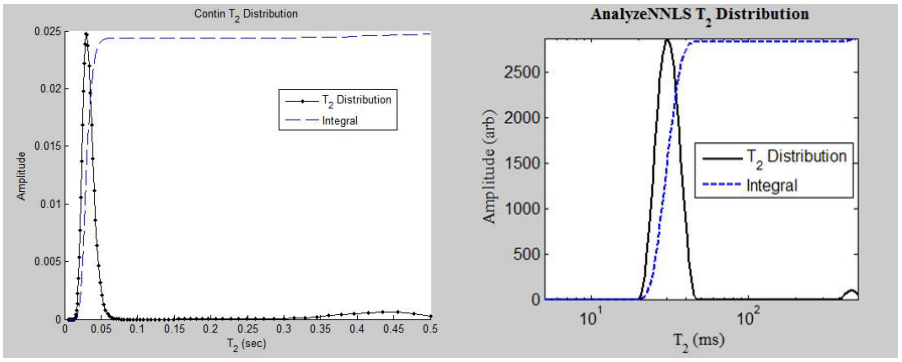

Figure 1. T_2 distributions of a murine liver specimen using CONTIN (left) and AnalyzeNNLS (right) algorithms demonstrate dominant, short T_2 peak (~40ms this example) and minor, long T_2 peak (~400ms this example).

Subsequent to the imaging experiments, the liver specimens were embedded in paraffin and serial sections of 5µm were cut. Sections were stained with hematoxylin and eosin as well as Masson's trichrome stains. A board certified pathologist reviewed the specimens to evaluate the extent of steatosis, inflammation, and fibrosis using commonly employed grading schemes. In addition to the subjective scoring, the trichrome stained specimens were digitized and digital image analysis (DIA) was used to determine the degrees of hepatic fibrosis, expressed as percentage area of fibrosis of the liver specimens.

The geometric mean T_2 (GMT2) values and FWHM values for the CONTIN results were plotted against both subjective assessments of hepatic fibrosis, steatosis, and inflammation as well as digital image analysis derived percentage area of fibrosis. Geometric mean T_2 (GMT2) values generated using the AnalyzeNNLS algorithm were similarly compared to histology. Pearson correlation coefficients (R) were derived using linear regression analyses.

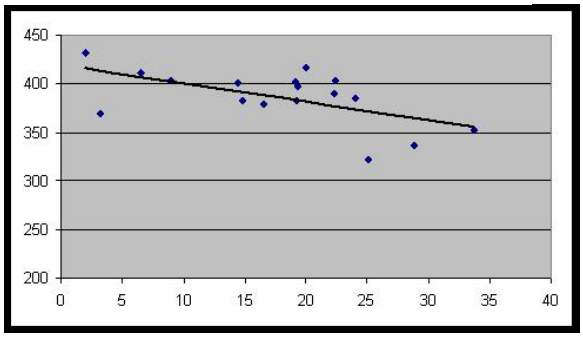

Figure 2. Scatterplot comparison of CONTIN-calculated T2 values (y-axis, ms) versus DIA assessment of percentage fibrosis area (x-axis) reveals a moderate correlation.

Results: The DDC diet induced significant hepatic fibrosis in the experimental group of mice, none of which died prior to planned sacrifice. The degrees of fibrosis assessed by the pathologist ranged up to the maximum score of 4 and DIA based percentage area of fibrosis ranged up to 34%. Minimal steatosis was seen; 4 of 15 experimental mice demonstrated any degree of steatosis greater than a grade of 0. Inflammation ranged from mild to severe in all experimental mice.

When applying both CONTIN and AnalyzeNNLS algorithms, in all cases, two distinct peaks were identified, termed here short and long T_2 components (Figure 1). Using the CONTIN algorithm, the dominant, short T_2 component had a mean GMT2 of 30.2ms (range, 24.5-35.3ms) and mean area fraction of 97.4% (range 95.1-98.9%). Using the AnalyzeNNLS algorithm, the short T2 component had a mean GMT2 of 30.6ms (range, 24.8-35.8ms) and mean area fraction of 97.3% (range, 94.9-98.8%). Using the CONTIN algorithm, the minor, long T_2 component had a mean GMT2 of 385.9ms (range, 321.5-431.3ms) and mean area fraction of 2.62% (range 1.13-4.94%). Using the AnalyzeNNLS algorithm, the minor, long T_2 component had a mean GMT2 of 434.2ms (range, 332.4-482.0ms) and mean area fraction of 3.09% (range 1.22-9.12%). Using the CONTIN algorithm, the mean FWHM of the dominant, short T_2 peak was 16.2ms (range, 11.6-19.6ms).

Using both CONTIN and AnalyzeNNLS, poor correlation between geometric means of the short T_2 components and subjective, pathologist scored and DIA derived degrees of hepatic fibrosis. Using CONTIN, moderate correlation between geometric means of the long T_2 components and subjective, pathologist scored (R=0.50) and DIA derived (R=0.58) degrees of hepatic fibrosis were seen (Figure 2). Using AnalyzeNNLS, poor correlation between the geometric means of the short components degrees of fibrosis were seen. Fair correlation was seen using AnalyzeNNLS between long T_2 components for subjective (R=0.34) and DIA (R=0.28) derived degrees of fibrosis. Increasing degrees of hepatic fibrosis were seen to result in an increase in FWHM values for the CONTIN algorithm. Based on scatterplot comparisons, fair correlation between FWHM and the pathologist-determined fibrosis score (R=0.39) and the percentage area of fibrosis by DIA (R=0.44) were seen. Poor correlations between degrees of hepatic steatosis or inflammation and the multiexponential T_2 derived parameters from either algorithm were found.

Conclusion: Two distinct T_2 components were seen in the murine liver samples; both geometric mean T_2 values of the minor, long T_2 component and the FWHM of the short T_2 component correlated with hepatic fibrosis. Studying hepatic microenvironments using multiexponential T2 analyses offers potential utility in the ongoing development of noninvasive assessments of hepatic fibrosis using MRI.

References: 1. Bjarnason TA, et al. J Magn Reson. 2010; 206(2):200-204. **2.** Provencher SW. Commun. Comput. Phys. 1982; 27:213-227.