MR elastography of non-alcoholic steatohepatitis in the rat

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Introduction

Non-alcoholic steatohepatitis (NASH) has an increasing worldwide prevalence. It is characterized by liver steatosis and inflammation leading to fibrosis, cirrhosis and hepatocellular carcinoma [1-3]. Elastography techniques [4, 5] have emerged as accurate methods to diagnose and quantify liver fibrosis. It has been shown that the elasticity of the liver increases with increasing degree of fibrosis. The aim of our study was to assess the role of MR elastography in staging NASH and to evaluate which parameters influence the visco-elastic measurements in this model of chronic liver disease.

Material and methods

Animals: Twenty-three Sprague-Dawley rats were fed a choline-deficient diet during two weeks (N=7), five weeks (N=8) and eight weeks (N=8) in order to induce NASH [6]. A group of five healthy rats was used as controls.

MR elastography: The rats were placed under anesthesia in prone position on a piezoelectric based driver which generated mechanical waves at 300 Hz. Images were obtained on a 7 T Bruker Pharmascan animal imager. The propagation of the waves was imaged with a modified spin-echo sequence as described in [7]. Eight transverse slices through the liver were acquired. The field of view was 53 mm, matrix size 64^2 leading to an isotropic voxel size of 830×830×830 μm^3. The sinusoidal motion was sampled with four dynamics. The full 3D displacement field was imaged inside the liver in the stationary wave regime (Fig. 1d, 1e, 1f) and used to calculate the elasticity (dynamic modulus Gd) and the viscosity (loss modulus Gl) (Fig. 1b, 1c) through a full inversion of the wave propagation problem. Respiratory motion was triggered via a sensor monitoring the abdominal motion.

Histological analysis: After imaging, the rats were killed and the livers were fixed in paraffin for histological analysis. The percentage of fibrosis and steatosis was determined with quantitative morphometry. Hepatic inflammation was scored according to Steiner [8]. Activation of the stellate cells secreting collagen was assessed with immunohistochemistry of alpha smooth muscle actin (aSMA).

Statistical analysis: The comparison between groups was performed with the non-parametric Kruskall Wallis test, followed by two by two comparisons with the Wilcoxon rank-sum test. The Spearman method was used for the correlations.

Results and Discussion

The elasticity (Gd) and viscosity (Gl) increased significantly after two-week diet and increased further at five weeks ($P < 0.05$). The visco-elastic parameters correlated significantly with the percentage of fibrosis, the score of inflammation and the score of aSMA (Gd and Gl versus fibrosis: $P < 0.001$, $r = 0.75$, and $P < 0.001$, $r = 0.75$, respectively, Gd and Gl versus inflammation: $P < 0.001$, $r = 0.64$, and $P = 0.001$, $r = 0.61$, respectively, Gd and Gl versus aSMA: $P < 0.001$, $r = 0.76$, and $P < 0.001$, $r = 0.77$, respectively).

At histopathology, severe hepatic steatosis was observed at two weeks ($P = 0.004$) and decreased at eight weeks ($P = 0.015$). Fibrosis appeared later, at five weeks ($P < 0.001$) and remained stable. The score of inflammation already increased significantly at two weeks ($P = 0{,}001$) as did the number of activated stellate cells, positive for aSMA ($P = 0{,}019$) (Fig. 2).

The results of the current study show that in NASH the visco-elastic parameters increase according to the degree of liver fibrosis. This is in accordance with previous studies [9]. In our study, however, the visco-elastic parameters already increased significantly after two-week diet, before fibrosis appeared. This increase might be explained by the significant increase of inflammation and stellate cell activation occurring at two weeks. This is in agreement with recent studies showing that inflammation and edema may increase the stiffness of the liver [10, 11].

Our results in the rat suggest that NASH could be detected by an increase of the visco-elastic parameters at MR elastography before fibrosis develops. This point should be further assessed in patients.

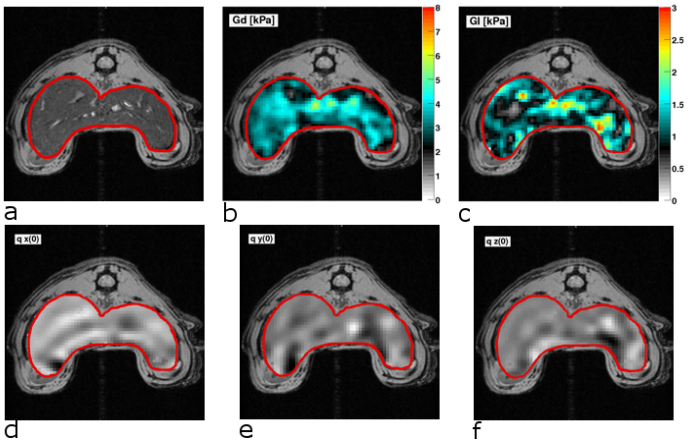

Figure 1: ROI (red line) delineating the liver on the anatomical slice (a), the Gd - Gl maps (b and c respectively) and the displacement q in the x- y- and z-directions (d, e and f respectively). These images show good penetration and propagation of the waves through the whole liver.

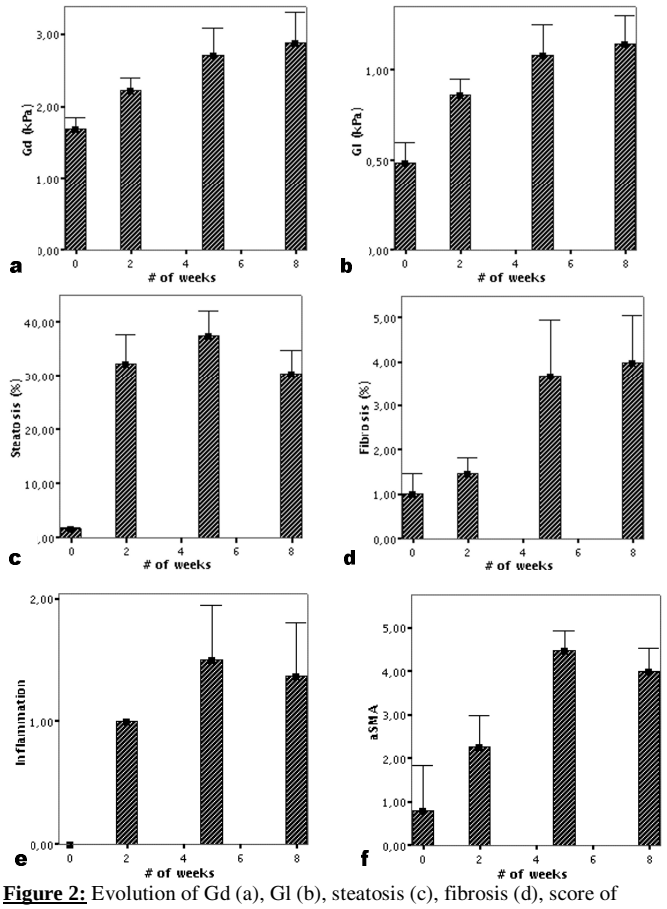

Figure 2: Evolution of Gd (a), Gl (b), steatosis (c), fibrosis (d), score of inflammation (e) and stellate cell activation (aSMA) (f) according to the number of weeks. All the parameters increased already after two weeks except for the percentage of fibrosis.

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