

# Multifrequency MRE of human liver specimen: Sensitivity of viscoelastic powerlaw constants to the collagen matrix in hepatic fibrosis

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**Background:** Elastography has been established for the clinical assessment of liver fibrosis. Particularly MR elastography (MRE) has become the most accurate noninvasive modality for staging hepatic fibrosis [1]. MRE uses shear vibrations visualized by MRI and analyzed by sophisticated inversion methods for generating maps of the distribution of viscoelastic parameters in the tissue [2]. Multifrequency MRE (MMRE) [3] is capable of analyzing the viscoelastic scaling behavior of the tissue leading to insight into the hierarchical arrangement of mechanical networks in biological tissue.

**Problem:** Despite the success of elastography in the clinical diagnosis, little is known about the relationship between macroscopic viscoelastic constants and pathophysiological mechanisms in the liver. Hepatic inflammation and steatosis [4] in interaction with blood flow conditions can influence the global mechanical response of the liver and impact the diagnostic power of elastography for staging hepatic fibrosis.

**Objective:** To measure structural, biochemical and functional parameters of human liver with different degrees of fibrosis, inflammation and steatosis and to correlate these pathologies to the tissue biomechanical properties revealed by ex-vivo wide-range MMRE and by static indentation experiments.

**Methods:** 16 patients with malignant hepatic lesions or liver cirrhosis assigned to the Department of Transplantation Surgery of our institution were included in this study. 10 patients were subjected to pre-surgery LiMax-liver function tests [5]. Immediately after resection, a cylinder of approximately 3.5 cm diameter and 2 cm thickness was cut from the resected tissue similar to what is displayed in Figure 1 for a bovine liver sample. For human tissue, scores of inflammation, fibrosis and steatosis were determined by histology. Furthermore, collagen type-I and type-III were quantified by Silver staining. MRE was applied in a large dynamic range from 200 to 1200 Hz as described in [6]. From multifrequency complex modulus data, viscoelastic constants  $\mu$  and  $\alpha$  were determined according to the springpot model. While  $\mu$  is related to stiffness,  $\alpha$  is a powerlaw exponent indicating changes in the geometrical arrangement of the viscoelastic lattice at multiple scales. Three samples could not be investigated by MRE due to hypointense signals. For testing static mechanical tissue properties, a hole of  $\varnothing$ 5 mm was drilled into the sample container's wall in order to allow the indenter tip ( $\varnothing$ 3 mm) stimulating the tissue by a linear loading ramp of 1 mm amplitude and 20 s period. 4 loading cycles were used for preconditioning. The stress-strain data were fitted by linear (ind.1) and quadratic (ind.2) functions. Sample #15 was too small (thickness < 1cm) for reliable static tests. After mechanical testing, all samples were analyzed for their amount of hydroxyprolin which is an established marker of collagen.

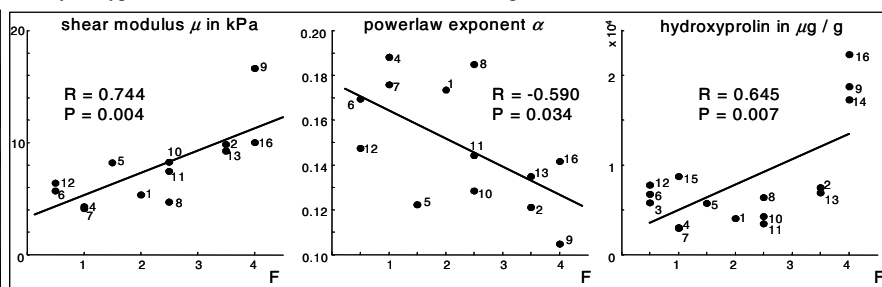
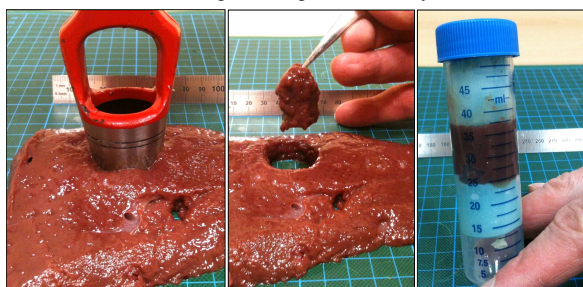


Fig.1: Sample preparation (cutting and placing in container).

Fig.2: The three parameters of our study which were correlated to the degree of hepatic fibrosis (F).

**Results:** Histologically proven scores of fibrosis (F), inflammation (inflam.) and collagen-type I (colla-I) are given in the table. In all samples, only insignificant amounts of collagen-type III could be detected by staining. Steatosis was <5% in all samples except in #13 which had a significant higher content of fat of >50%. LiMax liver function neither correlated with any histological parameter nor with the amount of hydroxyprolin (hprol. given in mg per g liver tissue). Also static indentation was not significantly correlated with histology although a trend (P = 0.062) of ind.2 with F was discernible. Elasticity  $\mu$  measured by MRE significantly correlated with F as shown in Fig.2 similarly to hydroxyprolin while  $\alpha$  decreased with F. There was no correlation between mechanical constants with inflam. or colla-I while  $\mu$  correlated well with hprol (P = 0.003).

**Discussion:** Our main findings can be summarized as follows:

- 1) In our limited cohort, global liver function was not correlated to inflammation and structural parameters such as the amount of collagen of the liver.
- 2) The degree of fibrosis is the major contributor to hepatic stiffness. The correlation of elasticity and F increases with higher excitation frequencies.
- 3) Probably for this reason, our static mechanical constants were less sensitive to F than  $\mu$ . Furthermore, it has to be mentioned that static indentation experiments are highly sensitive to sample geometry, boundary conditions and loading conditions. Therefore, a dynamic bulk method like MRE appears to be more suitable than static methods for mechanical testing soft tissues with irregular shape.
- 4) The decay of  $\alpha$  with F indicates an alteration of the geometry of tissue architecture with an increasing amount of collagen. Given a correlation between  $\alpha$  and mechanical network density, fibrogenesis causes substitution of the healthy hepatic network by fewer but mechanically firmer network elements.
- 5) Albeit less sensitive than  $\mu$ ,  $\alpha$  may be used in further studies as an additional diagnostic parameter for hepatic fibrosis.
- 6) The fact that compared to  $\mu$ , the total amount of hprol was less sensitive to F indicates the contribution of intra-collagen crosslinks to the effective stiffness.
- 7) The insignificant amount of hepatic fat in our cohort prevents further conclusions about the influence of steatosis to MRE parameters.

sample #	F	inflam.	colla-I	LiMax $\mu$ g/kg/h	hprol. mg/g	ind.1 kPa	ind.2 kPa	$\mu$ kPa	$\alpha$
1	2.0	1.0	1.0	NaN	4.0	2.1	31.7	5.3	0.17
2	3.5	1.0	1.5	460	7.5	6.7	101.6	9.9	0.12
3	0.5	0.5	1.5	NaN	5.8	0.9	21.6	NaN	NaN
4	1.0	0.5	1.5	NaN	3.0	2.6	25.3	4.3	0.19
5	1.5	1.0	1.5	321	5.8	3.0	54.4	8.2	0.12
6	0.5	0.5	1.5	NaN	6.8	0.8	20.2	5.7	0.17
7	1.0	1.0	1.0	NaN	3.0	0.8	9.2	4.1	0.18
8	2.5	2.0	2.0	476	6.4	0.8	10.5	4.7	0.19
9	4.0	1.5	1.5	249	18.7	3.4	57.0	16.7	0.10
10	2.5	1.5	1.5	259	4.3	1.8	34.4	8.3	0.13
11	2.5	1.5	1.0	307	3.5	0.7	8.2	7.5	0.14
12	0.5	0.5	2.0	270	7.8	1.4	28.3	6.4	0.15
13	3.5	1.0	2.0	304	6.9	1.2	19.9	9.3	0.13
14	4.0	2.0	1.0	74	17.3	4.0	100.7	NaN	NaN
15	1.0	1.0	1.5	219	8.8	NaN	NaN	NaN	NaN
16	4.0	2.0	NaN	NaN	22.3	1.3	23.8	10.0	0.14
bovine	NaN	NaN	NaN	NaN	4.1	0.2	5.0	4.7	0.16
mean	2.2	1.2	1.5	294	8.0	2.0	34.5	7.5	0.15
SD	1.3	0.5	0.4	115	5.8	1.6	29.9	3.4	0.03

**Limitations:** Our study is limited by the low number of samples, incomplete LiMax data and uncertain boundary conditions during indentation tests.

**Conclusion:** The increase of collagen in the liver due to fibrogenesis provides sensitivity of elastography for staging hepatic fibrosis. Springpot constants measured by multifrequency MRE provide insight into the transformation of the hierarchic mechanical system of the liver during fibrogenesis.

**Literature:** [1] Bonekamp J Hepatol 2009;50:17-35. [2] Muthupillai Nature Med 1996;2:601-603. [3] Asbach Radiology 2010;257:80-86. [4] Salameh, Radiology 2009;253:90-97 [5] Stockmann HPB (Oxford) 2010;12:139-146. [6] Riek J Biomech 2011;44(7):1380-1386