Prospective evaluation of liver steatosis comparing stereological point-counting biopsy analysis and 1H MRS

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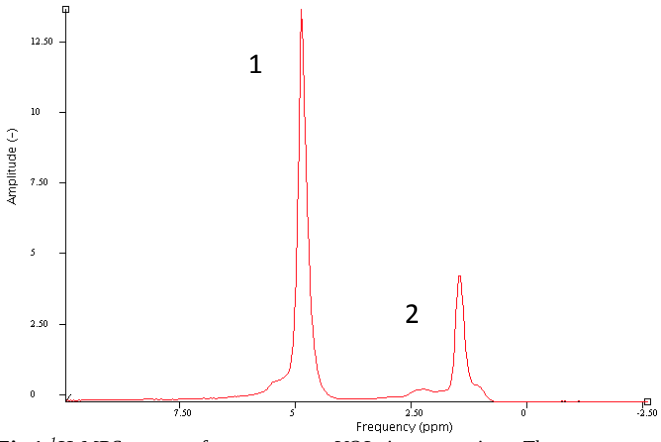

Fig.1 *[1]H MRS, mean of two separate VOIs in one patient. The resonances correspond to 1) water (6.0-3.3 ppm) and 2) triglycerides (2.7-0.4 ppm). Estimated HTCG in this patient was 20.85%.*

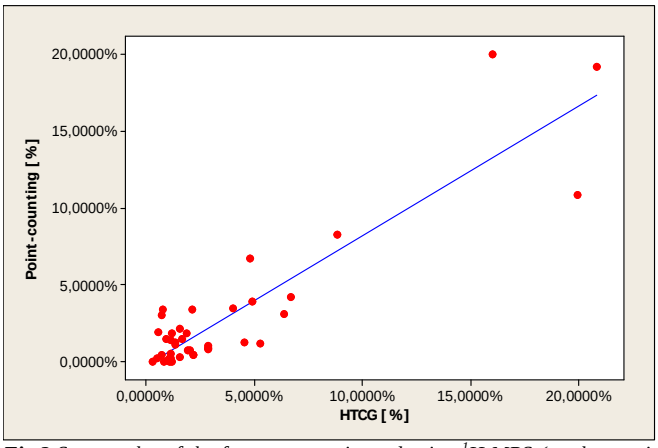

Fig.2 *Scatter plot of the fat content estimated using [1]H MRS (on the x-axis) and the biopsy determined fraction (y-axis). y=-0.00228 + 0.842x, r^2=0.816*

Introduction: Steatosis is a commonly measured attribute in liver biopsies both in clinical and experimental studies; the fat accumulation of a patient is often of interest in diffuse liver disease diagnosis. Stereological point-counting of the fat content in biopsies has been proposed as the preeminent quantitative measure [1]. Moreover ^{1}H magnetic resonance spectroscopy (MRS) is successful in estimating the fat content non-invasively [2]. The aim of this study was to prospectively compare fat estimation in the liver using stereological point-counting in biopsies and quantitative ^{1}H MRS.

Materials and Methods: In this prospective study 41 asymptomatic patients were studied between 2008 and 2010. The patient group consisted of 22 men (median age: 43, range: 20-66 years) and 19 females (median age: 59, range: 22-77 years). They were referred to our hospital for evaluation of elevated serum alanine aminotransferase (ALT) and/or alkaline phosphatase (ALP). Physical examination revealed no signs of liver disease and none of the subjects reported weekly alcohol consumption exceeding 140 g.

Liver biopsies were performed on an outpatient basis using a 1.6 mm Biopince needle (Medical Device Technologies Inc., FL, USA). A Nikon Eclipse E800 microscope with a Nikon DS-Ri1 digital camera was used for image capturing. In all, 5 images from each biopsy were captured and stored in a computer using the software NIS elements D (v3.2). The first field of view was chosen in the end of the biopsy closest to the end of the microscopic slide. After the first image had been grabbed, the next field of view was chosen by moving along the length axis of the biopsy 1.25 fields of view in order not to get overlapping images for evaluation. This procedure was continued until 5 images had been grabbed. A point grid, consisting of 221 crosses 35 µm apart, was superimposed on each image. The final magnification on the computer screen when counting was x400. The number of hits on fat vacuoles in hepatocytes (including both macro- and microvesicular) was counted. Hits on damaged tissue and larger areas with connective tissue were excluded. The results are given as the percentage of biopsy area with fat deposition.

A Philips Achieva 1.5 T MR-scanner (Philips Medical Systems, Best, The Netherlands) was used to measure the ^{1}H-MRS using a single detection element of a four-element SENSE body coil. Two MRS-volumes of interest (VOIs) were placed within the liver. The ^{1}H-MRS acquisition parameters were: PRESS volume selection 30x30x30 mm^3 VOI, repetition time = 1.5 s, echo time = 35 ms, dummy excitations = 2 and averages = 8. Acquisition of spectra and localizer images were performed using single breath holds.

jMRUI [3] was used for processing of ^{1}H-MRS using the AMARES algorithm with prior knowledge [4] for quantification of the water (6.0-3.3 ppm) and lipid (2.7-0.4 ppm) resonances (**Fig. 1**). Zero order phase correction was applied to the spectra prior to quantification. HTCG was calculated as previously described [2] using the mean of the resonances in the two VOIs, correcting for proton density, T_1 and T_2. T_1 was assumed to be 663 and 236 ms for the water and lipids respectively [5]. T_2 was assumed to be 39 and 58.5 ms for the water and lipids respectively [6].

Results: HTCG estimated using 1H MRS showed a significant Pearsson correlation with the point-counting biopsy results (rho=0.903, p<0.001), linear regression yielded r^2=0.816 (**Fig. 2**).

Discussion and Conclusions: In this prospective study the estimated fat content in the liver using quantitative ^{1}H-MRS correlates well with stereological point-counting of biopsies. Our results add to the evidence that proton MRS is a viable alternative to liver biopsies in terms of assessing the fat content, however it is not yet clear which of the two techniques is superior. Currently literature values for T_1 and T_2 is used for correction for relaxation effects; this could potentially be replaced with subject specific values in order to reduce patient and system bias. In part, the differences in derived fat content can be a consequence of the fact that MRS VOIs and biopsies were not being spatially correlated. Furthermore the MRS VOIs also cover several magnitudes larger volumes than a biopsy could ever do. There is also a potential variable population of fat vacuole sizes, *i.e.* there may be micro vesicles that ^{1}H MRS can capture but that are invisible in a light microscope. It would likely be highly useful to investigate such phenomena in detail in the future.

References: [**1**] Franzen L.E. *et al* Mod Pathol 2005;18:912-6. [2] Longo R *et al* J Magn Reson Imaging 1995;5(3):281-5. [3] Naressi A *et al* MAGMA 2001;12;141-52. [4] Vanhamme L *et al* J Magn Reson 1997;129:35-43. [**5**] Thomsen C *et al* Magn Reson Imaging 1994;12(3):487-95. [6] Lee SS *et al* J Magn Reson Imaging 2011;33(6):1390-8.