Supplementary Material to: Utility and cost evaluation of multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease

MRI Sequence Parameters

T1 Mapping

A T1 relaxation time map was acquired using the Shortened Modified Look Locker Inversion recovery (shMOLLI) sequence. Shimming was performed across the volume of the liver based on a GRE field map acquired during a single expiration breath hold. The ShMOLLI sequence samples the T1 recovery curve using single-shot steady state free precession (SSFP) acquisitions using the following parameters. TR 2.14ms, TE 1.07ms, flip angle of 35°, field-of-view optimised per patient in the range 400-450mm. Acquisition matrix 192x134-160, depending on patient, with GRAPPA acceleration of 2 with 24 reference lines, yielding a typical interpolated voxel size 0.9 x 0.9 x 8mm. Images were acquired 340ms after the ECG R-wave with delay shortened in those with a heart rate >75beats per minute.

Proton magnetic resonance spectroscopy

^{1}H Magnetic Resonance Spectroscopy was performed in a 30x30x30 mm voxel in the right lobe of the liver using a stimulated echo sequence (STEAM), TR/TE=3000/20 ms. 5 measurements each of water-suppressed and non-water-suppressed data were acquired across 2 expiration breath holds. Measurements were automatically phase and frequency corrected individually using TARQUIN.[1] The 5 FID's were then averaged before automated fitting in

TARQUIN using customised liver-specific metabolite basis sets for water suppressed and non-suppressed data.

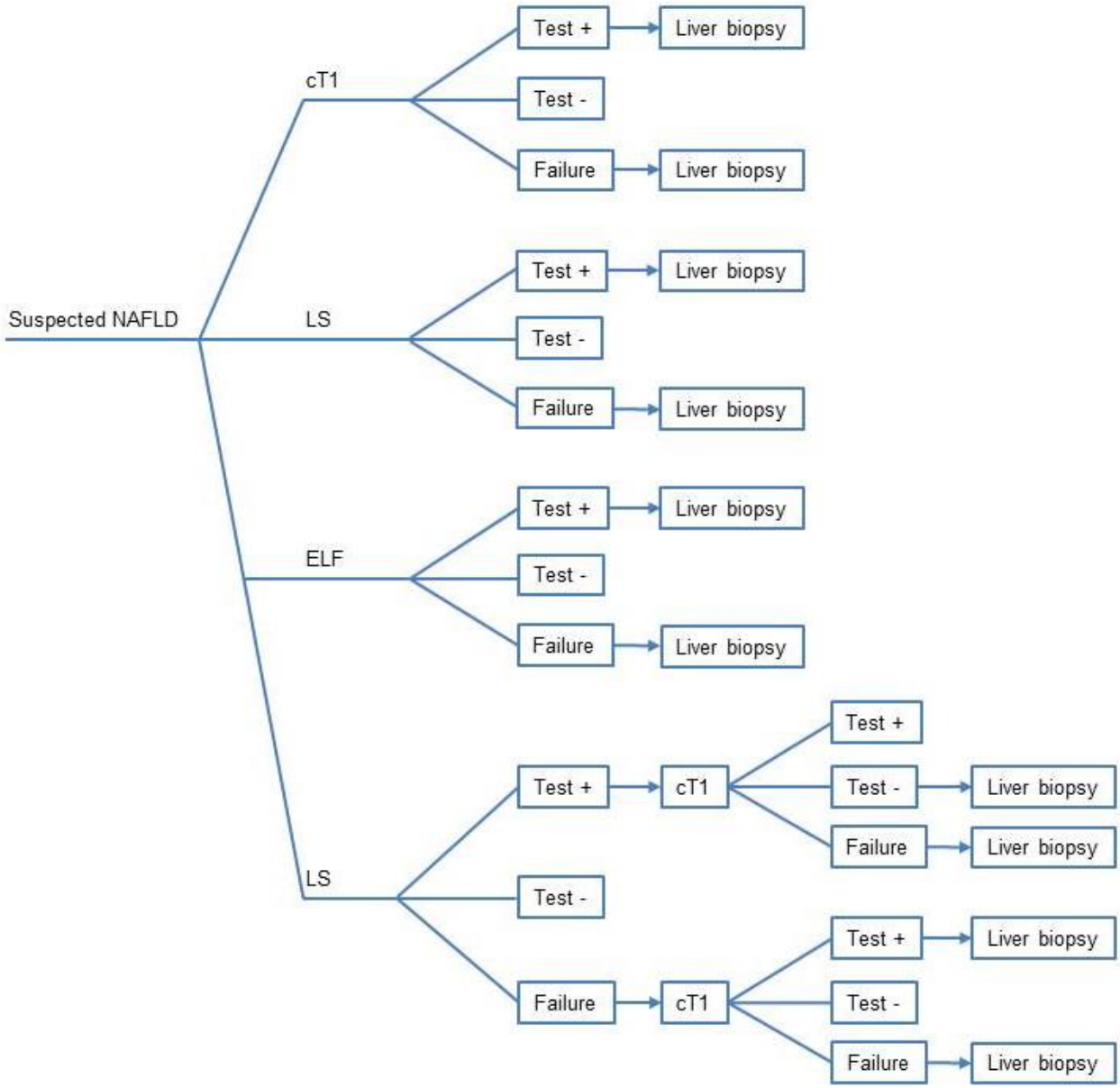
T2 mapping*

A multi-gradient-echo acquisition with RF spoiling is used to calculate a T2* map of the liver. The same field-of-view as in the T1 mapping sequence is used, with a matrix size of 192x128-160, depending on patient, slice thickness of 6mm and 2x GRAPPA acceleration, with the same delay after the R-wave before acquisition. The image is acquired in nine 4 segments with a TR of 26.5ms and flip angle of 20°. Echo times are selected as far as possible such that the signals from fat and water are in phase (TE = 2.46, 7.38, 12.30, 17.22 and 22.14 ms).

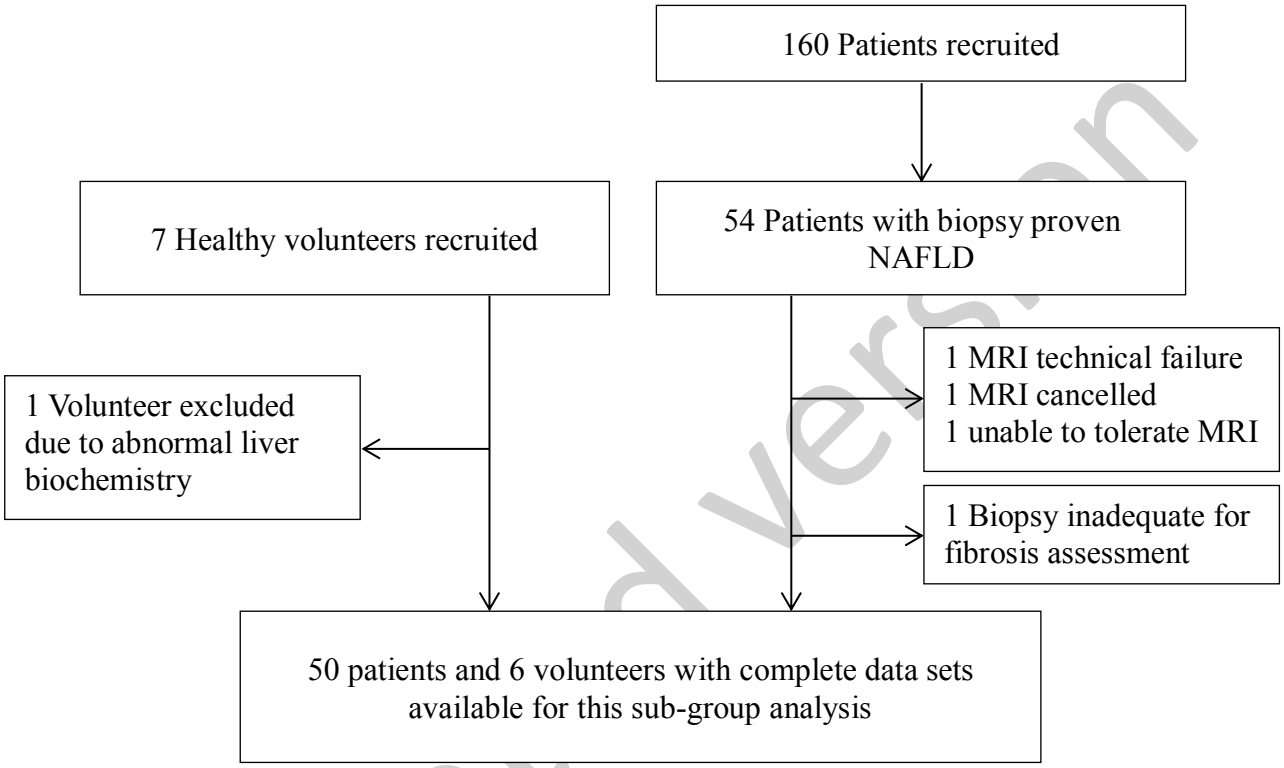
Modified Dixon Sequence

A further RF-spoiled multi-gradient-echo sequence was used to calculate the percentage fat content of liver tissue. Voxel size and acquisition parameters matched those of the T2* mapping sequence, but with echo times chosen to give alternating in- and out-of-phase fat and water signals. Calculation of fat fraction using this sequence is well described in the literature.[2]

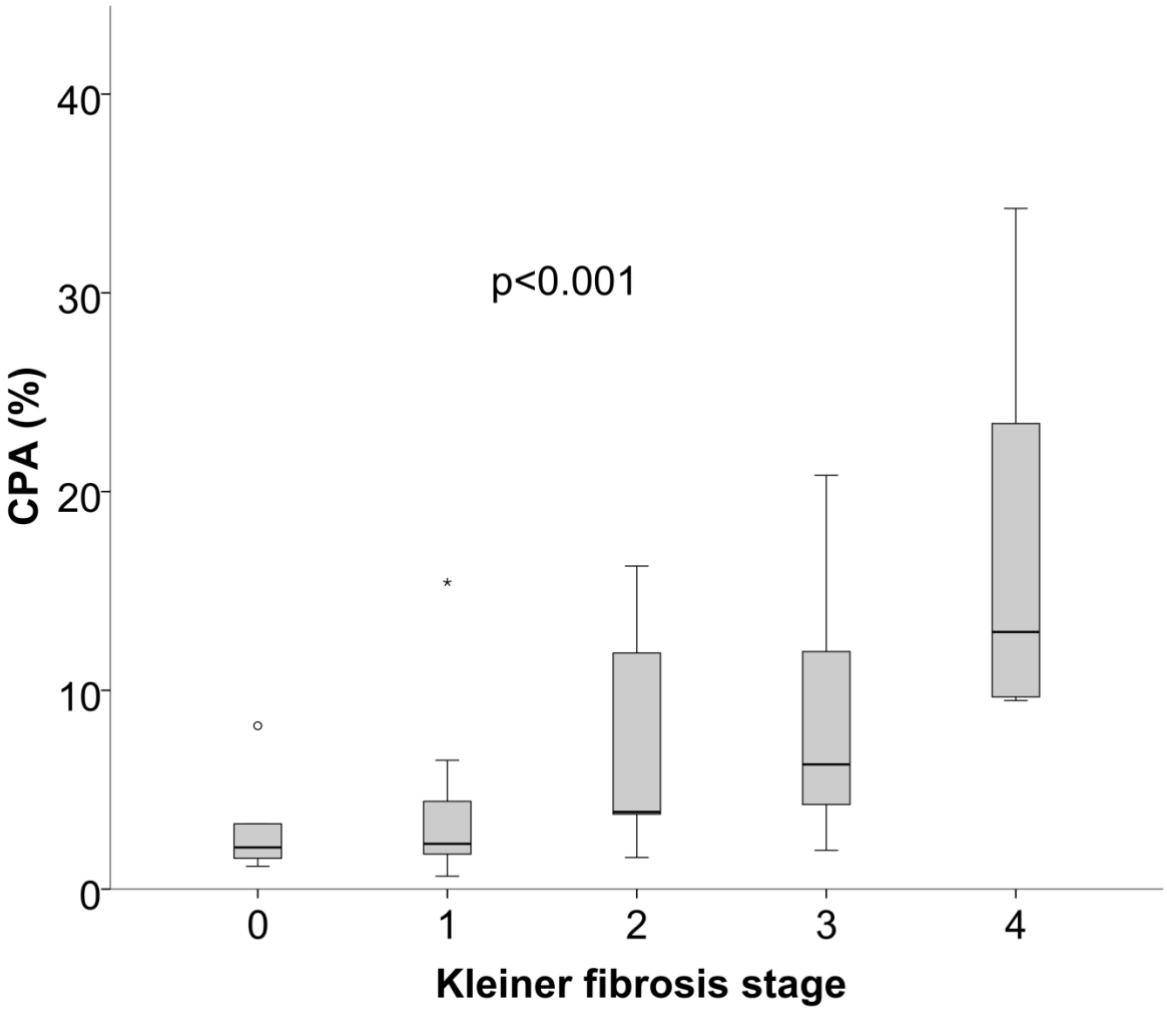
Supplementary Figures



Supplementary figure 1: Risk stratification pathway of patients with suspected NAFLD

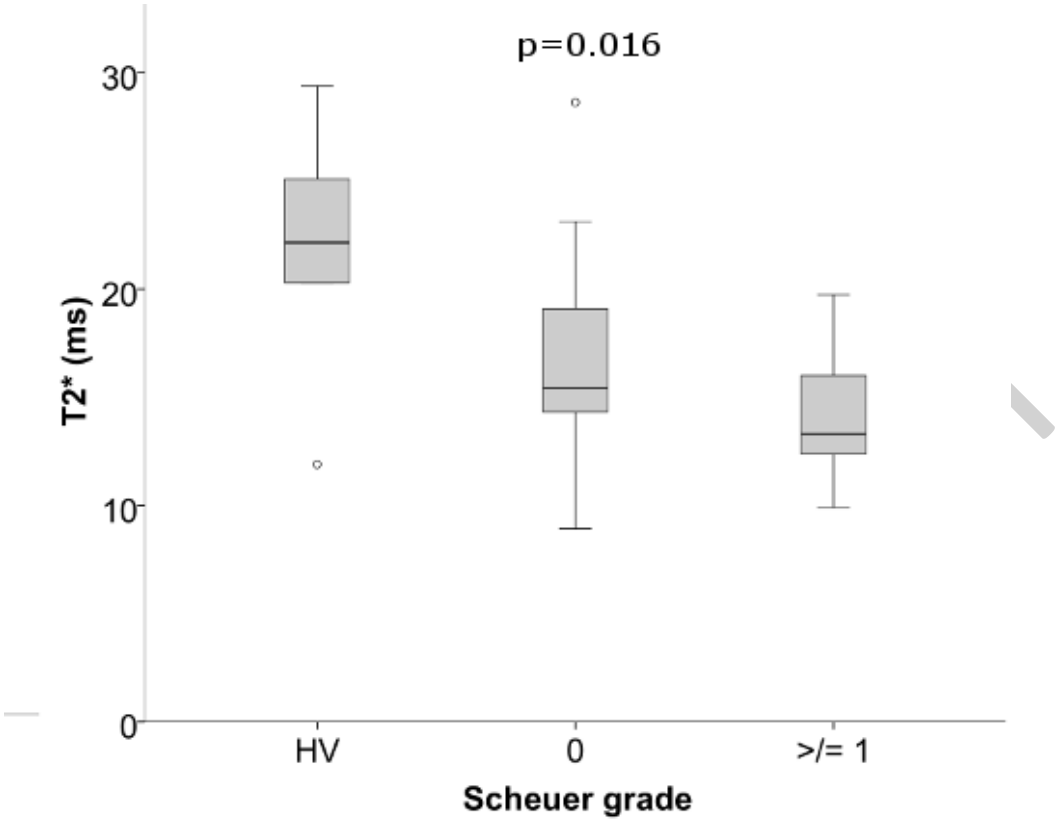


Supplementary figure 2: Study participant flowchart - 4 patients and 1 healthy volunteer were excluded from the final analysis.



Supplementary figure 3: The association of CPA and Kleiner stage in the study cohort.

P<0.001 by the Jonckheere-Terpstra test.



Supplementary figure 4: Relationship between Scheuer siderosis grade and T2*.

Supplementary data

Indication for liver biopsy

32 patients were diagnosed with NAFLD based on clinical assessment of risk factors, exclusion of other aetiologies and ultrasound findings consistent with hepatic steatosis. These patients had inconclusive non-invasive assessment of fibrosis and underwent liver biopsy for staging of fibrosis.

The remaining 18 patients were biopsied to make a diagnosis. These patients had undergone a range of non-invasive tests without a firm diagnosis being made.

Steatosis assessment

Comparison	Brunt vs. PDFF-Dixon	Brunt vs. PDFF-MRS	Brunt vs. CAP
Overall	**<0.001**	**<0.001**	**0.002**
HV vs. 1	0.211	0.312	0.090
HV vs. 2	**<0.001**	**<0.001**	**<0.001**
HV vs. 3	**<0.001**	**<0.001**	0.277
1 vs. 2	**0.011**	**0.004**	0.376
1 vs. 3	**0.002**	**<0.001**	1.000
2 vs. 3	1.000	1.000	1.000

Differences between groups in assessment of liver fat to accompany Figure 1. Overall significance by the Kruskal-Wallis test. Inter-group differences were assessed with post-hoc tests.

Grading of siderosis by multiparametric MRI

Seven out of 50 (14%) patients had grade 1 siderosis on biopsy and only 1/50 (2%) patients had grade 2 siderosis. Mean T2* in healthy volunteers, patients without siderosis on biopsy and patients with siderosis on biopsy (Scheuer grade ≥1) was 21.8 (±5.8), 16.7 (±3.7) and 14.1 (±3.1) milliseconds (ms) respectively (p=0.016) (Supplementary figure 4). AUROC for the differentiation of patients without and patients with siderosis on biopsy was 0.705 (0.498-0.912).

Scheuer Siderosis Grade		
0	42	84%
1	7	14%
2	1	2%
3	0	0%

Distribution of Scheuer siderosis grade on biopsy.

Comparison	**Scheuer vs. T2**
Overall	**0.016**
1 vs. 0	0.267
1 vs. HV	**0.012**
0 vs. HV	0.116

Differences between groups in assessment of liver iron to accompany Supplementary figure 4. Overall significance by the Kruskal-Wallis test. Inter-group differences were assessed with post-hoc tests.

Supplementary References

1. Wilson M, Reynolds G, Kauppinen RA, et al. A constrained least-squares approach to the automated quantitation of in vivo 1H magnetic resonance spectroscopy data. Magnetic Resonance in Medicine 2011;65:1-12.

2. Reeder SB, Cruite I, Hamilton G, et al. Quantitative Assessment of Liver Fat with Magnetic Resonance Imaging and Spectroscopy. J Magn Reson Imaging 2011;34:spcone.