Changes in Liver Volume in Patients with Chronic Hepatitis C Undergoing Antiviral Therapy

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Aim: Liver volumetric analysis has not been used to detect hepatic remodelling during antiviral therapy before. We measured liver volume (LV) changes on volumetric magnetic resonance imaging during hepatitis C antiviral therapy. **Methods:** 22 biopsy-staged patients (median [range] age 45^{19–65} years; 9F, 13M) with chronic hepatitis C virus infection were studied. LV was measured at the beginning, end of treatment and 6 months post-treatment using 3D T₁-weighted acquisition, normalised to patient weight. Liver outlines were drawn manually on 4 mm thick image slices and LV calculated. Inter-observer agreement was analysed. Patients were also assessed longitudinally using biochemical parameters and liver stiffness using FibroscanTM. **Results:** Sustained viral response (SVR) was achieved in 13 patients with a mean baseline LV/kg of 0.022 (SD 0.004) L/kg. At the end of treatment, the mean LV/kg was 0.025 (SD 0.004, $P = 0.024$ cf baseline LV/kg) and 0.026 (SD 0.004, $P = 0.008$ cf baseline LV/kg) 6 months post-treatment ($P = 0.030$ cf baseline, $P = 0.004$). Body weight-corrected end of treatment LV change was significantly higher in patients with SVR compared to patients not attaining SVR ($P = 0.050$). End of treatment LV change was correlated to initial ALT ($R^2 = 0.479$, $P = 0.037$), but not APRI, AST, viral load or liver stiffness measurements. There was a correlation of 0.89 between observers for measured slice thickness. **Conclusions:** LV increased during anti-viral treatment, while the body weight-corrected LV increase persisted post-antiviral therapy and was larger in patients with SVR. (J CLIN EXP HEPATOL 2016;6:15–20)

Hepatitis C virus (HCV) is a blood-borne hepatotropic RNA virus of significant worldwide public health concern.¹ Currently estimates indicate that there are 270–300 million people infected worldwide with the incidence of HCV expected to peak in the next 10–20 years.¹ Treatment of HCV aims to improve outcome by slowing or halting progression to cirrhosis and hepatocellular carcinoma (HCC), but serial biopsy during or following treatment is not considered necessary or ethical at present. Non-invasive methods of assessing pathological changes in the liver are being assessed, but are often

expensive, conceptually difficult or require specialist equipment.² At present, there is no accepted physiological description of any remodelling changes occurring during antiviral therapy, nor is there an accepted proxy to virological measurement to assess response to treatment.

Patients with chronic liver disease (CLD) frequently undergo imaging studies using magnetic resonance imaging (MRI) scanning primarily for the assessment of focal lesions. Volumetric analysis of the liver by MRI^{3,4} is applicable to many clinical settings.^{5–7} In operative planning particularly for partial hepatectomy prior to surgery³ for malignancy and for live-related liver donation, liver volume (LV) is useful in assessing the risk of inducing liver failure in the resection candidate⁸ or “small-for-size” syndrome in the graft recipient.⁹ Smaller LVs are seen in more advanced cases of fibrosis and in increasing Child–Pugh class of cirrhosis.¹⁰ In patients with cirrhosis and portal hypertension, a LV of 75% can be expected, compared to age-matched controls. LV may also be related to pre-fibrotic metabolic processes such as steatosis or hepatitis B.¹¹ Patients with non-alcoholic fatty liver disease (NAFLD) have increased LV which has been shown to decrease on intensive weight loss programs.¹² Furthermore, NAFLD is associated with faster disease progression in HCV and may contribute to the baseline LV prior to treatment. Some authors have suggested that it is useful to assess changes in volume over time as an indicator of therapeutic effectiveness and or disease progression.¹³

Keywords: hepatitis C virus, liver volume, magnetic resonance imaging, sustained viral response

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Abbreviations: ALT: Alanine aminotransferase; APRI: Aspartate transaminase to platelet ratio index; AST: Aspartate transaminase; CHC: Chronic hepatitis C; CLD: Chronic liver disease; CT: Computed tomography; EASL: European Association for the Study of the Liver; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; LV: Liver volume; MRI: Magnetic resonance imaging; NAFLD: Non-alcoholic fatty liver disease; NI: Necroinflammatory; SVR: Sustained viral response

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MRI is well established as an accurate means to measure LV.^{4,14} Unlike computed tomography (CT) scanning, MRI avoids the subject being exposed to ionising radiation, and the use of nephrotoxic contrast media is not necessary for volumetric analysis. CT volumetry has been employed in LV estimation in patients with acute liver failure,^{5,15} although this is likely due to a pragmatic choice of rapid scanning modality in these critically ill patients. Longitudinal measurement of LV during treatment for chronic HCV infection has never been performed previously, and MR would be a preferred platform to perform this readily understood and exportable potential longitudinal marker.

The purpose of this study was to measure and observe any changes in LV accurately in a cohort of patients undergoing therapy for chronic hepatitis C infection with pegylated interferon-alpha and ribavirin and assess the correlation of volumetric change with biochemical, virological and ultrasound transient elastography (FibroscanTM, Echosens, Paris, France) indices of treatment response to help understand putative hepatic remodelling processes during successful viral eradication.

METHODS

Patient Selection

Twenty-two patients with chronic hepatitis C (CHC) were prospectively recruited over a 2-year period from Imperial College Healthcare Trust with prior informed, written consent obtained from each subject. Ethical approval was obtained from the regional ethics committee in accordance with the 1975 Declaration of Helsinki, (ethics reference no. 06/Q041/10). Patients were studied at the beginning and 6 months after stopping treatment with pegylated interferon alpha 2a and ribavirin, the treatment time being genotype dependent with 24 weeks treatment given for genotypes 2 and 3, while genotypes 1 and 4 received 48 weeks treatment. Patients were included if they were aged 18–65 years, had evidence of replicating HCV infection on HCV RNA testing (Abbott Realtime HCV assay, Abbott Diagnostics, Illinois, USA), and had been referred for percutaneous liver biopsy for clinical indications. Patients were excluded if they consumed >20 g of alcohol per day; were obese (with a body mass index >30 kg/m²) or diabetic; if they were taking antiviral therapy; were co-infected with HIV or hepatitis B; were currently taking intravenous drugs, antihypertensive or lipid-lowering medications; had ongoing illness or had evidence of hepatic decompensation. Histological grading was performed by an experienced histopathologist using standardised scoring criteria. Sustained virological response (SVR) was defined as no detectable virus on quantitative RNA testing 6 months post-treatment. Length of treatment was decided by genotype as per European Association

for the Study of the Liver (EASL) guidelines.¹⁶ All patients completed the study.

MRI

Patients were scanned using a Philips 1.5 T AchievaTM MRI (Philips Medical Systems, Best, Netherlands). Scans were performed at baseline, 3 months, end of treatment and 6 months post-treatment. Using a SENSE surface body coil, TFE 3D T₁-weighted DRIVE Equilibrium sequence were performed in a single breath-hold following hyperventilation. The parameters were FOV 375 × 260, TR 7, TE 3.4, FA 15, 50 slices 8 mm/4 mm, thus resulting in 4 mm slice thickness. All the data were sent to one workstation (Viewforum version R4.2V1L2 [Philips Medical Systems, Best, The Netherlands]). The edge of the liver contour was manually drawn using the cursor by an observer with 14 years' experience in MR imaging (JAF). This process was repeated for each slice; approximately 50 per examination, a total of 3500 contours were drawn in total (Figure 1). The ViewForum gives an area in mm,² which was then multiplied by 4 to obtain a volume for the slice. These values were summed and divided by 1,000,000 to obtain a volume in litres. The LV was normalised to patient weight given the expected change in weight during antiviral therapy.

An exercise in reproducibility was also undertaken for LV. Two observers (one experienced radiographer (JAF) and one hepatologist (AZ)) analysed 46 randomised slices five times, over a disparate timeframe resulting in 230 liver areas being analysed. Further comparison was made by measuring and comparing the two observers drawing contours five times around five slices.

Non-invasive Markers of Liver Fibrosis

On the same day as MRI volume studies, all patients had serial standard blood liver biochemistry and both serum Enhanced Liver Fibrosis test (ELFTM) (Siemens Healthcare Global, Erlangen, Germany) and hepatic liver stiffness measurements using FibroscanTM (Echosens, Paris, France) as non-invasive markers of liver fibrosis.

Statistical Methods

Variables pre- and post-treatment were compared using paired *t*-testing and repeated measures ANOVA and % change in these variables was also assessed using one-way ANOVA. Coefficients of variability among measurements for the same patient were estimated and variability among observers was assessed using the intra-class correlation coefficient (ICC). Statistical significance was defined at the 95% level and all *P*-values calculated were two-tailed. Normality was assessed using the D'Agostino-Pearson test. Statistical analysis was performed using SPSS v 15 (SPSS, Chicago, USA) and MedCalc v 11.1 (MedCalc, Mariakerke, Belgium).

Figure 1 An example of liver contour drawing of 4 slices of a magnetic resonance imaging study.

RESULTS

Twenty-two patients (12M:10F) of median age 47 (19–65) years made up the study cohort. Eleven patients were current smokers and 7 patients were current alcohol users. The mean BMI was 25 (3.4) kg/m². The median fibrosis score on biopsy was 3 (1–6) and necroinflammatory (NI) score 4 (1–6) from biopsies of median length 26 (4–48) mm. Of these, only two patients had established cirrhosis and were well compensated with normal albumin levels and prothrombin times (Child grade A). The other 20 patients had pre-cirrhotic liver disease with good hepatic synthetic function. Twelve patients were genotype 1, two genotype 2, six genotype 3 and two genotype 4 with a median baseline viral load of 130417 (1404–3455391 copies/mL). The other baseline clinical and biochemical parameters are shown in Table 1 with their change during therapy.

Volumetry

Thirteen of the participants achieved sustained viral response (SVR), 6 months after finishing treatment. The mean LV/kg at the start of treatment for all participants

was 0.022 (SD 0.004) L/kg. Including all participants, the LV at the end of treatment was 0.025 (SD 0.004) L/kg (mean difference %, $P = 0.024$, Paired t test compared to baseline LV shown in Figure 2) and further increased to 0.026 (0.004) L/kg 6 months after the conclusion of treatment ($P = 0.008$ compared to baseline, $P = 0.034$, repeat measures ANOVA, linear trend). The change in LV was more pronounced in those patients who achieved SVR (MD + 0.004, $P = 0.008$, one way ANOVA). Volume change was not related to treatment duration or genotype ($P = 0.543$, repeated measures ANOVA) and body-weight corrected LV change was dependent on virological response ($P = 0.050$, repeated measures ANOVA).

Baseline LV was correlated to waist circumference ($R = 0.496$, $P = 0.016$). However, no further correlations were found with ALT, AST, APRI score, viral load, indices of necroinflammation or fibrosis, Fibroscan-measured liver stiffness or body mass index (BMI). However, the end of treatment LV was correlated to initial ALT ($R = 0.479$, $P = 0.037$), but not to initial APRI, AST or viral load. LV change was not dependent on the presence of cirrhosis at the start of therapy.

Table 1 Paired Change in Baseline Variables During Treatment Presented as Mean (SD). The Group Interaction was Assessed Using Repeated Measures ANOVA. ALT = alanine aminotransferase; GGT = gamma-glutamyl transpeptidase; Hb = haemoglobin; ELFTM = enhanced liver fibrosis score.

Variable	Pre-treatment	End of treatment	Paired <i>t</i> test	Group (SVR) Factor Interaction
ALT, iU/L	117 (103)	45 (39)	0.0018	0.297
GGT, iU/L	77 (65)	56 (40)	0.1393	0.821
Hb, g/L	140 (10)	120 (20)	<0.0001	0.074
AST, iU/L	79 (53)	48 (37)	0.0007	0.212
Platelets, $\times 10^9$ /mL	195 (64)	170 (65)	0.0189	0.354
ELF TM score	10 (1)	10 (2)	0.0467	0.782
Triglycerides, mmol/L	1 (1)	2 (1)	0.0001	0.030
Low density lipoprotein, mmol/L	2 (1)	25 (64)	0.1631	0.952
Fibroscan TM	10 (8)	11 (2)	0.7781	0.967
Liver volume, L	1.5 (0.3)	1.6 (0.3)	0.0276	0.115
Liver volume per kg body weight, L/kg	0.022 (0.004)	0.025 (0.004)	0.002	0.147

Figure 2 Comparison of liver volume in participants at baseline before the treatment and after 6 months of therapy. (A) Participants who did not achieve sustained viral response ($P = 0.438$); (B) Participants who achieved sustained viral response ($P = 0.020$).

There was no significant change in liver stiffness as measured by ultrasound transient elastography using FibroscanTM over the course of therapy (10 (8) kPa to 11 (2) kPa, $P = 0.778$ Paired *t* test) and no correlation between FibroscanTM change and LV change ($R = 0.196$, $P = 0.487$). However, significant change was noted with ELFTM serum testing (10 (1) to 10 (2), $P = 0.0467$, paired *t* test).

Reproducibility

Comparison of LV measurements between the two observers showed a mean difference of 1% which is not statistically significant. The mean standard error/% slice area for the second observer was 2–1.5. The mean (SD) area/slice measurement was 9256 (116) mm³ with the standard error over all the analysed slices 53 mm³. Thus, the % standard error/slice measurement was measured at 1.02 (± 0.18)%

with only four slices having a % standard error greater than 2%. The intra-class correlation coefficient between observer 1 and 2 was 0.707 (95% CI: 0.331–0.867), suggesting good agreement. However, Passing and Bablock regression while demonstrating an intercept not significantly different from zero (8225 (–2492–12392)) did show a deviation from linearity in the slope (0.574 (0.363–1.1101)), suggesting there may be errors in agreement dependent on slice area.

DISCUSSION

In this study, we have aimed to accurately measure and observe any variations in LV in a cohort of patients undergoing therapy for chronic hepatitis C infection with pegylated alpha interferon and ribavirin and assess the

correlation of volumetric change with standard and novel indices of treatment response. For the first time, we have demonstrated that the LV rises during antiviral treatment and is more pronounced when measured over a 12-month period in patients, who achieve SVR.

Volume increase could be interpreted as an indication of liver regeneration and/or recovery and this may be related to reduction in fibrotic load of the liver. Hepatic fibrogenesis is dominantly orchestrated by hepatic stellate cell activation, which describes the conversion of the normally quiescent vitamin A storage cells to 'myofibroblasts', which contribute to both structural and dynamic hepatic fibrosis. Resolution of myofibroblast activity is regarded as an important step in the reversal of inflammatory damage, initiated by the expression of extracellular remodelling signals, which contribute to fibrotic load.¹⁷ While the liver stiffness on FibroscanTM did not decrease, this is not necessarily the best test to determine very small changes in fibrosis given that there are a number of false positives related to an abnormal FibroscanTM including changes in liver perfusion.¹⁸ Furthermore, changes in fibrotic load, as they require the reversal of myofibroblastic activation, are likely to resolve over a longer time frame than during the study period, so the Fibroscan technique may not detect any small reduction in fibrotic load of the liver in the face of any hepatic perfusion/inflammatory changes.²

We observed a small, but significant increase in LV with SVR. This change is not related to baseline histological severity, suggesting that this is an effect of viral clearance. Furthermore, no positive correlation can be found in this study between the observed changes and FibroscanTM or ELF score. Further studies assessing the changes in LV with MR-measured perfusion techniques are required.

MRI is an expensive imaging modality and although commonplace in the developed world, demand on this particular resource is high. However, abdominal and liver studies are commonly performed on MRI due to its superior tissue contrast and lesion detection.¹⁹ Therefore, the addition of another fast breath-hold sequence assessing LV in a larger population could be considered, particularly given the difficulty in performing serial liver biopsies in this population. Both performing and post-processing of MR images involve highly trained personnel, while manually defining liver contours is time consuming, and we have shown that bias can be introduced when non-imaging trained staff perform contour analysis. It would be advantageous to develop automated techniques to define LV both currently and prospectively on acquired data sets. Novel registration techniques²⁰ are currently in development and automated methods may soon be available, when the limitations of breath-hold acquisitions are overcome. Furthermore, we did not assess functional LV in this study, which is possible using SPECT-CT.²¹ However, this is unlikely to be a source of bias, as no patients in this study had significant vascular abnormalities.

A particular strength of our study was the reproducibility of MRI LV assessment. We would recommend that this assessment is done by trained staff, given the findings from Passing and Bablock regression. This is in agreement with previous studies,¹⁴ which demonstrated that using MRI is a robust method for measuring LV and has the advantage of avoiding contrast media use and exposure of ionising radiation to participants. While multiple other modalities were employed to determine a potential mechanism of hepatic remodelling via non-invasive imaging our results were not conclusive in this regard. In future studies, it would be useful to measure LV changes during disease therapy, which in conjunction with the other imaging modalities discussed here, could further elucidate which mechanisms are involved hepatic remodelling of long-term fibro-inflammatory diseases.

CONFLICTS OF INTEREST

The authors have none to declare.

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