Quantitative Magnetic Resonance Imaging in Patients with Cirrhosis: A Cross-Sectional Study

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Cerebral magnetic resonance imaging was undertaken, at 3 Tesla field strength, employing magnetization transfer (MT) and diffusion-weighted imaging (DWI) sequences, in 26 patients with well-compensated cirrhosis, free of overt hepatic encephalopathy. Results were compared to those from 18 aged-matched healthy volunteers. Cerebral magnetization transfer ratios (MTR) were reduced in the frontal white matter, caudate, putamen and globus pallidus in patients with cirrhosis, compared to healthy controls, while the apparent diffusion coefficients (ADC) on DWI were significantly increased in the genu and body of the corpus callosum. An association between previous excessive alcohol consumption and both MTR and ADCs was noted, but this association was lost when controls were exercised for the severity of liver disease and psychometric impairment on multivariate analysis. Eight (31%) of the 26 patient had impaired psychometric performance consistent with a diagnosis of minimal hepatic encephalopathy. No statistically significant difference in regional cerebral MTRs or ADCs were found in relation to neuropsychiatric status, although there was a trend towards lower MTRs in patients with impaired psychometric performance. The alterations in MTR and ADC in the patients with functionally compensated cirrhosis are compatible with theories governing the genesis of hepatic encephalopathy, including changes in astrocyte membrane permeability, with subsequent redistribution of macromolecules.

Key words: apparent diffusion coefficients; diffusion-weighted imaging; hepatic encephalopathy; magnetic resonance imaging; magnetization transfer ratios

Hepatic encephalopathy (HE) is the term used to describe the spectrum of neuropsychiatric changes that can be observed in patients with cirrhosis. In the recent guideline published jointly by the European and American Associations for the Study of the Liver hepatic encephalopathy was defined as 'a brain dysfunction caused by liver insufficiency or portal systemic shunting' (AASLD/EASL, 2014; Vilstrup *et al*., 2014).

Clinically apparent or *overt* HE manifests as a neuropsychiatric syndrome encompassing a wide spectrum of mental and motor disorders (Weissenborn, 1998; Ferenci *et al*., 2002). Individuals with overt HE also show a wide spectrum of other abnormalities, including impaired psychomotor performance (Schomerus & Hamster, 1998) and disturbed neurophysiological function (Parsons-Smith *et al*., 1957; Chu *et al.*, 1997). The term *minimal* HE is used to describe patients with cirrhosis who are 'clinically normal', but who show abnormalities in neuropsychometric and/or neurophysiological performance (Ferenci *et al*., 2000).

The presence of HE, whether minimal or overt, is associated with significant impairment in the performance of complex tasks, such as driving (Bajaj *et al*., 2007; Schomerus *et al*., 1981; Wein *et al*., 2004); health-related quality of life (Groeneweg *et al*., 1998); earning potential (Schomerus & Hamster 2001); safety (Roman *et al*., 2011); neurocognitive function post-transplantation (Sotil *et al*. 2009); and, ultimately, survival (Amodio *et al.*, 1999; Bustamante *et al.*, 1999; Stewart *et al.*, 2007). The presence of HE also poses a substantial burden for caregivers (Bajaj *et al.*, 2011) and a significant financial burden on health-care systems (Poordad, 2007).

Currently, there is no accepted gold standard for the diagnosis of HE (Ferenci *et al*., 2002). Thus, the diagnosis of *overt*, or clinically apparent, HE is based on a careful and detailed neuropsychiatric history and examination and exclusion of other potential causes of neuropsychiatric abnormalities; collateral evidence may be obtained from psychometric and neurophysiological testing. The diagnosis of *minimal* HE relies on the exclusion of symptoms and signs of overt HE and the finding of impaired psychomotor performance and/or abnormal neurophysiology.

Cerebral imaging should be undertaken when patients with cirrhosis first present with neuropsychiatric abnormalities in order to exclude alternative diagnoses (AASLD/EASL, 2014; Vilstrup *et al*., 2014) and again later if there is any suspicion of alternative neurological pathology. Less certain, however, is the role of cerebral imaging in the diagnosis of HE *per se*, despite the fact that numerous cerebral imaging studies, using increasingly sophisticated technologies, have been undertaken in these patients over the last two decades (Alonso *et al*., 2014).

Bilateral, symmetrical T_1 signal hyperintensity of the globus pallidus is observed, using conventional MR techniques, in between 50 to 100% of patients with cirrhosis (Inoue *et al*., 1991; Pujol *et al*., 1996; Taylor-Robinson *et al*., 1995; Weissenborn *et al*., 1995). Signal hyperintensity has also been observed in areas adjacent to the globus pallidus in several studies (Inoue *et al*., 1991; Syh *et al.*, 1991; Taylor-Robinson *et al*. 1995; Weissenborn *et al*., 1995). No significant correlations have been observed between the cerebral T_1 hyperintensity and the severity of underlying liver disease in the majority of studies (Pujol *et al*., 1993; Rovira *et al*., 2001; Spahr *et al*., 1996; Taylor-Robinson *et al*., 1995; Weissenborn *et al*., 1995), although the pallidal hyperintensity lessens or resolves completely following liver transplantation (Pujol *et al*,. 1991; Pujol *et al*., 1993; Weissenborn *et al*., 1995). There is evidence of a positive relationship between cerebral

T_1 signal hyperintensity and the degree of portal systemic shunting (Inoue *et al.,* 1991; Kulisevsky *et al.,* 1992; Pujol *et al.,* 1993) and the hyperintensity has been shown to increase following insertion of transjugular intrahepatic portosystemic stents (TIPS) (Krieger *et al*., 1997). Greater difficulties have arisen in defining the relationship between the cerebral T_1 signal abnormalities and neuropsychiatric status. Nevertheless, there appears to be a consensus that cerebral T_1 signal changes can be observed in patients who are neuropsychiatrically unimpaired and that the intensity of the change does not correlate with the degree of neuropsychiatric impairment (Inoue *et al.,* 1991; Pujol *et al.,* 1991; Thuluvath *et al*., 1997; Taylor-Robinson *et al*., 1995; Weissenborn *et al*., 1995).

Several lines of evidence support the view that the pallidal T_1 signal hyperintensity observed in patients with cirrhosis results from the deposition of manganese (Alonso *et al*., 2014). Thus, increases in manganese concentrations have been observed in whole blood and CSF in these patients (Katsuragi *et al*., 1999; Kreiger *et al*., 1995; Spahr *et al*., 1996) and in pallidal tissue obtained at *post-mortem* (Krieger *et al*., 1995; Pomier-Layrargues *et al*., 1995; Rose *et al*., 1999). In addition, strong collateral evidence is provided by observations in individuals with chronic manganese toxicity, resulting from industrial exposure (Nelson *et al.,* 1993; Josephs *et al*., 2005) or long-term parenteral nutrition (Mirowitz *et al*., 1991), who show similar cerebral T_1 signal abnormalities which reverse with specific therapy or cessation of long-term nutritional support (Mirowitz *et al*., 1991; Mirowitz & Westrich 1992; Nelson *et al.,* 1993; Nagatomo *et al*., 1999).

Magnetization transfer (MT) is a quantitative MRI technique, based on interactions and exchanges between mobile protons in a free water pool and those bound to macromolecules. By using MR sequences with and without an off-resonance saturation pulse, MT allows calculation of an index, the magnetization transfer ratio (MTR). Decreases in the MTR indicate that protons bound to the brain tissue matrix have a diminished capacity to exchange magnetization with the surrounding free water. Thus, this index provides an estimate of the extent of tissue structure disruption and affords a potential window into the macromolecular environment that is not directly visible using conventional MRI techniques (Grover *et al*., 2006), because it provides additional contrast in MR images and can be used to better characterize cerebral white matter.

Taylor-Robinson and colleagues (Taylor-Robinson *et al*., 1995) were the first to use T_1-weighted MT imaging to study the brain in patients with cirrhosis. They found that while MT imaging highlighted the basal ganglia and showed a correlation between globus pallidus contrast and blood ammonia levels, MT contrast measurements did not correlate with the severity of liver dysfunction or the presence or degree of HE. Subsequently, Iwasa and colleagues reported their findings using the MTR index; they found a reduction in the ratio in the globus pallidus, which correlated with the reduction in functional hepatic reserve. (Iwasa *et al*., 1998) and also reported similar findings in the surrounding white matter (Iwasa *et al.,* 1999). Similar findings of a reduction in MTR have been reported by others (Balata *et al*., 2003; Cordoba *et al*., 2001; Iwasa *et al*., 1999; Rovira *et al*., 2001; Taylor-Robinson *et al*., 1995), with the exception of one group (Restuccia *et al*., 2004), who found no difference in MTR between patients and controls, but they used a different MR sequence to the majority of other groups. These changes have been ascribed to the presence of manganese, but also of low-grade cerebral oedema.

Diffusion-weighted imaging (DWI) is a powerful MR technique, which allows tissue structure to be

probed at the microscopic level, by quantifying the motion of water molecules. The usual image resolution capability of MR is 1-2 mm, but during the diffusion times used in DWI, typically 50 ms, water molecules move on average 10 μm (Le Bihan 2001) and interact with cell membranes, macromolecules and nerve fibres. Thus, DWI allows non-invasive, high resolution probing of brain structure, but does not interfere with the diffusion process itself. Diffusion of water molecules is an intrinsic physical process independent of the MR effect of field strength (Le Bihan *et al*., 2001). The two common quantitative parameters measured in DWI are the mean apparent diffusion coefficient (ADC), which is a measure of tissue water diffusivity, and the fractional anisotropy (FA), which is a measure of the overall directionality of water diffusion. The ADC is affected primarily by intracellular and extracellular volume change, extracellular tortuosity and intracellular water motion, referred to as "streaming" (Duong *et al*., 1998; Norris 2001). An increase in ADC is likely to occur when the mobility of water molecules in the extracellular space increases, for example, as a result of vasogenic or extracellular oedema (Ebisu *et al*., 1993; Schaefer *et al*., 2000; Schwartz *et al*., 1998).

Several cerebral DWI studies have been undertaken in patients with cirrhosis, but the results are inconsistent, primarily because the cohorts studied have been small and heterogeneous, particularly in relation to the severity of the liver disease and the degree of neuropsychiatric impairment, which was variously assessed, and the MR techniques which were not standardized (Kale *et al*., 2006; Khalek *et al*., 2014; Lodi *et al*., 2004; Sugimoto *et al*., 2008).

Thus, currently the consensus appears to be that while studies using structural and functional cerebral imaging techniques have undoubtedly helped unravel the pathophysiology of HE, they are not thought to offer anything diagnostically (Berding *et al*., 2009; Grover *et al*., 2006). Notably, however, all the cerebral MR studies undertaken in patients with cirrhosis to date have used magnetic field strengths of 1.5T. The improved signal to noise ratio offered by 3T field strengths might, at least theoretically, enhances any differences in MR characteristics not previously apparent.

The hypothesis informing this present study is that cerebral MRI, undertaken at 3 Tesla field strength, employing MT and DWI sequences will reveal pathology in the basal ganglia and other vulnerable regions of the brain in patients with cirrhosis, and particularly those with underlying HE, which is independent of other potential confounding variables such as the aetiology and severity of the underlying liver disease, alcohol misuse and current hydration status. The specific aim of this study was, therefore, to examine the relationship between changes observed in high field strength, multimodal MRI and the presence and degree of psychometric abnormalities in a group of well-characterized patients with cirrhosis and no clinical evidence of HE exercising controls for other potential confounders. MT was used to derive a quantitative measure of brain water content and membrane fluidity, and DWI to provide insight into intracellular and extracellular water changes and structural integrity.

Methods

Subjects

Patients were recruited sequentially from those attending outpatient clinics at the Imperial College

Healthcare Trust, London. The population comprised of 26 patients (19 men: seven women; mean [range] age 51 [37-64] yr) with biopsy-proven cirrhosis. The aetiology of the liver injury was determined using clinical, laboratory, radiological and histological variables, while its severity was assessed using the Pugh modification of the Child's grading system (Pugh *et al*., 1971) (Table 1). All patients were clinically stable at the time of the study with Child-Pugh Grade A cirrhosis. Patients were excluded if they were under 30 or over 70 years of age; if they had evidence of clinically overt hepatic encephalopathy or had suffered an episode of major hepatic decompensation within 7 days of the assessment date; had significant cardiac, respiratory or renal failure; insulin-dependent diabetes mellitus; cerebrovascular disease; epilepsy; a history of significant head injury or other conditions likely to affect cerebral function. Patients were also excluded if they had misused alcohol or drugs in the previous three months; if their manual dexterity was impaired; if they could not speak English or obey spoken commands; or were taking psychoactive medications, including hypnotic drugs.

Healthy volunteers were recruited, by advertisement, from amongst visitors and staff at Imperial College Healthcare Trust, London. The population comprised of 18 individuals (nine men; nine women; mean age 49.0 [34-64] yr). None had a history of liver disease, significant medical co-morbidities, drank alcohol in excess of 20 g daily, and none took prescription or over-the-counter medications.

Patient clinical and psychometric assessment

All patients underwent detailed, clinical and laboratory assessment. The aetiology of their liver injury was determined using clinical, laboratory, radiological and histological variables. The functional severity of their liver injury was assessed using Pugh's modification of the Child's grading system (Pugh *et al*, 1973) and the MELD score (Malinchoc *et al*, 2000). Psychometric performance was assessed, by a single operator, in a quiet room with constant light level, using the validated CDR® computer-based assessment battery (United Bioscience, Goring-on-Thames, United Kingdom). This system provides information on five domains: power of attention (PoA), continuity of attention (CoA), quality of episodic memory (QoEM), quality of working memory (QoWM) and speed of memory (SoM). The raw scores for each of these domains were compared to age-and sex-matched normative data from the CDR bank to generate Z scores for each individual domain and the sum of Z scores totaled (Table 2). Patients were classified as neuropsychometrically impaired, by CDR criteria, if the total Z score was ≤ -3 (Mardini *et al*. 2005). On the basis of this assessment, eight patients were classified as psychometrically impaired and hence showing evidence of minimal HE (Table 2)

MR imaging

Cerebral MRI was performed on a 3T Philips Intera^{TM} MR system (Philips, Best, Netherlands). Standard volumetric T_1-weighted sequences were performed with a three-dimensional (3D) imaging sequence: echo time (TE) 3.8 ms, repetition time (TR) 256 ms, 1 NSA, 256 image matrix, 25 cm field of view (FOV) and 2.0 mm slice thickness. T_2-weighted sequences were performed to exclude structural brain pathology, with the following sequence parameters: TE 80 ms, TR 3000 ms, 2 NSA, image matrix of 230, 23 cm FOV, and 3.0 mm slice thickness.

MT was obtained using a two-dimensional gradient-echo pulse sequence (TR 54.7 ms, TE 3.75 ms, flip angle 15 degrees, slice thickness 2mm, 1 NSA) with 20 slices positioned over the basal ganglia. DWI was obtained using a standard Philips 32 direction DTI sequence (Philips, Best, The Netherlands). The sequence was constructed to obtain information from an area as small and symmetrical as possible. The voxel size that the sequence probes is 1x1x1.2mm. Data were obtained in 32 non-collinear directions of sensitisation using single-shot echo planar imaging (TR 16000 ms, TE 51 ms, slice thickness 2 mm, 2 NSA, b=1000 s/mm^2). A SENSE factor of 2 was used to reduce image distortion. Philips PRIDE™ software was used to co-register the images, correcting for motion and eddy current distortion (Philips, Best, The Netherlands). ADC and FA maps were calculated using DTI Studio® version 2.1. ADC and FA values were recorded from specific regions of interest (ROIs) in the genu, body and splenium of the corpus callosum, anterior corona radiata (ACR) and posterior corona radiata (PCR).

MRI analysis

MTR maps were calculated, using ImageJ® version 1.32j, (www.imagej.nih.gov) with the formula $MTR=100(SI_0-SI_{RF})/SI_0$, where SI_{RF} is the signal intensity in the image employing an off-resonance RF pulse and SI_0 the signal intensity in the initial proton density image. ROIs were drawn around the (i) frontal white matter, (ii) head of caudate, (iii) putamen, (iv) globus pallidus and (v) thalamus bilaterally. The same area of ROI was used for each brain region between subjects.

The pallidal index (PI) was calculated as the ratio of the left/right averaged signal intensity in the globus pallidus, to the averaged signal intensity of frontal white matter on T_1-weighted imaging multiplied by 100 (Krieger *et al*. 1995). Signal intensities were measured using ROIs drawn version 1.32j, (www.imagej.nih.gov).

ADC and FA maps were calculated using DTI Studio version 2.1 (www.dsi-studio.labsolver.org). ADC and FA values were recorded from specific regions of interest (ROI) in the genu, body and splenium of the corpus callosum. These areas were chosen as they were anatomically highly conspicuous and hence, they were easily defined on this imaging sequence. A standardized area of ROI was used for the individual ROIs between different subjects.

Statistical methods

Data were tested for normality using the Shapiro-Wilk test. Between group comparisons were made with the Mann-Whitney U test. Correlations were made with the Spearman rank test. Bonferroni's correction was used to correct for multiple comparisons. Tests of significance were two-tailed. Correlation coefficients between regional MTRs on MT imaging, or ADC and FA on DWI and individual psychometric domains measured by the CDR® system were calculated using Spearman's rank test. Univariate linear regression was used to determine if age, sex, aetiology and severity of liver disease, history of alcohol misuse, individual laboratory variables including liver function tests and serum sodium concentrations and the presence of neuropsychometric impairment were associated with observed changes in the MTR and ADC. Statistical analyses were performed using SPSS version 16 (SPSS Inc., USA).

Ethics

Ethical approval was obtained from the Hammersmith, Queen Charlotte's & Chelsea Research Ethics Committee (ref 04/Q0406/161) and the study was compliant with the principles outlined in the 1975 Declaration of Helsinki on Human Rights. Local Research Governance approval and indemnity, was provided by Imperial College London. All subjects provided written informed consent.

Results

No structural cerebral abnormalities were observed, on standard T_2-weighted MRI in any of the patients or control subjects. There were no difference in brain volumes between the patients and the age-matched healthy volunteers on T_1-weighted volumetric imaging.

Magnetization transfer ratios

MTRs were significantly decreased in the frontal white matter, caudate, putamen and globus pallidus in the patients with cirrhosis (Table 3) even after correction for multiple comparisons. The greatest reductions in MTR were found in the frontal white matter (4%) (Figure 1) and the globus pallidus (5.8%) (Figure 2).

Diffusion-weighted imaging

The ADC was measured in nine brain regions. A significant increase in ADC was found in the genu and body of the corpus callosum in the patients with cirrhosis compared to controls (Table 4), even after correction for multiple comparisons. There was no significant difference in fractional anisotropy (FA) between patients and controls.

MRI associations: patient populations and degree of psychometric impairment

There were no statistically significant differences in MTR in any of the cerebral areas studied between the unimpaired patients and those classified as having minimal HE. However there was a non-significant between-group trend of reducing MTR between unimpaired patients and those with minimal HE, particularly in the frontal white matter and the globus pallidus (Table 3).

There was an association on univariate analysis between the presence of minimal HE and a reduction in regional cerebral MTRs, but this association was lost on multivariate analysis after correction for the severity of liver disease and a history of alcohol misuse. No association was found between the MT imaging data and the individual psychometric domains measured by the CDR® system.

ADC values tended to be higher in the genu and body of the corpus callosum in the unimpaired patients with cirrhosis, but the differences between values in the unimpaired patients and those with minimal hepatic encephalopathy were not statistically significant. No association was found between the diffusion-weighted imaging data and the individual psychometric domains measured by the CDR® system.

MRI: patient population by aetiology of liver injury

Patients' whose liver disease was caused by a combination of chronic HCV infection and alcohol misuse were excluded from the by aetiology analyses. There were no significant differences in regional brain MTRs between patients with alcohol-related cirrhosis and those with non-alcohol related cirrhosis considered together. However, there was a trend towards a lower MTR in those with alcohol-related disease.

There was no significant difference in MTRs between neuropsychometrically impaired and unimpaired patients within the alcohol-related cirrhosis and non-alcohol-related cirrhosis cohorts. There was no significant difference in ADC or FA, between impaired and unimpaired patients, within the alcohol-related cirrhosis and non-alcohol-related cirrhosis cohorts.

In univariate analysis alcohol misuse was the only variable associated with a reduction in MTR in the frontal white matter, head of caudate, putamen and globus pallidus and an increase in ADC in the genu and body of the corpus callosum.. However, after adjusting for the effects of the severity of liver disease and psychometric impairment in multivariate analysis these associations were not sustained. None of the other variables tested was associated with the observed changes in MTR and ADC.

Discussion

The main findings in the present study were that mean MTRs were reduced in the frontal white matter and basal ganglia structures in the brains of patients with cirrhosis, while mean ADCs were increased in areas of the corpus callosum.

There are several possible explanations for the reduction observed in MTR including damage to myelin or to the axonal membrane (Lexa *et al*. 1994; van Waesberghe *et al*. 1999), deposition of paramagnetic substances (Iwasa *et al*. 1998) and low-grade cellular/cerebral oedema (Balata *et al*. 2003; Cordoba *et al*. 2001). Damage to myelin or the axonal membranes is an unlikely explanation as (i) cerebral proton magnetic resonance spectroscopy studies (^{1}H MRS) studies in patients with cirrhosis have invariable shown no significant changes in the signal for n-acetyl-aspartate, a marker of neuronal integrity; and (ii) FA was unchanged, indicating structural integrity (Taylor-Robinson *et al*., 1996).

Iwasa and colleagues demonstrated a strong inverse relationship between the manganese concentration in manganese chloride phantoms and the MTR, thus supporting the hypothesis that manganese deposition in the brain may account for the observed reduction in MTRs. However, they found no association between the whole blood manganese levels and MTRs in the patients studied (Iwasa *et al*. 1998). *Post-mortem* studies have confirmed an association between *pre-mortem* T_1 hyperintensity and elevated *post-mortem* levels of manganese in the basal ganglia in patients who died with chronic liver disease (Krieger *et al.* 1995; Maeda *et al*. 1997). Given that the MT sequence was T_1-weighted, this would also be supportive of an association between MTRs and manganese deposition in the context of cirrhosis. The observation that the reduction in MTR rapidly improves after liver transplantation (Cordoba *et al*. 2001) may be supportive of the manganese deposition hypothesis, as cholestasis, which is also associated with pallidal manganese deposition, improves post-transplantation. However, the observation that in occupational manganese exposure the resolution in cerebral pallidal T_1 hyperintensity is much slower (Josephs *et al*. 2005) suggests that this is a more complex issue.

Low grade cerebral/cellular oedema is often cited as the explanation for reduced MTRs in patients with cirrhosis (Balata *et al*. 2003; Cordoba *et al*. 2001; Miese *et al*. 2006; Poveda *et al*., 2010; Rovira *et al*. 2001). This is supported by the finding of reduced intracellular levels of the cerebral osmolyte myo-inositol (mI), reported in ^{1}H MRS studies (Häussinger *et al*. 2000). This may result from expulsion of mI from cells, in order to try to compensate for cellular oedema.

In the present study, mean ADCs were measured in a total of nine white and grey matter structures. There was a statistically significantly increase in the mean ADCs in patients with cirrhosis compared to controls in two of these regions, although the ADC was higher in all of the nine regions studied. The two compartment model for the interpretation of diffusion suggests that the extracellular fluid, with relatively fast and unhindered motion of water molecules, is the main component of the overall diffusivity whereas the intracellular compartment, with its cellular structures, is characterised by restricted motion of protons and hence contributes less to overall diffusivity (Norris 2001). Thus, a finding of elevated ADCs would usually be attributed to an increase in extracellular fluid. However, it has been suggested that if the increase

in ADC is accompanied by a reduction in MTR, as in the present study, this could reflect minimal cellular oedema with an increase of membrane permeability and increased intracellular diffusivity, as well as changes in the viscosity of the cytoplasm (Lodi *et al*. 2004). Microscopically the most striking feature observed in the brains of individuals dying of chronic liver failure is proliferation of the astrocytes with development of enlarged nuclei, prominent nucleoli, margination of chromatin and accumulation of glycogen — changes referred to as Alzheimer type II astrocytosis (Butterworth, 2002). These changes are found particularly in the cerebral cortex, basal ganglia and cerebellum. In addition, the expression of glial fibrillary acid protein (GFAP), which is a major contributor to the filamentous structures within astrocytes (Eng *et al*. 2000), is reduced in astrocytes in the basal ganglia and cerebral cortex (Sobel *et al*. 1981). Thus, the astrocytes in HE demonstrate cytoplasmic change and as they occupy one-third of the cerebral volume, this may explain the observed reduction in mean MTRs and the increase in mean ADC observed in this patient population.

It has been suggested that some of the MR-measurable changes observed in patients with cirrhosis might be attenuated in those with a history of alcohol misuse. Thus, Miese *et al*. (2006) reported a significant decrease in the MTR of the thalamus, globus pallidus, putamen, and white matter in patients with non-alcohol-related cirrhosis which negatively correlated with the severity of HE. However, in patients with alcohol-related cirrhosis the MTR was significantly decreased in all regions assessed, but showed no correlation with the severity of HE. Likewise, in patients with non-alcohol-related cirrhosis, the ADC in the occipital white matter was not significantly altered, but showed a tendency toward increased values in the thalamus and in normal-appearing white matter in those with overt HE, whereas there were no significant differences in ADC in any of the examined regions in patients with alcohol-related cirrhosis. The authors' speculated that the discrepancy between the MRI findings in the patients with alcohol-related and non-alcohol-related cirrhosis might reflect the presence of alcohol-related microstructural change or alcohol-related changes in membrane lipid composition. Thus, alcohol-tolerant membranes in the brains of patients with alcohol-related cirrhosis might exhibit a systematically different MT ability than those of non-alcoholic patients with cirrhosis.

In the present study, there was a non-significant trend towards a greater reduction in MTRs in patients with alcohol-related cirrhosis and minimal HE. Alcohol misuse was an independent predictor of the reduction in MTR in the frontal white matter, head of caudate, putamen and globus pallidus; and of the increase in ADC in the genu and body of the corpus callosum. However, the significance of these associations was lost after adjusting for the effects of the presence of liver disease and psychometric impairment. There was no statistically significant difference in mean MTRs in patients in relation to the presence of psychometric impairment contrasting with the findings of others. (Cordoba *et al*. 2001; Restuccia *et al*. 2004; Rovira *et al*. 2001). However, this may be a reflection of the small number of patients with minimal HE within the group, supported by the fact that there was a tendency for MTRs to be lower in the patients with impaired psychometric performance. This could not have been addressed at enrolment, as the study design was to recruit unselected patients who met inclusion criteria without biasing interpretation of the MR findings by knowledge of their psychometric status. No really meaningful comparisons of the relationship between MR-measured variables and psychometric status in subgroups by aetiology could

be made as the subgroups numbers were very small

In conclusion, this study has demonstrated the utility of the MT and DWI sequences to detect differences in cerebral MTRs and ADCs in patients with cirrhosis and .provides further insights into the pathophysiology of HE, in particular the potential effects of changes in astrocyte membrane permeability, with subsequent redistribution of macromolecules. However, future studies which include concurrent functional MRI sequences (Ahluwalia *et al.*, 2014) may also shed further light on the meaning and context of the changes observed with MTR and DWI.

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Figure legends

Figure 1 Box plot of cerebral magnetization transfer ratio (MTR) in the frontal white matter in patients with cirrhosis (n=24) and age-matched healthy controls (n=15) (median and interquartile range values)

Figure 2: Box plot of cerebral magnetization transfer ratio (MTR) in the globus pallidus in patients with cirrhosis (n=24) and age-matched healthy controls (n=15) (median and range interquartile values)

Table 1 Characteristics of the 26 patients with cirrhosis, free of overt hepatic encephalopathy, undergoing cerebral MRI

Study number	Sex	Age (yr)	Aetiology of cirrhosis
1	F	52	Alcohol
2	F	51	Hepatitis C*
3	F	47	Autoimmune hepatitis
4	F	56	Hepatitis C
5	M	48	Alcohol + hepatitis C*
6	M	49	Alcohol
7	F	64	Autoimmune hepatitis
8	M	64	Alcohol
9	M	63	Hepatitis C
10	M	61	Alcohol
11	M	57	Cryptogenic
12	M	51	Alcohol +Hepatitis C
13	M	48	Hepatitis C
14	M	47	Primary biliary cirrhosis
15	M	46	Alcohol
16	M	38	Haemochromatosis
17	M	56	Alcohol + hepatitis C*
18	M	50	Hepatitis C*
19	M	39	Hepatitis C*
20	M	44	Alcohol
21	M	37	Hepatitis B
22	M	46	Alcohol + hepatitis C*
23	M	45	Haemochromatosis
24	F	62	Primary biliary cirrhosis
25	F	45	Alcohol
26	M	58	Hepatitis C*

* Hepatitis C RNA positive. All patients had compensated, Child's Grade A cirrhosis

Table 2 Psychometric performance and overall status assessed using the CDR® system in the 26 patients with cirrhosis, free of overt hepatic encephalopathy, undergoing cerebral MRI

Subject no	PoA	CoA	QoEM	QoWM	SoM	Total	Psychometric status**
	(Z score)*						
1	-1.47	-3.62	-2.62	-1.0	-14.07	-22.77	Impaired
2	-1.54	0.53	-1.45	0.46	-1.09	-3.1	Impaired
3	-1.21	0.9	-1.31	0.79	-1.9	-2.72	Unimpaired
4	-0.19	1.11	1.94	0.8	0.68	4.33	Unimpaired
5	-6.96	-1.53	-2.62	-1.91	-2.49	-15.51	Impaired
6	1.06	0.21	-1.43	-0.03	-1.71	-1.9	Unimpaired
7	-1.62	1.15	-0.09	0.94	-2.12	-1.75	Unimpaired
8	-1.82	1.15	-0.52	0.17	-0.54	-1.55	Unimpaired
9	-1.64	-0.19	1.22	0.76	-0.64	-0.49	Unimpaired
10	0.65	0.61	-0.26	0.94	0.59	2.53	Unimpaired
11	0.59	-0.15	-0.49	0.37	0.33	0.65	Unimpaired
12	-0.68	-0.65	0.07	0.9	-0.03	-0.39	Unimpaired
13	0.62	0.9	1.83	0.79	0.43	4.58	Unimpaired
14	0.86	-0.49	-0.29	-0.03	1.21	1.27	Unimpaired
15	0.46	0.56	1.2	0.24	-0.42	2.03	Unimpaired
16	0.76	0.86	0.05	1.05	-1.24	1.48	Unimpaired
17	-2.26	-0.91	0.33	0.8	-3.60	-5.63	Impaired
18	0.61	0.9	0.05	0.79	-2.96	-0.61	Unimpaired
19	-2.46	-0.92	-0.58	-0.94	-2.89	-7.78	Impaired
20	-0.1	-6.04	0.28	-1.28	-0.65	-7.79	Impaired
21	-3.17	1.16	1.65	1.05	-1.24	-0.55	Unimpaired
22	-0.11	0.21	-2.01	-0.16	-1.33	-3.41	Impaired
23	1.13	-0.03	0.58	1.06	0.81	3.55	Unimpaired
24	-2.23	0.35	-0.65	0.94	-4.61	-6.20	Impaired
25	0.81	0.92	0.55	1.06	-1.78	1.56	Unimpaired
26	-2.65	0.86	0.92	0.8	-2.0	-2.07	Unimpaired

Abbreviations: PoA= power of attention, CoA= continuity of attention, QoEM= quality of episodic memory, QoWM= quality of working memory, SoM= speed of memory

*Z scores represent the number and direction of standard deviations in the patient data compared to those from the healthy controls

** Psychometric performance was classified as impaired if the total Z score for all domains was ≤ -3

Table 3 Regional cerebral magnetization transfer ratios (MTR) on MRI in healthy controls and in patients with cirrhosis, by psychometric status

Brain region	MTR				Significance
	Healthy controls	Patients with cirrhosis			Controls *vs* All patients
	(n=15)	Total (n=24)	*Unimpaired (n=16)*	*Impaired (n=8)*	
Frontal white matter	57.68 (1.28)	55.36 (1.61)	*55.67 (1.5)*	*54.73 (1.75)*	******
Caudate	48.12 (1.52)	46.52 (1.39)	*46.70 (1.47)*	*46.16 (1.21)*	*
Putamen	48.48 (1.06)	47.04 (1.28)	*47.20 (1.25)*	*46.72 (1.38)*	***
Globus pallidus	52.79 (0.882)	49.71 (2.93)	*50.11 (2.46)*	*48.93 (3.77)*	******
Thalamus	53.27 (1.83)	51.93 (1.79)	*51.87 (1.88)*	*52.07 (1.71)*	NS

Data are expressed as mean and standard deviations.

*significance value between healthy controls and the patient entire population with cirrhosis, corrected for multiple comparisons; ****** = $p<0.0001$;*** = $p<0.005$; * = $p<0.05$; NS = non-significant. There were no significant differences between the neuropsychometrically unimpaired and the neuropsychometrically impaired patients

Table 4 Regional cerebral mean apparent diffusion coefficients (ADC) for healthy controls and patients with cirrhosis categorized as impaired or unimpaired by psychometric testing ($x10^{-3}$ mm^2/s).

Brain region	Mean ADC $x10^{-3}$ mm^2/s (SD)			
	Controls (n=16)	Patients with cirrhosis (n=25)	Unimpaired patients (n=17)	Impaired patients (n=8)
Genu of CC	0.809 (0.04)	0.845* (0.03)	0.845ϕ (0.03)	0.848 (0.03)
Splenium of CC	0.718 (0.03)	0.737 (0.04)	0.742 (0.05)	0.727 (0.03)
CC body	0.769 (0.03)	0.816***** (0.04)	0.812ϕϕϕϕ (0.03)	0.824 (0.4)
ACR	0.740 (0.04)	0.774 (0.04)	0.771 (0.04)	0.780 (0.06)
PCR	0.805 (0.04)	0.812 (0.05)	0.818 (0.05)	0.798 (0.03)
Caudate	0.698 (0.03)	0.720 (0.03)	0.715 (0.04)	0.730 (0.3)
Putamen	0.683 (0.02)	0.707 (0.04)	0.704 (0.05)	0.712^ (0.04)
Globus pallidus	0.709 (0.04)	0.724 (0.04)	0.722 (0.04)	0.728 (0.04)
Thalamus	0.721 (0.02)	0.734 (0.04)	0.732 (0.04)	0.741 (0.03)

CC= corpus callosum; ACR= anterior corona radiata; PCR= posterior corona radiata.p values are displayed for the various patient groups where values are significantly different from the healthy controls: patients with cirrhosis *$p<0.05$; *****$p<0.0005$; neuropsychometrically unimpaired patients: ϕ = $p<0.05$; ϕϕϕϕ = $p<0.001$; neuropsychometrically impaired patients ^ = $p<0.05$

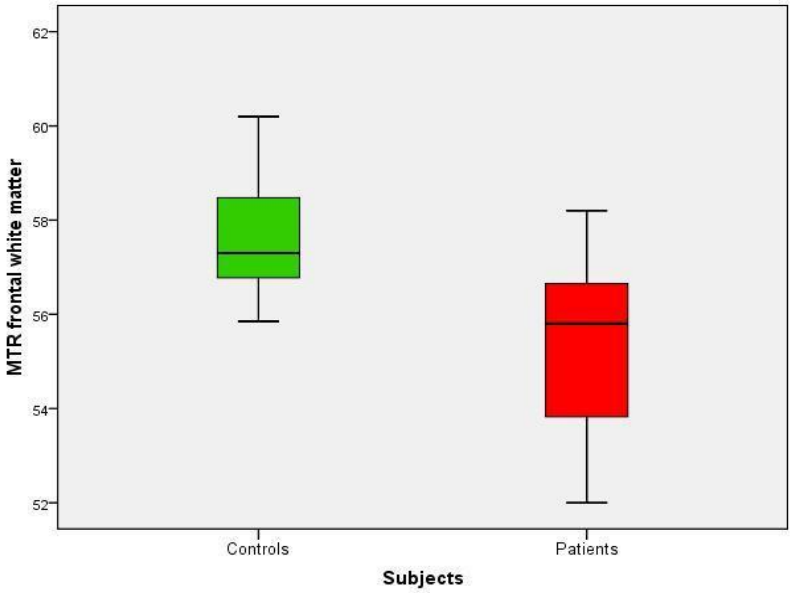
62
60
58
56
54
52
MTR frontal white matter
Controls
Patients
Subjects

Figure 1

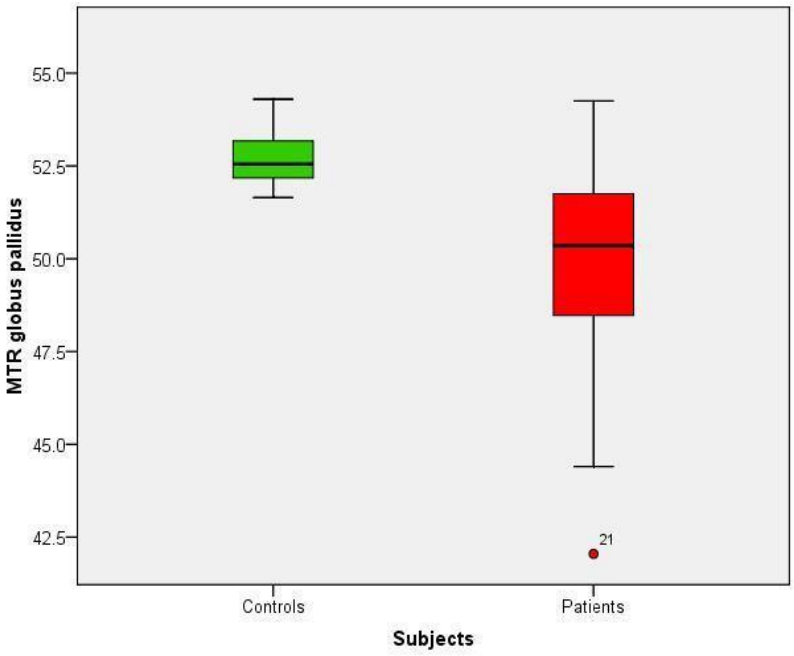
55.0
52.5
50.0
47.5
45.0
42.5
MTR globus pallidus
21
Controls
Patients
Subjects

Figure 2