Brain Imaging and Hepatic Encephalopathy

Neeral R Patel BSc, *Mark JW McPhail PhD MRCP,
Simon D Taylor-Robinson MD FRCP

Liver and Antiviral Center, Department of Medicine, St Mary´s Hospital Campus, Imperial College London, London, United Kingdom

*Address for correspondence:
Dr Mark JW McPhail
Liver Unit, Department of Medicine, 10th Floor QEQM Wing, St Mary's Hospital Campus,
Imperial College London, South Wharf Street, London, W2 1NY, United Kingdom
Tel: +44 207 886 6454
Fax: +44 207 724 9369
Email: mark.mcphail@imperial.ac.uk

Mr Neeral Patel
Liver Unit, Department of Medicine, 10th Floor QEQM Wing, St Mary's Hospital Campus,
Imperial College London, South Wharf Street, London, W2 1NY, United Kingdom
Tel: +44 207 886 6454
Fax: +44 207 724 9369
Email: neeral.patel06@imperial.ac.uk

Professor Simon D Taylor-Robinson
Liver Unit, Department of Medicine, 10th Floor QEQM Wing, St Mary's Hospital Campus,
Imperial College London, South Wharf Street, London, W2 1NY, United Kingdom
Tel: +44 207 886 6454
Fax: +44 207 724 9369
Email: s.taylor-robinson@imperial.ac.uk

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SYNOPSIS

Novel imaging techniques are allowing hepatologists to investigate the structural and functional neuropathology of hepatic encephalopathy (HE) in greater detail but only limited techniques are applicable to the clinic. Computed tomography and magnetic resonance imaging (MRI) can rule out other diagnoses and in the case of MRI, give certain key diagnostic features in widely available sequences. While increased brain water content is a hallmark of HE, the localisation of low-grade cerebral edema, the extent of regional swelling or atrophy and the different functional characteristics of affected brain regions continue to be debated. More specialised volumetric, diffusion-tensor, magnetization transfer, functional magnetic resonance imaging and magnetic resonance spectroscopy, in conjunction with positron emission tomography continue to enrich the investigative findings in HE. Nevertheless an internationally accepted diagnostic framework that includes an objective imaging test to replace or augment psychometry remains elusive. Quantitative MRI is likely to be the best candidate to become such a test and the utility of MR and nuclear medical techniques to the clinic and results from recent research are described in this article.

1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric disturbance affecting patients with acute liver failure (ALF), cirrhosis or non-cirrhotic portosystemic bypass (1). The clinical spectrum of HE extends from mild cognitive impairment, to coma and death (2). The majority of cases involve patients with minimal HE (MHE), which has been associated with an impaired ability to drive automobiles safely, reduced health-related quality of life and increased risk of hospitalization due to overt HE (3-5).

The disease process is multifactorial, with hyper-ammonaemia, gut-derived toxins, short and medium chain fatty acids, cerebral manganese deposition and relative deficiencies in circulating amino acids with consequent neurotransmitter imbalance commonly implicated. There is consensus that ammonia is central to the pathogenesis (6). Varying degrees of cerebral edema may result from the uptake of excess ammonia into astrocytes, with subsequent conversion to glutamine, which acts as a cerebral osmolyte, mitochondrial toxin and instigator of neurotransmitter instability (7-11).

Currently, the diagnosis of HE lacks standardization, particularly in MHE, which requires neuropsychiatric testing to detect cognitive impairment. Psychometric test results can be dependent on the patient's age, educational status, emotional affect and linguistic abilities. Evaluation varies between countries while in some forms of testing, considerable expertise and facilities to conduct an assessment are required (12). This perceived diagnostic difficulty leads to under recognition of this important clinical problem. Even when the presentation of HE is clinically overt, the grading and assessment of longitudinal change is subjective among clinicians, and is also prone to disagreement or even misdiagnosis (12).

The cerebral insults secondary to hepatocellular failure or portosystemic shunting result in structural and functional abnormalities in the brain, which imaging may detect and quantify. Suitable modalities include magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), computed tomography (CT) in conjunction with positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Table 1 and Box 1). These imaging techniques may allow the development of tools for the objective, reproducible and non-invasive diagnosis and monitoring of HE.

While stand-alone computed tomography (CT) is useful for determining gross structural lesions such as cerebral edema in ALF or other pathologies in patients with CLD, it is limited as a diagnostic and longitudinal research tool, owing to poor sensitivity and repeated exposure to ionizing radiation. MRI and PET/SPECT will thus be the focus for this article with particular focus on patients with HE secondary to cirrhosis, where most possibility for diagnostic doubt or pathophysiological disagreement exists.

2. Magnetic Resonance Imaging

MRI is currently the most frequently utilized imaging tool in HE research studies and even standard clinical sequences can give information supporting a diagnosis of HE. A clinical set of sequences when requesting an "MR Brain" study would usually consist of T_1- and T_2-weighted sequences as standard and a selection of more advanced T_2-weighted sequences such as Fluid Attenuated Inversion Recovery (FLAIR) or Diffusion-Weighted Imaging (DWI).

2.1 T_1-weighted MRI

Bilateral and symmetric hyper-intensity of the basal ganglia, using T_1-weighted MRI sequences in patients with cirrhosis is an observation with some clinical utility (Figure 1) (14-17). It has been postulated that excess circulating manganese is the cause of such hyperintensity, due to reduced hepatobiliary excretion as a result of liver failure, and subsequent deposition in the basal ganglia where blood flux is high (18,19). This theory has been corroborated by the correlation between blood and cerebrospinal fluid manganese levels with T_1-weighted hyperintensity; and also the normalization of basal ganglia hyperintensity and blood levels of manganese after liver transplantation (20-22).

However, the results of studies investigating the relationship between manganese-related pallidal hyperintensity and psychometric performance in HE are conflicting (23,24). It is therefore unclear whether T_1-weighted hyperintensity represents a manifestation of HE, or if it is an occurrence secondary to cirrhosis, cholestasis and/or porto-systemic shunting (25,26). The preferential deposition of manganese in the basal ganglia has been suggested to be an explanation for the Parkinsonian-like symptoms, which can arise as a result of HE (27). So while a useful adjunct this

lack of correlation with grades of HE makes a single T_1-weighted exam insufficient in MR Brain in patients with cirrhosis.

2.2 T_2-weighted MRI: Fast FLAIR Imaging

Fast FLAIR (Fluid Attenuated Inversion Recovery) T_2-weighted imaging represents a MRI sequence that has been shown to be sensitive for the detection of diffuse high intensity white matter lesions (WMLs) (28,29). WMLs may develop secondary to cerebrovascular small-vessel disease, which neuropathologically, are a combination of reversible edema and irreversible neuronal damage. (30). Minguez and colleagues noted WMLs were found to be reduced in volume and number after treatment with neomycin and/or branched-chain amino acids in conjunction with improved psychometric performance (31). To further investigate this observation, the same investigators compared WML volumes in patients with impaired cognition, pre- and post-liver transplantation (32). A significant reduction in WML volume was detected after liver transplantation (Figure 2), as well as a strong negative correlation between fast FLAIR T_2-weighted lesion load and psychometric score. Rovira and colleagues, and Cordoba and colleagues, measured high-signal intensity, along the corticospinal tract on fast FLAIR T_2-weighted images in patients with chronic liver disease, with subsequent signal normalization after liver transplantation. (33,34).

2.3 T_2-weighted MRI: Diffusion Weighted Imaging

Diffusion-Weighted MRI (DW-MRI) is a commonly available sequence often used in assessing neurovascular disease which assesses changes in the motion of water molecules, by defining the chemical interaction between water and cellular barriers (35). The diffusivity of water molecules can be quantified by calculating the apparent diffusion coefficient (ADC) and can distinguish between vasogenic (interstitial) and cytotoxic edema (36,37).

Several studies have consistently demonstrated a link between increased cerebral ADC (demonstrating interstitial edema) and patients with either MHE or overt HE; the degree of brain water diffusivity also correlating with grade of HE (37-40). DW-MRI studies have countered the traditional astrocytic swelling hypothesis, suggesting increased ADC values relate to purely extracellular water accumulation in low-grade cerebral edema, secondary to chronic HE (37,41-43). Theories that explain

this phenomenon include hyperammonemia-induced increased blood-brain barrier permeability and reduced glial fibrilliary acidic protein (GFAP) expression, a protein that regulates astrocytic permeability; reduced membranous GFAP in astrocytes has been previously linked increased diffusivity in the extracellular space (44-46). Discrepancies in studies where ADCs have been raised or lowered in clinically defined HE may be related to the onset of disease. Lower ADC values have been noted in patients with HE, secondary to ALF (and cytotoxic edema), whereas higher periventricular white matter and basal ganglia ADC in patients with CHE have been suggested to support a finding of interstitial edema (47).

Detection of water diffusion has improved in recent years with the development of diffusion tensor MRI (DT-MRI), a technique that provides detailed information on brain tissue structure. Fractional anisotropy (FA, where the fractional free or bound water component is estimated) maps can be assimilated to perform "tractography", a method of determining underlying brain anatomy and where edema is localised (48). Kumar and colleagues demonstrated increased mean diffusivity in the internal capsule and cortical gray and white matter of HE patients, but differential patterns of correlation between mean diffusivity and fractional anisotropy in the corpus collusum suggested that interstitial edema was the primary HE correlate in this study of 14 low-grade HE patients. While DWI is often used in clinical scanners, and we would recommend using T_1-weighted and FLAIR/DWI T_2-weighted sequences, the statistical requirements of DTI make it currently unsuitable for standard clinical use.

2.4 Volumetric MRI

Shah and colleagues correlated quantitative T_1 mapping of cerebral water content in the putamen, globus pallidus and occipital white matter with severity of HE (13). This was one of the first direct and quantitative measurements of increased regional cerebral water content with more severe grades of HE, but the imaging technique is highly specialised. Cerebral edema that occurs as a result of ALF can be routinely viewed using MRI (49), but is often low grade and is hence undetectable by radiologists in chronic HE. The question arises of whether a simple measure of total brain volume (BV) would be useful. Recent developments in software packages for brain volumetry allow small (<1% total BV) changes in brain size in HE to be quantitated, allowing precise measurements of BV

(50) on relatively standard T_1-weighted MR sequences (51-55). Single time-point determination of brain volume is considerably less accurate than when BV change is determined longitudinally, owing to the ability to co-register (align) images using the theoretically unchanged skull surface, thus allowing more robust determination of CSF and gray and white matter densities (50,56). Further structural information can be determined using voxel-based morphometry (VBM) where regional contribution to brain volume change can be calculated; this has been applied in many neuropathological scenarios (57).

A pilot study conducted by Patel and colleagues, was the first to utilize co-registered MRI techniques to determine changes in BV in chronic liver disease (58). This small scale investigation focused on six patients with MHE, and three patients who had been diagnosed with overt HE. The authors concluded that in patients treated with lactulose brain volume fell, in association with improved psychometric performance.

In contrast patients with HE often have risk factors for cerebral atrophy (such as age, alcohol abuse and possibly cirrhosis itself) and these effects couldalso contribute to neuropsychological impairment and apposite structural brain changes. Garcia-Martinez and colleagues investigated cognitive function, cerebral magnetic resonance spectroscopy and BV post-liver transplantation (59). Post-transplantation, BV was reduced in patients with prior HE, correlating with worse neuropsychiatric score in this group. This study, suggests that brain atrophy accrued prior to liver transplantation, but masked by low grade edema, may also play a role in cognitive dysfunction post-transplantation. Further evidence of the contribution of atrophy in patients with hepatic encephalopathy has recently emerged with the identification areas of atrophy throughout the cortex and white matter quantitated by VBM and correlating with the severity of encephalopathy (60). A lack of corroborative MR spectroscopy or more advanced water localisation sequences could not add further mechanistic data to this interesting observation, which requires validation from other groups.

2.5 Magnetization Transfer Imaging

Magnetization transfer imaging (MTI) improves image contrast as a consequence of the magnetic properties of free and bound protons (61). Free protons are present in water molecules, whereas

bound protons are fixed to macromolecules, such as proteins, lipids, carbohydrates and nucleic acids (62). Magnetization transfer between bound and free protons reduces the signal intensity observed in the resultant MR image (17,25). MTI also allows for the magnetic transfer ratio (MTR) to be quantified, which ultimately reflects brain parenchymal changes: a low MTR indicates neuronal damage as well as an increase in water content or membrane permeability (17,63).

Lower MTRs have been demonstrated in patients with HE, including a cohort who developed MHE secondary to extra-hepatic portal vein obstruction (64-66). A study by Cordoba and colleagues verified this by concluding MTR normalisation post-transplantation reflected the correction of low-grade cerebral edema in patients with MHE, and that the basal ganglia and white matter are initial targets for water accumulation (67). A study by Miese and colleagues that utilized both MTI and DW-MRI to investigate cerebral edema in HE, found that both reduced MTR and raised ADC were correlated with HE grade in non-alcoholic patients (40). However, in patients continuing to drink to alcohol to excess, no such correlation was established, suggesting chronic alcohol mis-use may independently cause cerebral oxidative damage in this group, although as has been noted above, the effect of atrophy on these measurements may be underestimated (68).

2.6 Functional MRI

Functional MRI (fMRI) measures changes in deoxyhaemoglobin concentration (a substance with paramagnetic properties, relative to tissue) that occurs as a result of the rise in blood oxygenation during neuronal activity (69). The subsequent blood oxygen level dependent (BOLD) contrast highlights areas of activity in the brain.

fMRI has the benefit of being a non-invasive and safe investigatory tool in low grade HE (as these patients can follow instructions in the scanner), that is particularly useful in longitudinal studies as no radioactive marker injections are required (35). Zafiris and colleagues demonstrated that MHE is associated with impaired coupling between visual judgment areas (70). A study conducted by Zhang and colleagues compared brain fMRI data in 14 patients with cirrhosis and 14 healthy volunteers (71). An incongruous word reading task and incongruous color-naming task (testing for attention and interference) highlighted various cerebral areas on fMRI: there was greater activation of the bilateral

parietal and prefrontal cortices in the patients with cirrhosis (72). In a separate study, Zhang and colleagues concluded there was reduced functional connectivity in the right middle frontal gyrus and left posterior cingulate cortex, (part of the default-mode network [DMN)).. This highly interconnected, metabolically active and well described area of the brain is vital for the preservation of attention and is worthy of further study into whether this abnormal activation is related to hyperammonemia or low-grade cerebral edema (73-75).

2.7 Magnetic Resonance Spectroscopy

MRS has been utilised in HE investigations since the 1980s, although it has been more widely used in recent times as a consequence of improved sequence development and higher field strength to resolve metabolite signals. MRS also allows for the investigation of HE at the molecular level, by studying cerebral tissue *in vivo* in whole-body clinical magnets (typically at 1.5-3.0T) (76).

Various nuclei can be used to determine metabolite changes in HE, the most common clinically-used being proton (^{1}H) and 31-phosphorus (^{31}P) MRS. ^{1}H MRS allows for the quantification of metabolites such as choline (Cho), creatine (Cr), *N*-acetyl aspartate (NAA), glutamine (Gln) and glutamate (Glu) or the unresolved combination (Glx), as well as osmolytes such as *myo*-inositol (mI) and taurine. ^{31}P MR spectra allow definition of phosphomonoester (PME), inorganic phosphate (Pi), phosphodiester (PDE), phosphocreatine, γATP, αATP and βATP resonances (or also more correctly termed nucleoside triphosphate resonances [NTP], as they also contain contributions from cytosine triphosphate, guanosine triphosphate and uridine triphosphate, in addition to the overwhelming proportion from adenosine triphosphate, [ATP]) (77). These resonances provide information on cell membrane turnover with cell membrane precursors measured in the PME resonance and cell membrane degradation products measured in the PDE resonance, while information on high energy phosphate metabolism and intracellular pH is available from the Pi, PCr and NTP resonances (78-80). Ammonia is not detected by MRS due to rapid interchange with water (81).

The characteristic spectral appearance of HE on *in vivo* ^{1}H MRS adds further evidence to the astrocyte swelling hypothesis demonstrated by a reduction in intracellular mI and Cho, and a concurrent increase in Gln and Glu (Figure 3) (77,82). Furthermore, these characteristic metabolic

changes have been shown to correlate with psychometric performance (23). This suggests that cells expel osmolytes, such as taurine, mI and Gln, in the face of an osmotic water load. *In vivo* ^{1}H MRS allows the degree of intracellular osmolyte homeostasis to be detected and monitored sequentially and to give an indirect indication of cell swelling in a non-invasive way. The technique is also open to following response to therapeutic intervention. Recent sequence development using 2-dimensional spectroscopy allows improved resolution of 1-dimensional ^{1}H MRS and resolution of glutamine and glutamate (Figure 4) (83).

The interpretation of ^{31}P MRS is complex *in vivo*, since many of the resonances are multi-component and are not easily separated into their constituents at clinically-used magnetic field strengths. Overall, however, changes do appear to reflect alterations in bioenergetic pathways, and glucose utilisation (since sugar phosphates also contribute to both the PME and PDE resonances), as well as giving an indication of phospholipid membrane synthesis and degradation and of cell membrane fluidity (77,84). Consensus on ^{31}P MRS studies has been hampered by small study sample sizes and inconsistencies in MRS protocols between centers (35). Studies conducted by Taylor-Robinson and colleagues have demonstrated reductions in the PME/ βATP and PDE/ βATP which correlate with reduced choline, as verified by parallel ^{1}H MRS on the same subjects (77,84,85). These changes were thought to represent reduced glucose utilisation in HE, as components of the glycolytic pathway contribute to the ^{31}P MRS spectrum *in vivo*. However, a change in membrane fluidity is equally possible. ^{1}H *in vivo* MRS is more likely to be developed for clinical use but normal ranges and diagnostic thresholds for key metabolites are not yet agreed.

3. Nuclear medicine: Positron Emission Tomography and Single Photon Emission Computed Tomography

PET involves the measurement of the concentration of positron emitting radioisotopes from the body. Co-registered CT or MR tomographic imaging provides structural information, while, depending on the radioligand used, functional data are available in the form of glucose and oxygen metabolism, blood flow, amino acid metabolism and rates of amino acid incorporation into proteins, acid-base balance and membrane transport (86). Positron-emitting radionuclides that are used in PET include: ^{11}C, ^{18}F, ^{15}O, and ^{13}N (87).

Lockwood and colleagues have used ^{18}F-fluorodeoxyglucose (FDG) PET to investigate functional changes in chronic HE (88,89). They demonstrated a reduction in glucose metabolism in the anterior cingulate gyrus, which may reflect the attention deficit found in many HE patients on neuropsychometric testing and in fMRI studies. Alterations in cerebral blood flow (CBF) have also been established using FDG PET and ^{15}O PET, where poor neuropsychiatric performance correlated with reduced blood flow in all cortical areas; temporal lobe CBF was found to be most discriminatory between HE patients and healthy volunteers (88).

Brain imaging with ^{13}N-ammonia, has been used to assess cerebral ammonia metabolism in (i) healthy subjects, (ii) subjects with mild liver disease, but with no evidence of cirrhosis, (iii) subjects with cirrhosis with and without HE, and (iv) subjects with malignant neoplasms with metastases in the liver (90-94). However, the results of these studies are somewhat conflicting, particularly with respect to interpretation of blood-brain barrier (BBB) permeability in HE. This is probably owing to differences between research groups in the tracer kinetic modeling approach used to determine parameters of cerebral ammonia metabolism quantitatively. Different expert opinions exist over which conclusions should be drawn out of conflicting results in PET studies of ammonia metabolism and BBB permeability. Some proponents suggest that further studies, including larger numbers of patients and using standardized analysis techniques are necessary, in order to provide consensus and easy methodology to clarify the relationship between BBB permeability and ammonia toxicity (95).

Changes in different neurotransmission systems have been demonstrated in HE patients using radiotracer methods. Increased benzodiazepine receptor binding, decreased dopamine receptor binding and decreased binding to serotonin transporters have been shown (Figure 5) (96). These changes correspond to symptoms observed in HE (depression of neuropsychological function and extrapyramidal symptoms) and suggest possible targets for treatment (97-99). Studies of neuronal activity have been used to shed light on the pathophysiology of HE, while imaging of a potentially crucial process in HE, neuroinflammation, using the radioligand C-11-PK11195 to detect peripheral benzodiazepine receptors (PTBR), which are present on a number of cell types, including activated microglial cells, is gaining increased attention (100-102).

Molecular imaging using SPECT requires a molecular marker that is labelled with a radionuclide, which results in the emission of gamma ray photons or high-energy X-ray photons (103). Cerebral blood flow is most commonly assessed by using ^{99}Tc or ^{133}Xe radioactive tracers. Despite SPECT being more readily available and cheaper than PET, the latter is preferred in functional imaging studies due to its superior spatial and temporal resolution (86). Previous studies have demonstrated increased cerebral blood flow in the basal ganglia in patients with MHE, suggesting increased ammonia delivery to these areas, resulting in astrocytic dysfunction and cognitive alterations (104,105). This is in agreement with a ^{1}H MRS study by Taylor-Robinson and colleagues that demonstrated Glx concentration was highest in the basal ganglia (82). However, SPECT studies that have investigated HE have been thwarted by small study sizes, limiting the conclusions that can presently be drawn from the use of this functional imaging tool.

4 Summary

In a patient with liver disease and neurological impairment, a CT scan and/or preferably, MRI of the brain can rule out other diagnoses and provide some corroborative evidence of HE. More research-based modalities such as MRT, DTI, MRS, PET and SPECT have provided valuable insight into the pathogenesis of HE, but have not been transformed into widely available diagnostic tools. Prior to this transformation, there must be consensus with regards to uniformity of study protocols, imaging sequences and analysis methodologies, both in MRI/MRS and in PET/SPECT, where promising functional data are emerging. In the future, a quantitative MR technique is most likely to give objective, reproducible and longitudinal diagnostic information in this common but under recognised complication of liver disease.

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Imaging technique	Availability	Findings in Hepatic Encephalopathy	Recommended in the clinic
CT Brain	Standard	Cerebral edema in ALF Rules out some common differential diagnoses	Yes
MRI brain			
T_1-weighted	Standard	Basal ganglia hypersensitivity (not HE specific) Cerebral oedema in ALF Quantitative brain volume by research statistical methods	Yes (not in ALF)
T_2-weighted FLAIR	Standard	White matter lesions in cases of low grade HE	Yes (not in ALF)
DWI	Standard	High ADC in CHE Low ADC in ALF	
Diffusion tensor imaging	Research	Increased mean diffusivity in corticospinal tracts	No
Magnetisation Transfer	Available in specialist centres	Low MTR in white matter	No
^{1}H MR Spectroscopy	Available in specialist centres	High Glx (glutamine/glutamate) Low myoinositol and choline in basal ganglia and frontal white matter	No
Functional MRI	Research	Deactivation of the default mode network including the anterior cingulate	
Nuclear medicine			
PET	Research in HE	Reduced glucose uptake in the anterior cingulate	No
SPECT	Research in HE	Increased blood flow to the basal ganglia	No

Table 1: Approach to different imaging modalities in HE in clinical and investigative use

- CT Brain can demonstrate edema in ALF and assists in the differential diagnosis of neurological impairment in cirrhosis
- MR Brain is not recommended routinely in ALF but can assist in the diagnosis of HE in cirrhosis where it is the preferred method of brain imaging
- Quantification of cerebral metabolites or brain water is possible but not yet widely used diagnostically
- PET/SPECT are powerful but expensive research tools

Box 1: Clinical and research imaging techniques applied in the diagnosis of hepatic encephalopathy

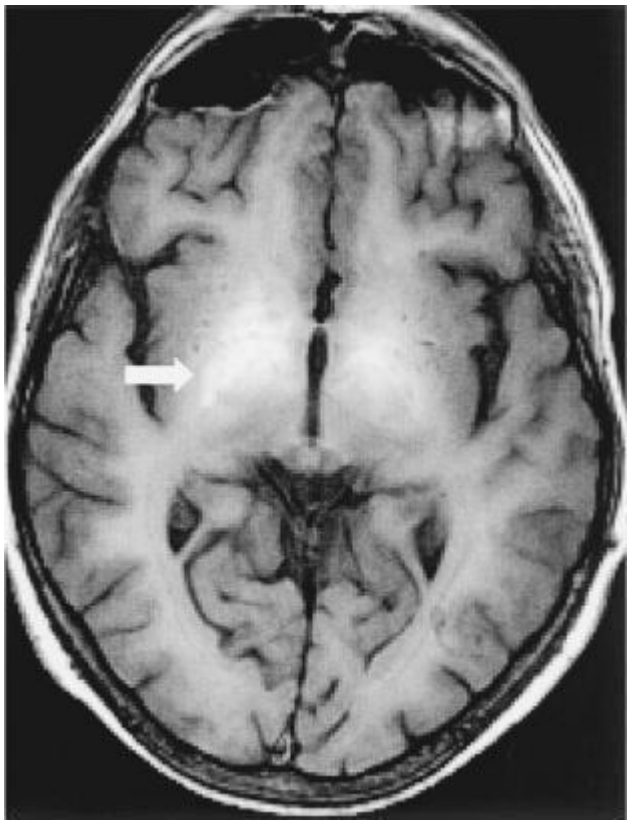


Figure 1: T_1-weighted magnetic resonance image of the brain of a patient with cirrhosis. The arrow demonstrates the area of pallidal hyperintensity. Reproduced from Córdoba J, *et al*. ^{1}H magnetic resonance in the study of hepatic encephalopathy in humans. Metab Brain Dis 2002;17(4):415. With permission from Springer Inc.

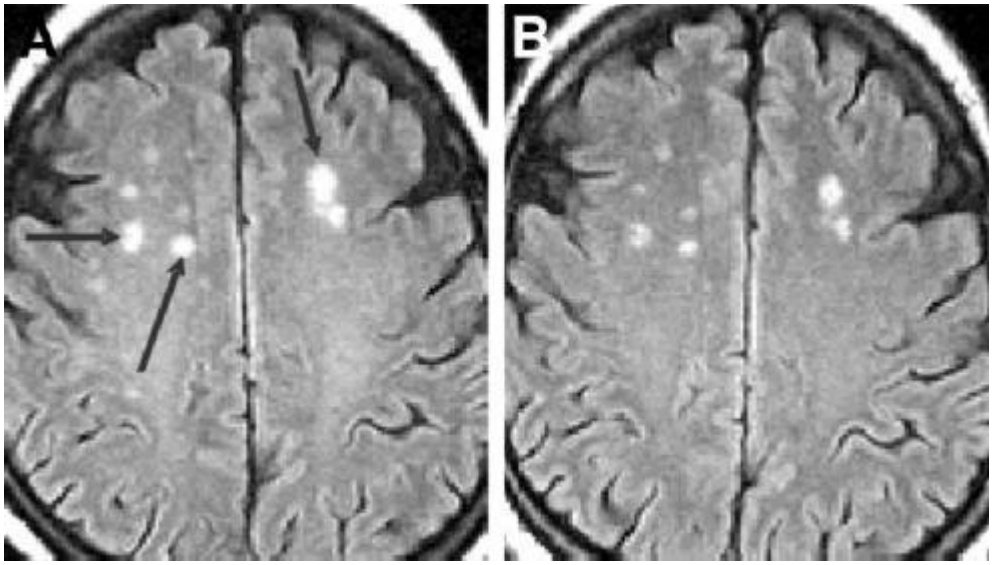


Figure 2: A. Baseline fast-FLAIR MRI image of a hepatitis C patients prior to liver transplantation. Focal lesions can be visualized in the subcortical white matter (arrows). B. Same MRI study 6 months post-liver transplant in the same patient; there is a noticeable decrease in the size of focal white matter lesions. Reproduced from Rovira A, *et al*. Decreased white matter lesion volume and improved cognitive function after liver transplantation. Hepatology 2007;46(5):1485. With permission from John Wiley and Sons Inc.

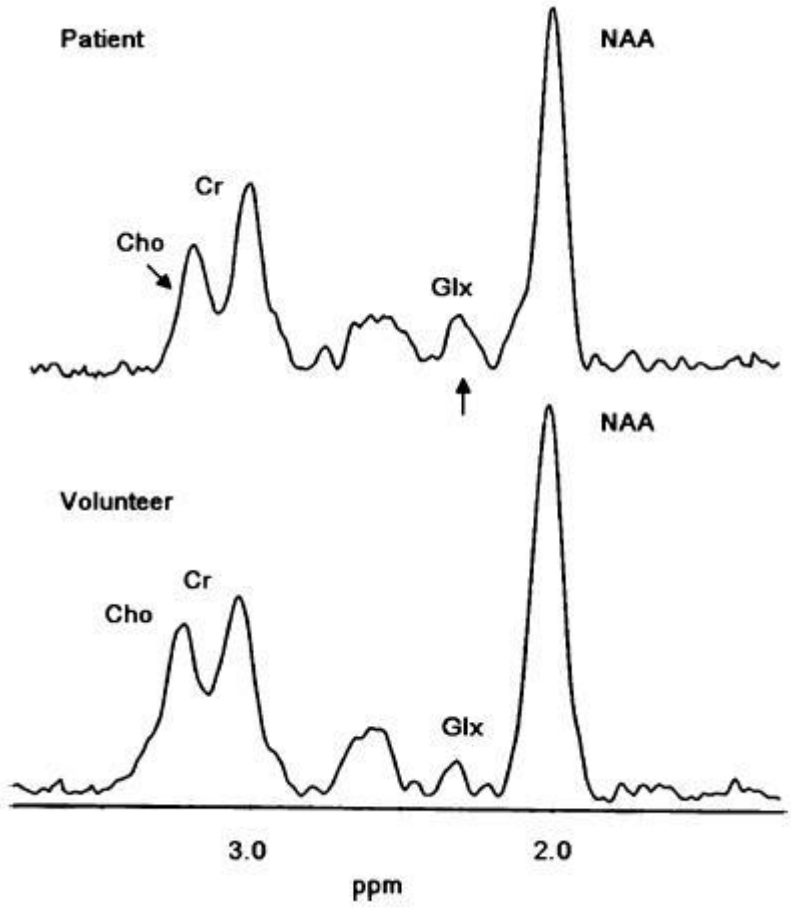


Figure 3: ^{1}H MR spectra from the basal ganglia of a patient with chronic hepatic encephalopathy and healthy volunteer, demonstrating decreased choline/creatine ratio and increased glutamate/glutamine resonance in the CHE patient. Cho: choline; Cr: creatine; Glx: glutamine/glutamate; NAA: N-acetyl aspartate. Reproduced from Taylor-Robinson SD, *et al*. Regional variations in cerebral proton spectroscopy in patients with chronic hepatic encephalopathy. Metab Brain Dis. 1994 Dec;9(4):347-59 With permission from Springer Inc.

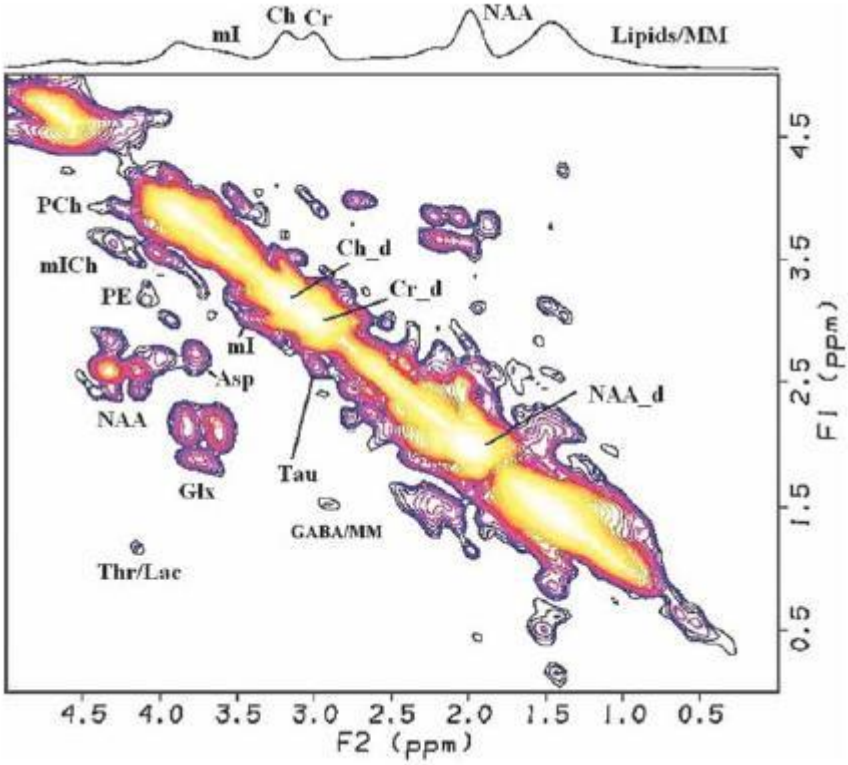
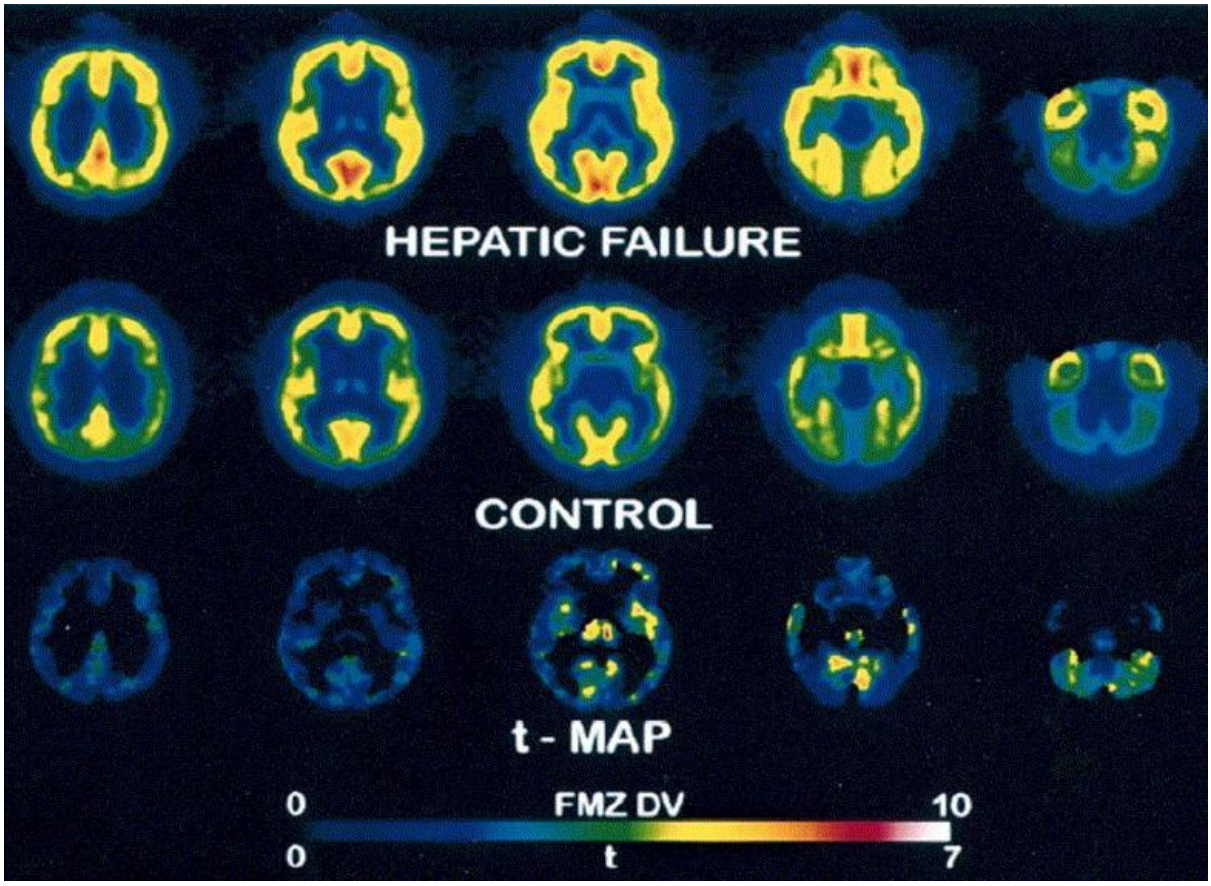


Figure 4: The 2D-COSY spectrum from the occipital lobe of a 51-year-old patient with minimal hepatic encephalopathy. Cr: creatine; Ch: choline; Glx: glutamate/glutamine; NAA: N-acetyl aspartate; Asp: aspartate; PE: phosphoethanolamine; PCh: phosphocholine; mI: myoinositol; Tau: taurine; ThrLac: overlapping cross peaks of threonine and lactate; GABA: gaba-aminobutyric acid; mICh: overlapping cross peaks of *myo*-inositol and choline; MM: macromolecules. Reproduced from: Singhal A *et al*. Two-dimensional MR spectroscopy of minimal hepatic encephalopathy and neuropsychological correlates *in vivo*. Journal of magnetic resonance imaging 2010;32(1):35. With permission from John Wiley and Sons Inc.



Fi Figure 5: Analyses of benzodiazepine binding in patients with hepatic failure (top row), control (middle row), and voxel-by-voxel comparison of between-group differences (bottom row). The greatest changes in distribution volume of flumazenil was seen in the cerebellum. Reproduced from MacDonald GA, *et al*. Cerebral benzodiazepine receptor binding *in vivo* in patients with recurrent hepatic encephalopathy. Hepatology 1997;26(2):277. With permission from John Wiley and Sons Inc.

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