Brain Edema in Chronic Hepatic Encephalopathy

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Brain edema is a common feature associated with hepatic encephalopathy (HE). In patients with acute HE, brain edema has been shown to play a crucial role in the associated neurological deterioration. In chronic HE, advanced magnetic resonance imaging (MRI) techniques have demonstrated that low-grade brain edema appears also to be an important pathological feature. This review explores the different methods used to measure brain edema *ex vivo* and *in vivo* in animal models and in humans with chronic HE. In addition, an in-depth description of the main studies performed to date is provided. The role of brain edema in the neurological alterations linked to HE and whether HE and brain edema are the manifestations of the same pathophysiological mechanism or two different cerebral manifestations of brain dysfunction in liver disease are still under debate. *In vivo* MRI/magnetic resonance spectroscopy studies have allowed insight into the development of brain edema in chronic HE. However, additional *in vivo* longitudinal and multiparametric/multimodal studies are required (in humans and animal models) to elucidate the relationship between liver function, brain metabolic changes, cellular changes, cell swelling, and neurological manifestations in chronic HE. (J Clin Exp Hepatol 2019;9:362–382)

Brain edema is defined as an excessive accumulation of fluid (chiefly water) in the intracellular or extracellular spaces of the brain, which occurs on the background of an osmotic gradient. The pathological process is a complex phenomenon to measure and characterize, because it can be the result or effect of a certain disease or cerebral injury, but can also cause pathology or aggravate an existing disease process. The measurement of brain edema can be used to aid diagnosis and/or to measure targeted treatment effects. It is now well accepted that brain edema is a common feature associated with hepatic encephalopathy (HE).

Net fluid entry to the brain from the vascular compartment (vasogenic edema) increases the brain volume, raises intracranial pressure, and potentially leads to fatal brainstem compression in the most severe, acute form.[1] Vasogenic edema mainly occurs because of a breakdown of the tight endothelial junctions that make up the blood-brain barrier (BBB),[2] while a disruption in cellular metabolism impairs functioning of the sodium and potassium pump in the glial cell membrane and causes accumulation of osmotically active molecules, leading to cellular retention of sodium and water and consequently to cytotoxic edema.[2–4] Although cytotoxic edema refers to intracellular swelling (an isolated fluid shift from the interstitial to the intracellular, cytosolic compartment with no net fluid entry to the brain), it can also occur following an increase in permeability (not physical breakdown) of the BBB. It is not unreasonable to assume that this pathological process is accompanied by some degree of net brain edema.[1,2] This astrocytic swelling, accompanied by a shift of fluid from the interstitial/intravascular compartment to the intracellular (astrocytic) compartment, can lead to detrimental effects. The molecular mechanisms leading to astrocyte swelling are not yet fully understood and are believed to be linked with osmo-sensitive or stretch-sensitive intracellular signaling cascades, involving $[Ca^{2+}]_i$ transients, aquaporins (AQPs) and volume-regulated anion channels.[5–7] Astrocytes have a strategic perivascular location and high

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Abbreviations: ADC: apparent diffusion coefficient; ALF: acute liver failure; AQP: aquaporins; BBB: blood-brain barrier; BDL: bile duct ligation; CNS: central nervous system; Cr: creatine; CSF: cerebrospinal fluid; DTI: diffusion tensor imaging; DWI: diffusion-weighted imaging; FLAIR: fluid-attenuated inversion recovery; Gln: glutamine; Glx: sum of glutamine and glutamate; GM: gray matter; HE: hepatic encephalopathy; 1H MRS: proton magnetic resonance spectroscopy; Ins: inositol; Lac: lactate; LPS: lipopolysaccharide; MD: mean diffusivity; mIns: myo-inositol; MRI: magnetic resonance imaging; MRS: magnetic resonance spectroscopy; MT: magnetization transfer; MTR: MT ratio; NMR: nuclear magnetic resonance; PCA: portocaval anastomosis; tCr: total creatine; tCho: total choline; TE: echo time; WM: white matter

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water permeability, and therefore their membrane is believed to be the main source of water entry in the brain.[1] Moreover, water transport is the primary function of the main AQPs (plasma membrane water-transporting proteins) in the central nervous system (CNS). AQP-4 is expressed in astrocytic feet, lining the microcapillary endothelial cells of the BBB, and it is involved in water movement, cell volume regulation, cell migration, and neuroexcitation.[6,8] Accordingly, increased expression of AQP-4 has been shown to correlate with the development of brain edema in several diseases.[1,6]

Pathologically speaking, HE is characterized by astrocyte swelling, leading to brain edema. In acute HE (encephalopathy associated with acute liver failure [ALF][9]), brain edema occurs in the majority of patients to some degree and contributes to increased intracranial pressure, which can lead to brainstem herniation in the most severe cases.[10–13] In chronic HE (encephalopathy associated with cirrhosis and portal hypertension/or portal-systemic shunts[9]), magnetic resonance imaging (MRI) techniques have demonstrated that low-grade brain edema appears also to be an important pathological feature, even though intracranial hypertension is rarely observed[2,14–19] (for more details please see Tables 1–3). Edema in acute HE is believed to be mainly cytotoxic,[10,11] whereas in chronic HE, low-grade edema is also associated with Alzheimer type II changes as a morphological counterpart of astrocyte swelling.[20] It is important to emphasize that labeling a particular case of edema as "vasogenic" or "cytotoxic" cannot be rigidly applied, since it is unusual for only one of the two mechanisms to exist in isolation.[21] Overall, one type of edema will gradually lead to the development of the other type. This is also the case in HE where the two types of edema might coexist.[2,22] Nevertheless, knowledge of the relative contribution of these two mechanisms in the various phases of edema development might be useful in understanding the dynamics of brain edema and theoretically, in designing useful means of clinical management.

There are many studies implicating brain edema in the pathogenesis of HE; in patients with acute HE, brain edema has been shown to play a crucial role in the associated neurological deterioration.[13] Patients who have cirrhosis with chronic HE may present with some degree of brain edema,[14–19] but it is not known if this is a universal finding. In addition, the correlations/associations between brain edema and neurological damage in chronic HE are not yet clearly established, with some studies showing a correlation and others not (for more details please see Tables 1–3). This leads to the controversial question as to whether brain edema can be considered a valid endpoint in the evaluation of HE.[1,23] By extension, in rats with bile duct ligation (BDL), a type-C model of HE, brain edema, and HE are present.[24] Other studies suggest that brain edema is not implicated in the pathogenesis of HE; in BDL rats, brain edema was also shown to be absent[25] with no modifications in their neurological status 4–6 weeks after surgery,[26,27] while lipopolysaccharide (LPS) injection was shown to increase water content[26] and alter the level of consciousness in these rats[26] (for more details please see Tables 1 and 2). Moreover, in rats with portocaval anastomosis (PCA), a type-B model of HE (encephalopathy associated with portal-systemic bypass and no intrinsic hepatocellular disease[9]), brain edema is not present.[24,28] Finally, in rats with ALF, it was shown that motor tract function did not improve following attenuation of brain edema with the hypertonic solution, mannitol,[27] while an acute injection of ammonia to PCA rats led to severe alterations of motor tract function, without the development of brain edema.[27] It has been suggested that these discrepancies might be model specific (HE type A vs B vs C), since cerebral edema differs in terms of the temporality of the disease.[1,2,29] In chronic HE, there is sufficient time for effective compensation and stabilization of the osmolyte shift to counteract the osmotic imbalance induced by the astrocytic accumulation of glutamine. In acute HE, the natural history of the syndrome is rapid and does not allow the system to compensate for metabolic changes.[29] Moreover, in advanced chronic HE, there might be little room for activating additional volume-regulatory mechanisms against future challenges of cell volume (such as infection or neuroinflammation), which might explain the kinetics of HE occurrence and the episodic or persistent appearance of clinically overt cerebral edema in end-stage liver disease.[30] Nevertheless, all these assumptions remain to be determined.[1] Moreover, these results raise the question as to the role of brain edema in the neurological alterations linked to HE and whether HE and brain edema are the manifestations of the same pathophysiological mechanism or of two different cerebral manifestations of brain dysfunction in liver disease. It has been also postulated that brain edema may be a predisposing factor in the development of HE or a terminal complication.[1,2]

METHODS TO MEASURE BRAIN EDEMA *EX VIVO* AND *IN VIVO* IN ANIMAL MODELS AND HUMANS WITH CHRONIC HE

Several methods have been used to measure brain water content and consequently brain edema either *ex-vivo* or *in vivo*. Some of these methods will be briefly described below, and a summary of the main results published to date are listed in Tables 1–3.

***Ex vivo* measurements** of water content using dissected tissue from sacrificed animals (no studies on human HE patients) are performed using the dry/wet weight technique or the specific gravity method.[1,31,32] The advantage

Table 1 Summary of the Main Results Published to Date Using Different *Ex Vivo* Techniques in Chronic HE Animal Models.

Animal model	Subjects (n)	Method	Brain region	Type of measurement	Findings			Comments	Ref
					Edema	Type of edema Cell type	Other		
BDL rats	8	Gravimetry, 3 weeks post-BDL	CC, 2mm^2	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	No change in **water content** = 79.73±0.12%	No change in **plasma** and **brain ammonia** (122±70 μmol/L in plasma and 0.29±0.18μmol/g in brain of BDL)	133
	9	GFAP staining	FC, PC	*Ex-vivo*, end point	Indirect indication	Direct evidence, astrocytes	No changes in **GFAP** staining in BDL rats	Mild impairment of motor coordination and a ↓spontaneous motor activity in BDL rats	
Sham rats	8-10	HPLC – osmolytes		*Ex-vivo*, end point					
		Behavior studies					Minor and non-significant changes in brain **Gln** and **Ins**	LPS: ↑brain water content and Alzheimer type II astrocytes	
BDL rats Sham rats	7 6	Gravimetry, 4 weeks post-BDL	FC, CC – 2mm^2	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	No change in **water content** = 79.9±0.27%	↑ **plasma** (168±14μmol/L) and **brain** (1.0±0.36μmol/g) **ammonia** No neurological modifications in BDL rats	26
		Ex-vivo ^{1}H MRS, *no information on quantification*		*Ex-vivo*, end point			↓ **Gln, NAA**	*Among the very few reports showing a* ↓***Gln***	
		Electron microscopy Assessment of level of consciousness		*Ex-vivo*, end point		Direct evidence-cytotoxic edema, astrocytes	Partially collapsed microvessel Intact BBB	Minimal water accumulation in astrocytic, perivascular tissue LPS injection ↑brain water content and lead to a deterioration of tin the conscious level	
BDL rats Sham rats	6 6	Gravimetry, 6 weeks post-BDL	FC, 2mm^3	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	↑**water content** = 79.46±0.28% (BDL) vs 78.35±0.17% (sham)	↑ **arterial** (119.7±15.2μM) and **CSF** (128.4±36.7μM) **ammonia** HA does not induce OS independently nor brain edema	24
		Locomotor activity					Allopurinol treatment decreased arterial ROS and brain edema but did not improve liver function nor fully restored locomotor activity-edema is not the only cause of HE	In combination systemic OS and HA stimulate an ↑**water content** Systemic OS is a result of primary liver injury	
BDL rats Sham rats	7 6	Gravimetry, 6 weeks post-BDL	FC, 1mm^3	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	-no significant change in **water content** between BDL and sham rats	*Exact water content difficult to assess from the graph = 81.5-82.5% (BDL)* LPS injection ↑brain water content	25

Table 1 (*Continued*)

Animal model	Subjects (n)	Method	Brain region	Type of measurement	Findings			Comments	Ref
					Edema	Type of edema Cell type	Other		
BDL rats Sham rats	*No indication on number of rats was found*	Gravimetry, 6 weeks post-BDL	FC	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	↑**water content**	*Exact water content was difficult to assess from the graph = 78-79% (BDL)* Correlations: No correlation between **CSF ammonia** and **brain Gln** Correlation between CSF ammonia and brain Lac ↑**brain Lac** and not **Gln** is a key factor in pathogenesis of brain edema together with impaired compensatory osmoregulatory mechanisms	95
		Ex vivo ^{1}H MRS, *no information on quantification* *Ex vivo* fluorescence		*Ex-vivo*, end point			↑**Gln, Glu, Tau** ↓**Ins** ↑sum of osmolytes ↑**brain Lac**, ↑**CSF ammonia** AST-120 and DCA treatments ↓ brain edema, Lac but not brain Gln Only AST-120 ↓ CSF ammonia		
BDL rats Sham rats	6 groups (6/group) 3 groups (6/group)	Dry weight technique, 4 weeks post-BDL	50 mm^2 wet FC	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	No change in **water content** in BDL rats ↑water content in shams +HD and shams+LPS ↑water content in BDL+HD and BDL+HD+LPS	↑**arterial and brain ammonia** in HD and BDL rats; and ↓ after OP (±infliximab) ↓arterial ammonia with OP may prevent LPS induced worsening of HE and brain edema. *Exact values of water content and ammonia were difficult to assess from the graphs*	134
		Assessment of level of consciousness					↓ water content after administration of OP and OP + infliximab		
BDL rats Sham rats	9 groups (6-8/group) 2 groups (7/group)	Dry weight technique, 4 weeks post-BDL *Ex vivo* ^{1}H MRS, *no information on quantification*	50 mm^2 wet FC (GM)	*Ex-vivo*, end point	Direct, absolute assessment of water content	N/A	↑**plasma ammonia** in BDL rats (67±6 to 186±20 μmol/L) ↑**water content** in BDL rats No change in **brain Gln** in BDL rats ↓ **brain mIns** in BDL rats OP treatment: ↓brain water content and plasma ammonia, no change in brain Gln or mIns,	*Exact values of water content were difficult to assess from the graphs (~76% in Shams and ~78% in BDL)*	135

Abbreviations: Frontal cortex (FC), Cerebral cortex (CC), parietal cortex (PC), gray matter (GM), oxidative stress (OS), reactive oxygen species (ROS), blood brain barrier (BBB), hepatic encephalopathy (HE), cerebrospinal fluid (CSF), lactate (Lac), glutamine (Gln), taurine (Tau), inositol (Ins), myo-inositol (mIns), glutamate (Glu), lipopolysaccharide (LPS), hyperammonemia (HA), glial fibrillary acidic protein (GFAP), bile duct ligation (BDL), ornithine phenylacetate (OP), oral ammonia absorbent engineered activated carbon microspheres (AST-120), dichloroacetate (DCA), proton magnetic resonance spectroscopy (^{1}H MRS), high protein/ammoniagenic diet (HD). *Authors personal comments are in italics in the comments row.*

of these two techniques is that both of them allow a direct/absolute estimation of the water amount in the brain. However, these techniques do not provide any information on the type of edema and they are endpoint measurements. Therefore, no longitudinal measurements on the same animal are possible. Table 1 presents a summary of the results published to date on type C HE animal models, while more details on these two techniques can be found in the published literature.[1,2] The gravimetry technique appears to be most widely used and to have some advantages, such as a better specificity, together with the possibility of being able to use a smaller quantity of samples.[1,2] However, at the time of writing, there are only a few published studies using these techniques, and the results appear to be controversial. At 3 or 4 weeks post-BDL, no increase in water content was measured in BDL rats using the gravimetry technique, while an increase in water content at 4 weeks post-BDL was measured using the dry-wet technique (from ~76% in sham operated rats to ~78% in BDL rats). At 6 weeks only, one group measured an increase in brain water content using the gravimetry technique (from 78.35 ± 0.17% in sham-operated rats to 79.46 ± 0.28% in BDL rats), while others did not observe this (please see Table 1 for more details).

***In vivo* measurements of water content** use several MRI or magnetic resonance spectroscopy (MRS) techniques, which have the main advantage of being non-invasive and thus allowing studies on the same individual longitudinally. The phenomenon of nuclear magnetic resonance (NMR) is based on the interaction of magnetic moments of nuclei of different atoms within the main (static) magnetic field (B_0, usually expressed in Tesla). The magnetic moment of nuclei is associated with a nuclear spin (a form of angular momentum) characterized by a value called a spin number. The nucleus is defined by its number of protons and neutrons and its total nuclear spin. Nuclei with an odd number of protons or neutrons possess a non-zero spin and magnetic moment. Some of these nuclei have a spin number of ½ (e.g. ^{1}H, ^{31}P, ^{13}C, and ^{15}N), which is favorable for applications of magnetic resonance.[33] MRI is mainly focused on imaging the hydrogen nucleus (^{1}H) of water, since water is present in high concentrations in biological tissues, and ^{1}H is the most sensitive nucleus in terms of high natural abundance (>99.9%) and intrinsic sensitivity (high gyromagnetic ratio), leading to a high signal-to-noise ratio. MRI techniques are presently available to detect subtle functional or structural changes in the human brain. The only MRI method allowing a direct *in vivo* water content measurement is brain water mapping, and this technique appears to be able to detect changes of approximately 1% in total brain water content, but it lacks specificity in relationship to the etiology of the water accumulation.[34] Indirect or relative information regarding the content of water in the brain can be obtained using magnetization transfer (MT), diffusion-weighted or diffusion-tensor imaging (DWI or DTI), fast fluid-attenuated inversion recovery (FLAIR) MRI methodologies and MRS. All these techniques can provide some evidence of increased water content in HE, but they lack specificity in drawing conclusions about absolute water content changes, in addition to elucidating the origin of these perturbations in the brain. Therefore, these changes provide insight and pointers toward pathological mechanisms but are mainly interpretable simply as imaging manifestations of brain edema.[1,14–18,35]

Volumetric MRI in Chronic HE

MRI-based brain volumetry has been used in chronic HE to identify volume changes in a quantitative manner (total brain volume and/or specific brain regions) from T_1-weighted structural MR images (Table 3). These volumetric methods are mainly based on brain segmentation (separation into non-brain and brain tissue, with the latter being sub-segmented into gray matter [GM], white matter [WM], and cerebrospinal fluid [CSF]).[36] As the position of the patient and, possibly, the shape and size of the brain are likely to have changed between examinations, co-registration is needed in longitudinal assessments, and this involves several MRI head images as a starting point. Advanced software packages can align or register brain images and delineate or segment tissue boundaries between CSF, cerebral WM, and GM.[37] The final images can then be used for volumetry or morphometry measures.[36,38]

Qualitative visual assessment of cerebral edema on MRI is usually only possible in ALF.[39] In minimal chronic HE, quantitative assessment of small percentage volume changes is only possible with advanced brain mapping software packages, where the conflicting effects of alcohol or age-related atrophy are assessed alongside the resultant changes in brain size due to HE. Several software packages are available for performing brain segmentation and volumetry/morphometry (including FSL software library, 3D slicer, SIENA, and SIENAX).[36,40–45] More details on the methodology behind brain volumetry in the context of HE can be found in the published literature.[14,15,17,18,46]

The main volumetric MRI results obtained in chronic HE are summarized in Table 3. Some studies have shown a decrease in brain volume in HE[47,48] mainly in GM while others have not.[49–52] In addition, a relationship between brain volume and HE was sometimes observed.[47,53] It is important to underline that functionally well-compensated patients with cirrhosis showed no brain volume changes. There are a few reasons that could explain these discrepancies: the small number of studies performed to date and the small percentage volume changes associated with chronic HE, where the usage of higher magnetic fields might be more illuminating. The changes in brain volume measured in chronic HE were mainly associated with brain

Table 2 Summary of the Main Results Published to Date Now Using Different *In Vivo* MRI/MRS Techniques in Chronic HE Animal Models.

Animal model	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema	Type of edema Cell type	Other		
BDL rats **Sham rats**	8 6	7T	^{1}H MRS, PRESS, TE=12ms 7 metabolites quantified using LCModel, absolute quantification using water as internal reference DTI, 20 directions and 4 b-values (0-1000 s/mm^2)	6.5x6.5x6.5mm^3 - No brain region specific VC, SC, MC, Hip, Tha, HypoT, Str, NC	*In vivo* Longitudinal @ 4, 5, 6 weeks post-BDL	Indirect indication In LPS – indication of intra and extra cellular edema supported by no changes in ADC	N/A	↑**Gln** ↓**Glu, tCho, tCr, NAA and Ins** No change in **Lac** -No difference in **ADC** values between BDL and sham operated rats and neither in water content using gravimetry (Table 1)	*Statistical changes are between-group over the entire time course with LPS injections as last time point and not by individual time points* LPS injection ↑water content in brain (gravimetry-Table 1)	25
BDL rats	7	9.4T	^{1}H MRS, SPECIAL, TE=2.8ms 18 metabolites quantified using LCModel, absolute quantification using water as internal reference Changes post-BDL always compared to those before BDL (week 0)	4x7.5x6.5mm^3 - No brain region specific	*In vivo* – longitudinal @ 0, 4, 8 weeks post-BDL	Indirect indication	N/A	↑**Gln** and **plasma NH_4^+** post-BDL ↓**Ins, tCho** @ 8 weeks post-BDL ↓**Glu, Asp** @ 8 weeks post-BDL ↑Sum of main brain organic osmolytes @ 8 weeks post-BDL	Positive correlation between brain Gln and plasma NH_4^+ Brain Gln showed stronger correlations than plasma NH_4^+ with the rest of metabolites	96

Abbreviations: visual cortex (VC), sensorimotor cortex (SC), motor cortex (MC), hippocampus (Hip), thalamus (Tha), hypothalamus (HypoT), striatum (Str), nucleus accumbens (NC), lactate (Lac), glutamine (Gln), taurine (Tau), inositol (Ins), glutamate (Glu), total choline (tCho), total creatine (tCr), N-Acetylaspartate (NAA), aspartate (Asp), lipopolysaccharide (LPS), bile duct ligation (BDL), diffusion tensor imaging (DTI), proton magnetic resonance spectroscopy (^{1}H MRS), apparent diffusion coefficient (ADC), SPin ECho, full Intensity Acquired Localized (SPECIAL), point resolved spectroscopy (PRESS), echo time (TE). *Authors personal comments are in italics in the comments row.*

Table 3 Summary of the Main Results Published to Date Using *In Vivo* MRI/MRS Techniques in Chronic HE Patients.

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Liver cirrhosis of different origins HE I+HE II =overt HE	13-HE-0 12-MHE 10-HE I 3-HE II	1.5T	Fast absolute measurement of cerebral water content, TAPIR – T_1 measure QUTE – quantitative T_2* image Psychometric testing	Pu, CR, OWM, FWM, OC, FC, Tha, GP, CN, AL, PL	*In vivo* - Single point	Direct, absolute assessment of water content (%)	N/A	-↑**0.4% water** in HE-0, ↑0.8% in MHE, ↑**2.1% in overt HE** – WM (FWM, OWM) -No significant water content changes in GM, however 1.9%↑ in GP for overt HE	Correlation between CFF and WM water content	34
Mild chronic HE Controls	3 7	1.5T	^{1}H MRS, STEAM, TE=30ms, quantification of 5 metabolites using the scanner data analysis package and ratios to tCr	Midparietal cortex, WM+GM, 12.5-27cm^3	*In vivo* - Single point	N/A	N/A	-trend of ↑**Gln** and ↓**Cho** and Ins	-no statistics due to small number of patients	136
Liver cirrhosis of different origins Controls	5-no HE 10-mHE 11-overt HE 14	1.5T	T_1 weighted images 2D CSI, TE=130ms quantification of 3 metabolites using ratios to Cr Psychometric and EEG testing	BG, temporal and occipital cortex	*In vivo* - Single point	N/A	N/A	- ↑**Glx/Cr** and ↓**tCho/Cr** in patients - no change in NAA/Cr - stronger ↑**Glx/Cr** in BG - stronger ↓**tCho/Cr** in occipital cortex	- patients with no HE – normal spectra - patients with overt HE – abnormal spectra	137
Liver cirrhosis of different origins	4-no HE 7-mHE 15-overt HE	1T	T_1 weighted SE images T_1 weighted MT images	BG	*In vivo* - Single point	N/A	N/A	Hyperintensity of GP in 17 patients, and a difference between noHE vs mHE vs overt HE Hyperintensity of Pu in 5 patients	Relationship between T_1 contrast in GP and blood ammonia	138
Liver cirrhosis of different origins Controls	24-no HE 4-mHE 4-HE I 6-HE II 1-HE IV 20	2T	Routine T_1 and T_2 weighted images ^{1}H MRS, PRESS, TE=30ms, quantification of 4 metabolites using a Marquardt curve-fitting algorithm and ratios to Cr Neuropsychological tests	PWM, OGM (2.5cm)^3	*In vivo* - Single point	Indirect indication based on ↓**mIns/Cr** and ↑**Gln/Cr**	assumption -Astrocytes swelling	Asymptomatic (no HE) patients GM: -↓**mIns/Cr** Subclinical (mHE), overt HE(HE I-IV) GM: -↓**mIns/Cr,** ↑**Gln/Cr** -↑**NAA/Cr** only in over HE Asymptomatic and subclinical HE WM: -↓**mIns/Cr** Overt HE (HE I-IV) WM: -↓**mIns/Cr,** ↑**Gln/Cr,** ↓**tCho/Cr**	Correlation between Gln in GM and plasma ammonium (r=0.62) No MRS differences between no HE and mHE MRS differences between mHE and overt HE ↑**Gln** and ↓**mIns** with HE grade	139
Liver cirrhosis of different origins Controls	8-HE 0 7-HE I 2-HE II 13	1.5T	^{1}H MRS, STEAM, TE=30ms, quantification of 4 metabolites using peak integration and ratios to Cr Neuropsychological tests	PWM, 18ml	*In vivo* and longitudinal: 30-60 days after LT or 2weeks after a low protein diet	N/A	N/A	-↓**mIns/Cr** and **tCho/Cr** in HE - no change in Glx/Cr - no MRS changes observed with diet - no MRS changes 30-60 days after LT	Correlations: mins/Cr and ammonia with the neuropsychological data	140
Liver cirrhosis of different origins	6-mHE 3-overt HE	1T	Coregistered 3D T_1 weighted images Semiautomated contour and thresholding program Neuropsychological tests, EEG	whole brain and ventricles	*In vivo* , longitudinal: 6weeks after lactulose (n=7), before and 24h after TIPSS	Indirect indication of low-grade brain swelling	N/A	No structural abnormalities on T_1 weighted images Change in brain and ventricular size after treatment: ↓**brain,** ↑**ventricles** and **improved psychometric** testing (n=3); ↑**brain,** ↓**ventricles** and **worsen psychometric** testing (n=2)	Blood ammonia (66-98 μmol/L - mHE; 85-130 μmol/L- overt HE) No correlations between MRI, HE and liver function	88

Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Liver cirrhosis of different origins Controls MHE	24-MHE 5-no HE 5-HE I 5-HE II 18 10	1.5T	DTI, single shot EPI dual SE sequence, b-value of 1000 s/mm^2, 10 directions, MD and FA measured Neuropsychological tests	CC, RIC, LIC, CN, Pu, FWM, OWM	*In vivo* Longitudinal: 3weeks after lactulose in 10 MHE and 10 controls	Indirect indication ↑**MD** suggestive of ↑interstitial brain water	Assumption	No HE - ↑**MD** in CN MHE - ↑**MD** in CC, RIC, LIC, CN HE - ↑**MD** in CC, RIC, LIC, CN, Pu, FWM, OWM -no changes in **FA** - ↓**MD** in MHE after lactulose treatment and no change in FA	**MD** ↑ from no HE to gr 2 HE-suggestive of increased water with HE grades Correlations between NP and MD in CC, RIC. Correlations between NP and MD in CC. Extracellular migration of macromolecules during the cellular osmoregulatory response may result in ↑ acculmulation of extracellular fluid	29
Viral liver cirrhosis Controls	7 –no HE 6-HE I 1-HE II 12	1.5T	DWI, b-values:0, 300, 600,900 s/mm^2	CN, Pu, GP, OWM, FWM, PWM, Tha	*In vivo* - Single point	Indirect indication of cytotoxic brain edema	Assumption	↑**ADC** in all brain regions except Tha Patient with HE II showed the highest ADC values No differences in ADC between no-HE and HE I Ammonia and related Gln accumulation might contribute to changes in water motility and content	Correlation between venous ammonia and ADC values in deep gray and WM regions, except CN An increase in cell volume reduces the influence of restriction effects on intracellular diffusion pathways leading to ↑ADC	64
Liver cirrhosis of different origins	9-HE 0 6-mHE 6-HE I	1.5T	T_1 weighted images ^{1}H MRS, STEAM, TE=18ms, quantification of 5 metabolites using peak integration and ratios to Cr ^{13}N –ammonia and FDG PET Psychometric examination	BG, PWM, FGM, 8cm^3	*In vivo* - Single point	N/A	N/A	MRS changes significant if patients divided into Child classes but not in HE classes -↓**mIns/Cr** in all 3 brain regions from Child A to C -↓**tCho/Cr** in BG, GM from Child A to C -↑**Glx/Cr** in BG, WM from Child A to C -↑**NAA/Cr** in WM from Child A to C	No controls Correlations: -psychometric HE score with Glx/Cr in BG -venous plasma ammonia with MRS in WM -cerebral glucose utilization with mIns/Cr	141
Liver cirrhosis of different origins	27	1.5T	T_2 weighted, FSE Fast FLAIR images Neurologic assessment	WM	*In vivo*, longitudinal: before and after LT	Indirect indication of brain edema	N/A	-focal lesions were identified on the T_2 weighted images before LT compatible with small-vessel brain disease in 19 patients - after LT (6-14 months)– average of 21.7% decrease of Wm lesion volumes	No association between WM lesion, age, cause of cirrhosis, Child-Pugh score or laboratory findings Correlation: WM lesions and percent improvement in overall cognitive function	90
Cirrhotic patients with HE	3	No detail	FLAIR images	WM	*In vivo*, longitudinal	Indirect indication of brain edema	N/A	-supratentorial focal and diffuse WM lesions compatible with small-vessel brain disease which reduced with improvement of HE	- these changes were associated with brain edema and support the participation of BBB in the pathogenesis of brain edema in HE	89

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Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Liver cirrhosis of different origins Controls	20-no HE 10-mHE 24	1.5T	DWI, single shot EPI sequence Neuropsychological tests	Pu, GP, Tha, posterior cingulate GM, FWM, PWM	*In vivo* - Single point	Indirect indication of minimal cellular edema		-↑ **ADC** in mHE in WM compared to no HE -no difference in noHE compared to controls for ADC values	Correlations: ADC in WM with venous ammonia; ADC in WM and neuropsychological tests minimal cellular edema with an increase of membrane permeability and increased intracellular diffusivity, as well as changes in the viscosity of the cytoplasm	65
Liver cirrhosis of different origins Controls	33-mHE 30	1.5T	Proton density, T_2 weighted images T_1 weighted images, MPRAGE sequence ^{1}H MRS, 2D L-COS, TE=30ms, quantification of 13 metabolites using Felix NMR software and ratios to Cr Neuropsychological tests	GP Occipital and prefrontal lobe, 27cm^3	*In vivo* - Single point	N/A	N/A	-↑GP signal intensity -↑**Glx/Cr** in both brain regions -↓**mICh/Cr, mIns/Cr** and **Ch_d/Cr** in both brain regions	Correlations between NP tests and MRS ratios mICh – most discriminant variable	142
Liver cirrhosis and overt HE Controls	41 16	1.5T	T_2 weighted, FSE T_1 weighted, SE DTI, single shot EPI sequence, 6 noncollinear directions, 11b-values (0-7500s/mm^2), mono and bi-exponential fitting Neuropsychological tests	PWM, corticospinal tract	*In vivo*, longitudinal: before and 1 year after LT (n=24)	Indirect indication of increased brain water content based on ↑**MD**	assumption interstitial edema	-↑**MD** for fast diffusion in PWM which returned to normal after LT -↓**FA** that increased after LT -↑**MD** for fast and slow diffusion in corticospinal tract, only fast MD returned to normal after LT -↓ **fast FA** in corticospinal tract with a persistent decrease after LT	- edema is reversible after LT but some microstructural changes might persist along the corticospinal tract as suggested by evolution of FA - extracellular edema - PWM - mixed edema -corticospinal tract No association between DTI parameters and neuropsychological tests	22
Viral cirrhosis Controls	28 28	3T	3D FLAIR sequence Brain volume, vertex based shape analysis – FIRST/FSL software Total intracranial volume – Gaser's VBM5 toolbox with SPM5 Neuropsychological tests	DGM (NC, Amy, CN, Hip, GP, Pu, Tha)	*In vivo*, single point	N/A	N/A	↓ volume in CN and Pu - a smaller volume was proportional to the severity of the disease -shape alteration in Pu, CN and GP	Correlations: decreased DGM volume with poorer cognitive results	47
Multiparametric studies / Multimodal studies										
Non-alcoholic cirrhosis Controls	24 (16 with mHE) 8	1.5T	T_2 weighted, FSE T_1 weighted, IR SE MT, 2D GE ^{1}H MRS, STEAM, TE=20ms, quantification of 5 metabolites using AMARES and ratios to tCr Neuropsychological tests	PWM; FWM Parietal WM, 8cm^3	*In vivo*, single point	Indirect indication of low grade intracellular swelling (↑ water content) based on ↓**MTR**	assumption	No changes in T_2 weighted images ↑T_1 signal intensity in BG and GP index ↓**MTR** in PWM and FWM ↑**Glx/Cr** in mHE only in PWM ↓**mIns/Cr** and **Cho/Cr** in all patients in PWM No changes in NAA/Cr	Correlations: MTR with Glx/Cr; MTR with GP index	56

Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Nalc cirrhosis without overt HE (70% mHE) After LT Controls	24 11 10	1.5T	T_2 weighted, FSE T_1 weighted, IR SE MT, 2D GE ^{1}H MRS, STEAM, TE=20ms, quantification of 5 metabolites using AMARES and ratios to Cr Neuropsychological tests	PWM; FWM Parieto-occipital WM, 8cm^3	*In vivo* Longitudinal: before and after LT at 1 month and 1 year	Indirect indication of low grade edema (↑ water content) based on ↓**MTR**	N/A	No changes in T_2 weighted images ↑T_1 signal intensity in BG ↓**MTR** in PWM and FWM ↑**Glx/Cr** in mHE only ↓**mIns/Cr** and **Cho/Cr** in all patients No changes in NAA/Cr After LT: improvement in MTR; normalization of ^{1}H MRS findings with a lower normalization for mIns/Cr; slower normalization of T_1 hyperintensity in GP; neuropsychological impairment showed a rapid improvement	Correlations between MTR and Glx/Cr and plasma osmolarity Glx/Cr and mIns/Cr correlated with liver and neuropsychological function No correlation between MTR and neuropsychological function Low grade edema and mHE are associated with ↑Gln –manifestations of metabolism of ammonia	57
PBC stage I-II PBS stage III-IV Controls	14 4 11	1.5T	SE proton density image MT ^{1}H MRS, PRESS, TE=135ms, quantification of 3 metabolites using the scanner software (Philips)	 GP, CN, Pu, Tha, FWM 8cm^3, in BG and WM	*In vivo* - Single point	N/A	N/A	↓**MTR** in GP No changes in ^{1}H MRS	Correlations between MTR and fatigue and MTR and blood manganese MTR changes are not a consequence of HE but rather of altered manganese homeostasis	143
Liver cirrhosis Alcoholics Nonalcoholics Controls	 26 16 18	1.5T	^{1}H MRS, STEAM, TE=20ms, 5 metabolites quantified using LCModel and ratios to Cr MT, 2D GE images DWI, single shot SE EPI, b-values: 0-500-1000 s/mm^2, 3 directions Neuropsychologic examination	Left OWM and BG, 8cm^3 Tha, pons, OWM, GP, Pu, CN Tha, pons, OWM	*In vivo*-single point	Indirect indication of ↑water content based on ↓**MTR**	N/A	Nalc group in BG: ↓**mIns/Cr**, **Cho/Cr** and ↑**Glx/Cr** Nalc group in OWM: ↓**mIns/Cr** and ↑**Glx/Cr, NAA/Cr** Alc group in BG: ↓**mIns/Cr**, **Cho/Cr** and ↑**Glx/Cr** Alc group in OWM: ↓**mIns/Cr** and ↑**Glx/Cr** MRS changes were significant for overt HE and similar in GM and WM ↓ **MTR** in both groups No change in **ADC** only a small trend of ↑ with increasing HE	Correlations in Nalc: mIns/Cr and Glx/Cr with HE in both regions and MTR with HE *Other correlations are presented* No correlations in Alc group MR differences between Alc and Nalc –possible microstructural lesions due to chronic alcohol abuse	144
Liver cirrhosis of different causes and overt HE Liver cirrhosis without overt HE Controls	24-overt HE 9 9	1.5T	DWI, b-values: 0-500-1000 s/mm^2 MT, 3D GE images ^{1}H MRS, TE=31ms, no sequence mentioned, 5 metabolites quantified using AMARES and ratios to Cr	GP, Pu, Tha, Hip, CR, PGM, PWM 2x2x2cm^3, PWM	*In vivo* Longitudinal:24h after diagnosis and 5 days after resolution of HE episode	Indirect indication of ↑water content/low grade edema based on ↓**MTR and ↑Glx/Cr, ↓Ins/Cr**	assumption	-No change in **mean ADC** between HE and non-HE patients ↓**MTR** in non-HE ↓↓**MTR** in HE in GP and PGM **Glx/Cr** –median =1.8 controls, 2.4 non-HE and 4.4 in HE. **Ins/Cr** – similar between HE and non-HE but lower than controls 5 days after no change in MTR, Glx/Cr, Ins/Cr but a ↓**ADC** in PGM	Correlation between MTR and Glx/Cr in WM in HE patients ↓**ADC** 5 days after – water flux from extracellular to intracellular compartment Brain regional difference – WM stronger water increase Small number of patients	145

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Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Liver cirrhosis no evidence of overt HE	24	1.5T	Proton density and T_2 weighted FSE T_1 weighted SE imaging - Brain volume – SIENAX from FSL ^{1}H MRS, PRESS, TE=30ms, metabolites quantified using LCModel and ratios to Cr Neuropsychological assessment (n=52)	Parieto-occipital WM, 8cm^3	*In vivo*, single point: 6 to 12 months post LT	N/A	N/A	Improvement in neuropsychological tests after LT except for 7 patients Brain smaller volume showed poorer function on motor tests **Bain metabolites were in normal range**	MRI and MRS data only after LT HE has an effect on cognitive function after LT, likely because it results in neuronal and brain volume loss	53
Stable liver cirrhosis of different causes (no-HE+mHE)	13	3T	3D T_1 weighted, T_2 weighted and FLAIR DTI, EPI, 2 b values:0-1000s/mm^2, 6 directions ^{1}H MRS, PRESS, TE=36ms, 6 metabolites quantified using QUEST/ jMRUI and water as internal reference Psychometric tests: PHES, CDRS	WM Frontal WM, 8cm^3	*In vivo* Longitudinal at 0, 140 and 170 min after ingestion of amino acid capsules	Indirect indication of in changes in brain water compartmentalization based on ↑**trADC**	N/A	No change in the CDRS after challenge ↑**trADC (9%)** after the challenge ↓**Ins** after challenge, no change in **Gln, Glu, NAA, Cr, Cho** **No change in brain volume**. Ammonia can directly drive changes in water distribution. No vasogenic mechanisms[146]	No controls Correlations: changes in trADC vs blood ammonia, changes in blood ammonia vs brain Gln, changes in trADC and brain Ins Glial swelling and redistribution of extra-intracellular water during HA – likely mechanisms of edema in HE[146]	51
Liver cirrhosis of different causes Controls	6-HE II 10-HE III 2-HE IV 8	3T	Proton density and T_2 weighted FSE and fast FLAIR T_1 weighted imaging DWI, single shot EPI, 4 b values:0-3000s/mm^2 ^{1}H MRS, PRESS, TE=30ms, 5 metabolites quantified using LCModel and ratios to Cr HE patients: lactulose and rifaximin-severity grades were lower for the MRI	PWM, corticospinal tract WM-parieto-occipital region, 8cm^3	*In vivo* –first 5 days after hospitalization Longitudinal – 6 weeks later (n=14)	Indirect indication of extracellular edema based on ↑**ADC** which returned to normal after 6 weeks	assumption	↑**ADC** in patients vs controls ↑**Gln/Cr** in HE patients vs controls (2.4±0.78 vs 0.22±0.08) ↓**Ins/Cr** and **Cho/Cr** No change for Glu/Cr and NAA/Cr ↓**ADC,** ↓**Gln/Cr** and ↑**Ins/Cr** after 6 weeks in patients recovering after HE **ADC** in PWM similar to controls but ↑ in corticospinal tract 6 weeks after	Correlations: Gln/Cr with HE grades, Gln/Cr and blood ammonia ↑ADC in patients with dehydration, ↓Ins/Cr in patients with hyponatremia Brain edema does not seem to be directly responsible for the neurological manifestation	23
Well-compensated liver cirrhosis of different causes and previous mHE Controls	22 21	3T	Volumetric imaging – 3D T_1weighted sequence, SIENA – FSL software FSL fMRI, visuomotor task ^{1}H MRS, PRESS, TE=36ms, 4 metabolites quantified using ratios to Cr Psychometric testing: CDRS, PHES	8cm^3, left BG	*In vivo* Longitudinal: 4weeks after LOLA	N/A	N/A	**No change in brain volume** No change in activation after visual task before and after LOLA Greater activation in motor task after LOLA **No Change in Glx/Cr, Cho/Cr, Ins/Cr, NAA/Cr** pre and post-LOLA	Improvements in CDRS and PHES after LOLA Correlations between the fMRI and psychometric tests	52

Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Liver cirrhosis with mHE	20	3T	DTI, single shot SE EPI, b=1000s/mm^2, 60 directions, FA, MD –FSL tool ^{1}H MRS, PROBE, TE=35ms, 4 metabolites quantified using LCModel and ratios to Cr fMRI, 2 tasks: N-back and inhibitory control tests Cognitive testing	12 ROI – e.g. FWM, pWM, CC, IC, EC, cingulum ACC; pGM, rpWM, 8cm^3	*In vivo* Longitudinal: before and 8 weeks after rifaximin treatment	N/A	↑**FA**, no change in MD, imply cytotoxic edema correction	No changes in **MD** Small ↑**FA** in 5 ROIs after rifaximin **No metabolite changes** before and after rifaximin Higher activation in some brain areas after rifaximin	Improvement in cognitive tests after rifaximin Improvement in WM integrity after rifaximin *No control or placebo group*	93
Liver cirrhosis with mHE or HE I Controls	30 16	3T	^{1}H MRS, MEGA-PRESS, TE=68ms, 4 metabolites quantified using LCModel and ratios to Cr Fast absolute measurement of cerebral water content[34] Psychometric tests	Occipital lobe, sensory and motor cortex–"hand knob", 27cm^3 each	*In vivo* - single point	Direct, absolute assessment of water content (%)	N/A	↑**Gln/Cr** in mHE and HE 1 in both voxels ↓**Ins/Cr** in mHE and HE 1 in both voxels compared to controls ↑**GSx/Cr** in mHE and HE 1 ↓**GABA/Cr** in mHE and HE1 in occipital lobe **No change in water content** MEGA-PRESS sequence was optimized for GABA and not glutathione.	Correlations: Gln/Cr with blood ammonia and CFF; Ins/Cr with ammonia and CFF, ↑GSx/Cr with ammonia *Several other correlations are mentioned* Edema is only marginally responsible for symptoms of covert HE	147
Liver cirrhosis Alc (n=46) Nalc (n=102) No Controls	 19-no HE 27-HE 48-no HE 44-HE	1.5T Two sites	T_1 weighted images (MPRAGE) -VBM using FSL-VBM DTI, single shot SE EPI, b=1000s/mm^2, 30 directions, FA, MD, CS –FSL tool ^{1}H MRS, PRESS, TE=35ms, 4 metabolites quantified using LCModel and ratios to Cr	13 ROI – e.g. FWM, pWM, CC, IC, cingulum ACC; pGM, rpWM, 8cm^3	*In vivo* Longitudinal: 1 year after	Indirect indication of interstitial edema based on ↑**MD and CS**	assumption	GM density reduced in Alc vs Nalc Alc vs Nalc: ↓**FA,** ↑**MD,** ↑**CS** in all ROI HE status affects Nalc (FA and CS) Alc vs Nalc: ↑**Glx,** ↓**Ins** (rpWM, ACC), ↓**Ins** (pGM) no HE: ↑**Glx,** ↓**Ins** HE: no difference In Nalc HE: ↑**Glx** in all 3 regions	No changes in brain metabolites 1 year later	148
Liver cirrhosis Controls	7-no HE 7-mHE 6	3T	T_2 weighted, FLAIR and T_1 weighted images (MPRAGE/SPGR sequence) DWI* MT* Neuropsychological tests Blood ammonia and cytokines	FWM, PWM, IC, BG	*In vivo* Longitudinal: 8 weeks after lactulose and rifaximin treatment	Indirect indication of low-grade brain edema in mHE based on ↓**MTR**	N/A	Diffuse atrophy–47.9% of patients Hyperintensity in BG-60.8% of patients No **DWI** results ↓ **MTR** in mHE in FWM, PWM, IC and BG compared to controls ↓ **MTR** in mHE compared to non HE – PWM, IC, BG ↑**MTR** after treatment except for BG in mHE No change in MTR in no HE after treatment	Correlations: -IL-6 with MTR in PWM and IC -ammonia with MTR in PWM -NP with MTR in PWM, IC -no correlations after treatment ↑ammonia in mHE and noHE with mHE>no HE ↑IL-1 and IL-6 in mHE	48

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Table 3 (*Continued*)

HE type	Subjects (n)	Magnetic Field (B_0)	Method	Brain region	Type of measurement	Findings			Comments	Ref
						Edema measurement	Type of edema Cell type	Other		
Cirrhotic patients of different causes	26	3T	Volumetric imaging – 3D T_1weighted sequence, T_2 weighted sequence		*In vivo*-single point	Indirect indication based on ↓**MTR** and ↑**ADC**	Assumption	**No change in total brain volume** ↑**ADC** in genus and body of CC No difference in FA	Trend of ↓MTR in mHE compared with other patients in FWM in GP Trend of ↓MTR in patients with alcohol-related disease ↓MTR and ↑ADC might demonstrate cytoplasmic changes of astrocytes Changes in astrocytes membrane permeability /redistribution of macromolecules	50
Controls	19		DTI, single-shot EPI sequence, 32 directions, b=1000s/mm^2, ADC and FA measured, DTI Studio software MT, 2D GE, ImageJ software Psychometric testing	Genu, body and splenium of CC, ACR, PCR FWM, Pu, GP, Tha, CN				↓**MTR** in GP (5.8%), FWM (4%), CN, Pu, 8 patients had mHE		
Well-compensated liver cirrhosis of different causes	22	3T	Volumetric imaging – 3D T_1weighted sequence, FMRIB software (FSL) T_2 weighted sequence DTI, single-shot EPI sequence, 15 directions, b=1000s/mm^2, ADC and FA measured, DTI Studio software MT, 2D GE, ImageJ software ^{1}H MRS, PRESS, TE=36ms, 5 metabolites quantified using AMARES and ratios to Cr Psychometric testing	FWM, Pu, GP, Tha, CN Genu, body and splenium of CC 15x15x15mm^3, left BG	*In vivo* Longitudinal: 4weeks after LOLA	N/A	N/A	**No change in total brain volume** No change in ADC or FA nor in their relation to neuropsychiatric status ↓**MTR** in GP, Tha in patients with cirrhosis ↓**MTR** in FWM only in mHE No change in metabolite ratios 7 patients out of 22 had mHE	Psychometric performance was improved in 4 mHE patients after LOLA. No other changes were found after LOLA	49
Controls	22									

atrophy,[15] but these findings require validation by other groups and additional studies using different multiparametric MRI techniques.

Magnetization Transfer Imaging in Chronic HE

MT was developed as a technique for manipulating tissue contrast for better image visualization on MRI,[54,55] also allowing an indirect measurement of bound and free water compartments in the brain. MT can be affected by variations in a variety of factors, including heavy metal concentration, membrane fluidity, and total water content.[49,50,56] Of note, MT pulse sequences allow measurement of MT ratios (MTRs), which represent a quantitative tissue characteristic, reflecting the behavior of normally MR-invisible protons, bound to intracellular macromolecules. MTR measurement can detect alterations in brain water content that may not otherwise be seen using standard MR techniques. From a technical perspective, magnetization can be transferred between bound and free water pools bi-directionally through direct interaction between spins, transfer of nuclei, or through direct chemical means. Under normal circumstances, MT is the same in both directions, but MT pulse sequences can be designed to saturate the magnetization in the bound pool, leaving the free pool mostly unaffected. Such saturation of the bound pool causes a substantial reduction in the amount of the magnetization. Consequently, there is little transfer of the magnetization back to the free pool, with the MR longitudinal relaxation time reduced as a consequence.

In chronic HE, MTR values have shown an overall trend toward decrease and appear to be one of the most consistent MRI findings as shown by the majority of the studies presented in Table 3. The decrease in MTR values has been demonstrated to be present in several brain regions and has been reported to be small in magnitude (around 10%).[16] Therefore, the main interpretation of this decrease includes the presence of low-grade astrocytic/cerebral edema which might also be linked to alterations in membrane permeability and cytoplasmic structure and to subsequent shifts in the distribution of macromolecules and intracellular water, with subtle alterations in intracellular and extracellular edema.[16,49,50,56,57] Several other hypothesis have also been put forward. These are linked to damage to myelin or to axonal membrane and deposition of paramagnetic substances.[50] In addition, some interesting correlations were reported by some studies between MTR values and MRS findings, the globus pallidus index, blood ammonia levels, and serum manganese concentrations, while the correlations with the neuropsychological tests are controversial (Table 3). Additional multiparametric MRI and multimodal studies would be useful to establish a clear link between MTR values and their brain regional dependence, HE severity, MRS-measurable metabolites, and other important findings in chronic HE.

Diffusion-Weighted/Diffusion-Tensor Imaging in Chronic HE

DWI/DTI is a MR technique allowing quantification of water molecule movement.[58–61] Water molecule diffusion follows the principles of Brownian motion. Unconstrained, water molecule movement is random and equal in all directions. This random movement is described as "isotropic". However, motion of water molecules in structured environments is restricted due to physical surroundings and is described as being "anisotropic" (unequal in all directions). In the brain, the microstructure within GM and WM restricts water molecule movement. On average, water molecules tend to move parallel to WM tracts, as opposed to perpendicular to them.[59] The molecules' motion in the x, y, and z planes and the correlation between these directions is described by a mathematical construct known as the diffusion tensor.[62] In mathematics, a tensor defines the properties of a three-dimensional ellipsoid, the diffusion tensor describing the magnitude, the degree of anisotropy, and the orientation of diffusion anisotropy. For the diffusion tensor to be determined, diffusion data in a minimum of six non-collinear directions are required. This process is known as DTI. This technique collects detailed information allowing insight into the microstructure found within an area of interest within the brain, whose characteristic features are on the same length scale as the micrometer scale displacement of water molecules. These features may be used to map and characterize the three-dimensional diffusion of water as a function of spatial location. Factors calculated include the mean diffusivity (MD), degree of anisotropy, and direction of the diffusivities.[62] MD is a measure of water diffusivity, dependent upon the surrounding chemical environment and the presence of obstacles to movement at a cellular and subcellular level. In parallel, using differently-weighted DWI images, a measure of diffusion can also be calculated. The different images can be mapped to create an apparent diffusion coefficient (ADC) image.[63]

In chronic HE, where less obvious water shifts may be occurring, there is, nevertheless, a mild increase in ADC in patients with cirrhosis, even when HE may not be clinically overt, as in minimal HE.[64] Even though the majority of previously published studies observed an increase in ADC (or MD), the overall interpretation of the diffusion data is difficult and sometimes controversial (Table 3). It is important to note that some studies were unable to report any change in ADC (or MD) values. The overall agreement appears to be linked to an increase in water content. However, some authors tend to believe that this increase in ADC is related to an increase in extracellular water content, others to astrocytes swelling while some

believe that it reflects minimal cellular edema with an increase of membrane permeability and increased intracellular diffusivity, as well as changes in the viscosity of the cytoplasm.[65] The very basic interpretation of a two compartment model with intracellular or cytotoxic edema (linked to a decrease in ADC) and extracellular or vasogenic edema (linked to an increase in ADC) is not straightforward and is simplistic in its interpretation. As previously mentioned, it is rare for one of the two mechanisms to exist in isolation, and sometimes cytotoxic and vasogenic edema might coexist. DWI/DTI remains an indirect probe, because extracting quantitative metrics, characterizing the underlying tissue microstructure requires modeling of the diffusion signal. The limited specificity of DTI metrics and the need for biophysical modeling of the tissue to achieve specificity is discussed in the published literature.[66]

Proton Magnetic Resonance Spectroscopy in Chronic HE

In vivo localized proton magnetic resonance spectroscopy (^{1}H MRS) is complementary to MRI and is a powerful technique to investigate brain metabolism of rodents and humans non-invasively and in a longitudinal manner.[67,68] It provides a spectrum as a readout, consisting of peaks at different resonant frequencies. In single voxel MRS, spectra are acquired from a well-defined volume, positioned in a specific brain region, using a combination of band-selective radiofrequency pulses and magnetic field gradients.[33,69] ^{1}H MRS is one of the most sensitive techniques, and nearly all brain metabolites contain hydrogen nuclei. An important number of biologically relevant metabolites can be observed and quantified *in vivo* within minutes. This technique can detect low molecular weight metabolites at concentrations as low as 0.5 mM.

Reliable quantification of the concentration of known metabolites and the extension of the number of quantifiable metabolites represent the main goal of *in vivo* ^{1}H MRS.[70–74] Accurate and precise quantification of brain metabolites is challenging and depends on hardware performance, pulse sequence design and adjustment, data processing, and quantification strategies. The choice of data processing software is very important, since many algorithms depend on user input, which might lead to inaccuracies. Moreover, published recommendations encourage the usage of quantification algorithms where metabolite concentrations are determined by fitting the measured *in vivo* ^{1}H MRS spectrum to a linear combination of spectra of individual metabolites (the metabolite basis set).[67] In clinical settings, metabolite concentration ratios are often used (mainly ratios to total creatine [tCr]); however, absolute metabolite concentrations are more valuable especially when tCr might change.

^{1}H MRS was among the first techniques which provided indications of the presence of low-grade cerebral edema in chronic HE by reporting changes in brain organic osmolytes (an increase in glutamine [Gln] concentration, together with a decrease in myo-inositol [mIns] that partially compensates for increased intracellular osmotic pressure).[30,75] The glial localization of these osmolytes suggests a disturbance of astrocyte volume homeostasis.[30,75,76] However, the information provided by ^{1}H MRS is an indirect evidence of astrocyte swelling.

A detailed description of the main findings using ^{1}H MRS in chronic HE in human patients can be found in Table 3. In clinical settings, the MRS acquisitions were performed at magnetic fields of 1.5T-3T and echo times (TEs) ≥20 ms, leading to the quantification of few metabolites (e.g. the sum of glutamine and glutamate [Glx], tCr [sometimes also simply called creatine {Cr}], total choline [tCho] and myo-inositol or inositol [mIns or Ins]). It is interesting to note that the stronger changes in brain metabolites (Glx/Cr, mIns/Cr, and tCho/Cr) were observed in overt HE, while in minimal HE, the decrease in mIns/Cr was observed more often than an increase in Glx/Cr. Finally, in functionally well-compensated liver cirrhosis, no significant changes were measured. This raises the question as to whether few metabolite changes occur in well-compensated liver disease patients, or if these changes are very small, and thus they are not detected at lower magnetic fields. Therefore, nowadays the availability of high magnetic fields (≥7T), together with MRS acquisitions at shorter TEs (<10–20 ms) might offer opportunities to better quantify and understand brain metabolites changes in chronic HE. Using this methodology, both in animal models and humans, about 19 brain metabolites can be quantified in the brain: glutamate, Gln, aspartate, γ-aminobutyrate, and glycine (neurotransmitters and associated metabolites); glucose, lactate (Lac), Cr, phosphocreatine, and alanine (markers of energy metabolism); taurine and mIns (markers of osmoregulation); phosphocholine, glycerophosphocholine, phosphoethanolamine, N-acetylaspartate, and N-acetylaspartylglutamate (markers of myelination/cell proliferation); and ascorbate and glutathione (antioxidants).[67,70,71,73,77,78] Table 3 also presents some interesting correlations between MRS changes and other MRI or blood parameters. In addition, some brain regional differences were observed in brain metabolites, but this observation requires further validation.

To date, brain water mapping[34] is the direct method for absolute quantification of water content *in vivo* in humans. In animal models, a multimodal approach is desired combining *in vivo* and longitudinal measurements with an *ex vivo* technique assessing the absolute brain water content. This combination provides additional information on the temporal resolution of the onset of brain edema by monitoring the progression of the syndrome longitudinally. None of these techniques provides information on

the type of the edema or which cell is involved. Therefore, using parallel electron microscopy or a similar technique would be very useful in animal models.

BRAIN EDEMA AND HE TREATMENTS

Drug therapy for HE largely focuses on removal of bacterial-derived toxins and manipulating gut flora levels, but underlying precipitating factors, such as gastrointestinal hemorrhage, infections, electrolyte disturbance, renal insufficiency, the use of psychoactive drugs, and the presence of constipation and the advent of ALF must be investigated and treated accordingly.[79] Published studies suggest that probiotics, non-absorbable disaccharides (lactulose and lactitol), and non-absorbable antibiotics (such as rifaximin) can be useful in treating HE and may have an effect on brain water content.[80–86] The MRI/MRS results of some studies using different treatment strategies are detailed in Table 3.

Non-absorbable disaccharides include lactulose and lactitol, which are well-known for their laxative effects; they also reduce the colonic pH and decrease gut mucosal uptake of glutamine.[87] This reduces synthesis and absorption of ammonia. There has been one study demonstrating a small reduction in brain volume in patients with chronic HE on lactulose[88] using a co-registration technique while another study observed a reduction in MD using the same treatment.[29]

Changes in T_2 FLAIR WM lesions and ventricular volumes have been studied in chronic HE patients[89] and following liver transplantation.[90] Moreover, an improvement in MTR and MD was also observed after liver transplantation,[22,57] while normal MRS spectra were also acquired after liver transplantation.[53]

Rifaximin is a minimally absorbed oral antibiotic with few adverse effects, no reported drug-drug interactions, and a low risk of inducing bacterial resistance.[91] A multicenter trial published in 2010 found that HE remission was prolonged in rifaximin-treated patients, the drug exhibiting a protective effect, and reducing hospitalization rates.[92] Ahluwalia et al. demonstrated a reduction in fractional anisotropy (but not in MD), along with significant improvement in cognition, including working memory, after rifaximin treatment in a group of 16 minimal HE patients, indicating an effect on brain water content.[93]

OVERALL PATHOGENIC MECHANISMS

In the brain, glutamine synthesis is largely confined to astrocytes.[94] In case of liver disease or shunting, brain ammonium accumulation increases astrocytic Gln, raising intracellular osmotic pressure and leading to astrocyte swelling and brain edema.[1,30,71,76,95–99] It is generally accepted that in hyperammonemia, excess glutamine compromises astrocyte function and morphology[76] and thus participates in the development of HE. Although the relationship between cause and effect, leading to HE, and the related spectrum of neurological symptoms remains unclear, ammonium and glutamine appear to be a common thread in the complex and multifactorial model of HE pathogenesis, since both precipitate a cascade of metabolic events that will ultimately result in the neurological disturbance. Ammonium triggers not only the increase in glutamine which will consequently perturb astrocyte metabolism and increase the intracellular osmotic pressure but also a series of signaling events: oxidative stress, activation of transcription factors, signaling kinases, mitochondrial permeability transition, and alterations in the neuronal processes growth.[3,30,97,99–110] Moreover, increased astrocytic Gln can lead to the opening of the mitochondrial permeability transition pore[111,112] and interfere with glutamatergic neurotransmission.[113] More details about Gln-related hypotheses, related evidences, and controversies can be found in study by Brusilow et al.[76] In addition, other pathogenic mechanisms are also involved in HE: inflammation, alterations in neurotransmission, cerebral energy disturbances, Lac accumulation, and probably others more.[114–130]

Even though astrocyte swelling and consequently brain edema are believed to act as a mediator in the neurological manifestations in HE, their pathophysiological role remains elusive. In the past years, several hypotheses have been elaborated regarding the relationship between brain metabolism changes, cellular changes, and cell swelling/edema in HE. The authors of the "osmotic gliopathy theory"[76] suggested that there is an initial pronounced osmotic stress in the astrocytes due to increased glutamine synthesis. With time, there is a gradual compensation as reflected by decreased organic osmolytes, and this compensation is accompanied by increased water in the extracellular space. However, this compensation cannot be complete since there is evidence that astrocyte swelling occurs, which may be more pronounced in the more severe disease. The Trojan horse hypothesis[105,131] is another mechanism by which glutamine is considered to contribute to the pathogenesis of HE. It postulates that glutamine is transported into mitochondria, where it undergoes hydrolysis thus yielding high levels of ammonia and finally resulting in deleterious effects (e.g. induction of the mitochondrial permeability transition and oxidative/nitrative stress leading to astrocyte dysfunction and cell swelling). More details about this theory and related controversies can be found in study by Brusilow et al. [76] The transporter hypothesis postulates that increased Gln synthesis coupled with a partial suppression of SNAT3- and SNAT5-mediated efflux of Gln from astrocytes results in an accumulation of Gln in the astrocytic compartment leading to osmotic stress.[132]

It is believed that small increases in astrocytes water content may have an important impact on astrocyte morphology, function, and gene expression despite the absence of clinically overt increases of intracranial pressure in chronic HE.[75] For example, prolonged osmotic and/or metabolic stress has been shown to cause production of reactive oxygen species, mitochondrial permeability transition, and inflammatory signals, which have physiological and pathophysiological consequences.[1] Altered astrocyte function eventually leads to deranged neuroglial communication and neurotransmitter system imbalance, which will impact synaptic plasticity and oscillatory cerebral networks, thus enabling a pathological environment characterizing HE.[30]

CONCLUSION

Although some of the discussed studies established a link between brain edema and alterations in cognitive function, the role of brain edema as a neuropathological feature/consequence or cause of HE remains controversial. It was speculated that different degrees of astrocyte swelling or brain edema might have different effects on cerebral function.[2] In addition, brain edema might act synergistically with other pathogenic factors or only be a predisposing or precipitating factor in the development of HE. The *in vivo* MRI/MRS studies were very helpful in the process of evaluating brain edema in chronic HE and in improving our understanding of the pathophysiological alterations in HE. As can be seen form Tables 1–3, there is an overall tendency in using multimodal (more than two MRI/MRS techniques) and multiparametric (MRS/MRS studies combined with neurological tests, biochemical analysis) approaches. However, additional *in vivo*, longitudinal, and multiparametric/multimodal studies are required (in humans and animal models) to elucidate the relationship between liver function, brain metabolism changes, cellular changes, cell swelling/edema, and neurological manifestations in chronic HE. The brain regional difference in chronic HE also remains an open question.

CONFLICTS OF INTEREST

The authors have none to declare.

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