COGNITIVE FUNCTION AND LIVER TRANSPLANTATION:

IMPLICATIONS OF HEPATIC ENCEPHALOPATHY

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Doctoral Thesis

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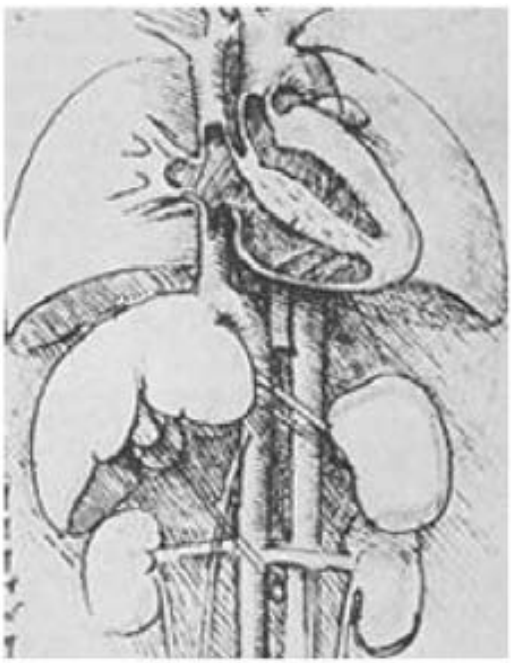
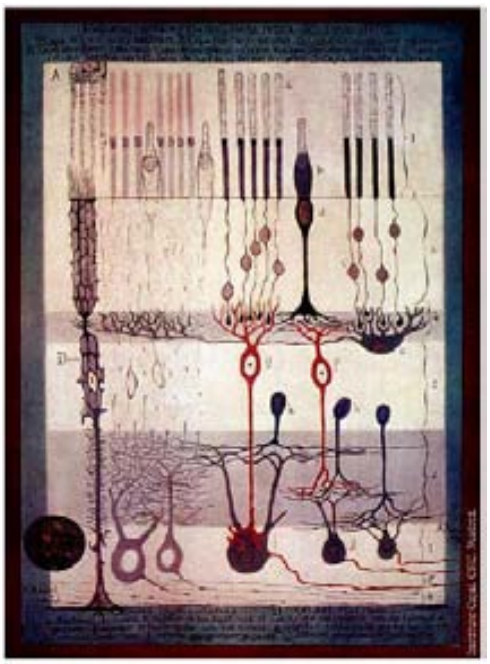
Department of Medicine

Universitat Autònoma de Barcelona

Barcelona, 2011





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PhD studies of Dr Rita Garcia Martinez were funded by grant CM07/00109 from Instituto de Salud Carlos III

This work was funded by the project FIS 05/0226 and CIBEREHD. Both are supported by Instituto de Salud Carlos III.

And I didn’t hesitate to follow the path of vocation and learning

When I felt full of comprehension and love,

Even when the steps seemed to be too short and the way immense.

I no he dubtat a seguir el sender de la vocació i aprenentatge

Quan m’acompanyava la comprensió i l’amor,

Tot i que els passos semblaven curts i el camí immens.

E non dubidei de seguir o camiño da vocación e a aprendizaxe

Cando tiña ó meu carón a comprensión e o amor,

Aínda cando os pasos asemellaban ser moi curtos e longuísima a estrada.

Y no he dudado en seguir el sendero de la vocación y el aprendizaje

Cuando me acompañaba la comprensión y el amor,

Aún cuando los pasos parecían cortos y el camino inmenso.

Preface

“Nothing worthwhile happens overnight. You have to put time and love to build relationships, careers, spiritual connections. When you hurry the process, you stagnate.

Or worse still, your impulsiveness can lead to build something with weak foundations, which will collapse over time.

Today, do not hurry the important things. Practice patience and perseverance to make sure your creations can be strong and stay a long time”

“Nada que valga la pena sucede de un día para otro. Tienes que poner tiempo y amor en construir relaciones, carreras, conexiones espirituales. Cuando apresuras el proceso, te estancas.

O peor aún, tu impulsividad puede llevarte a construir algo con cimientos débiles, que se derrumbará con el tiempo.

Hoy, no apresures las cosas importantes. Practica la paciencia y la perseverancia para que tus creaciones puedan ser fuertes y permanecer un tiempo largo"

Acknowledgments

This thesis is the culmination of a journey that began several years ago. Many people contributed to this dream, today a reality, in one way or the other. Juan Cordoba, the supervisor of this research, enthused me and made the challenges of this path possible. Through the different stages of this PhD, he guided me with patience and perseverance. I had the privilege of finding in him a brilliant scientific support and at the same time I discovered his dedication, honesty and generosity, virtues which I admire in a doctor and friend. I really think that he was the best supervisor I could have wished for.

I would like to highlight that this work would have not been possible without the help of the team that constitutes the “Servicio de Medicina Interna-Hepatologia” of Vall d’Hebron Hospital. Its tradition and the effort of all the members make successful teamwork possible. I would like to thank all of them for their lessons and their collaboration: Jaume Guardia, Rafael Esteban, Victor Vargas, Juan Ignacio Esteban, Maria Buti, Lluis Castells, Lluis Viladomiu, Antonio Gonzalez, Joan Genescá, Beatriz Minguez, and also Maria Vila, Eli, Pilar Tena, Lourdes, Chus and many other colleagues.

This thesis is the result of the cooperation with the co-authors of these studies. The experience, work and wise advice of Drs Rovira, Alonso, Jacas, Aymerich and Huerga have been a source of inspiration and made this research feasible.

One of the best things of this period has been sharing my path with colleagues and friends who were always there to help in the hard hours and celebrate the successes. To Laia and Maria, to Salva and Iñigo, to Isabel, Macarena, Jose, Laura Muntaner, Santi, Marc, Lluis, Jordi, Laura Millán and

many others of you who made these years more enjoyable, easy and fascinating.

I want thank to my parents José and Rosa and my sisters Patricia and Lucía, for the comprehension to my dedication to this work and to my profession and because with you I learned the values of life: respect and generosity, freedom and commitment. Thank you very much to all my family for being my strength and support.

To all my friends Serafín, Sofía, Ángeles, Cris Diz-Lois, Mariló, Mari, Patu, Yoli, Cris Dominguez, Eli, Montse, Sheila, Susana, Mireia, Claudia, Virginia and many others who fill my life with amazing moments and who love me without restrictions.

I would also like to thank Professor Rajiv Jalan for giving me the opportunity to join his research group in London. His enthusiasm makes me believe that everything seems to be possible and I really appreciate the possibility to develop a wonderful project under his supervision. To my new colleagues in London for helping me in my new beginning: María, Liliane, Abeba, Vikram, Nisha, Jane, Naina, Bala, Karla, Giovanni, Pamela, Fatma, Gautam, Nathan, Raj and to the entire group for making me feel part of this team. And especially to Yalda, for her help and support with English translation.

I realize that there are many people I did not mention but it certainly does not mean that they have not been important in this journey. I would like to extend my gratitude to all those who one day came into my life and stayed, because they are part of me and my work. To everyone: thanks.

Abbreviations

AHCD	Acquired Hepatocerebral Degeneration
BV	Brain Volume
CHESS	Clinical Hepatic Encephalopathy Staging Scale
DWI	Diffusion-Weighted Imaging
FLAIR	Fast Fluid-Attenuated Inversion Recovery
HE	Hepatic Encephalopathy
HESA	Hepatic Encephalopathy Scaling Algorithm
LT	Liver Transplantation
MELD	Model for End Stage Liver Disease
MR	Magnetic Resonance
MT	Magnetization Transfer
NAA/Cr	N-Acetyl-Aspartate/Creatine
SIRS	Systemic Inflammatory Response Syndrome
TIPS	Transjugular Intrahepatic Portosystemic Shunt
WMLs	White Matter Lesions

Contents

1. Summary

Historically, hepatic encephalopathy has been considered a reversible neuropsychiatric syndrome with the astrocyte as the pathological target. Implementation of liver transplantation showed the ability to improve cognitive and neuroradiological abnormalities related to liver failure and on the other hand revealed persistence of neuropsychological deficits in some cases. In addition, neuroimaging studies performed in recent decades unfolded brain atrophy in patients with chronic hepatic encephalopathy. Thus, last years pointed that hepatic encephalopathy may cause structural brain injure and sequels.

This research investigates the outcome of neuropsychological function and brain structure following successful liver transplant. Specifically, the effect of hepatic encephalopathy and its outcome have been analyzed. Other variables with suspected influence in the cognitive function were also evaluated.

These objectives were assessed by a dual approach of neuropsychological tests and magnetic resonance in longitudinal studies.

A heterogeneous cognitive outcome was found after one year of successful liver transplantation. Several pre-transplant conditions can impair the pos-transplant neurological function. Hepatic encephalopathy has been linked to a persistent damage (predominantly in psychomotor function) in addition to alcohol

abuse and diabetes mellitus. Besides, hepatic encephalopathy was associated to a decreased brain volume evoking loss of brain tissue. Spectroscopic analyses suggested neuronal loss with the smaller brain volume.

At long-term, cognitive function remained stable unless de novo neurological diseases cause cognitive decline. In fact, small vessel cerebrovascular disease was associated with loss of memory in those patients with accumulation of cardiovascular risk factors such as diabetes mellitus and arterial hypertension.

Some factors implicated in neurological injury may not be appropriately investigated due to methodological issues as immunosuppressive drug effects or perioperative ischemia. Additionally, the fact that other factors (e.g. alcohol, diabetes) affect cognitive function make difficult to define the damage due to hepatic encephalopathy. Despite these limitations, the present research strongly suggests that hepatic encephalopathy is associated with permanent structural injury and loss of neurons. This concept is supported by other lines of evidence such as activation of mechanism of cell death (oxidative/nitrosative stress, energy impairment and inflammation) in hepatic encephalopathy. Besides, neuronal loss has been previously demonstrated in other neurological diseases associated with liver failure as cerebellar degeneration and acquired hepatocerebral degeneration (non-Wilsonian).

It is important to note that persistent damage is mild and on average, cognitive function after liver transplant remains within normal range.

In order to establish a causal relationship further investigation is required. However, the results of this research can have important implications.

From a pathogenic perspective, the classical consideration of hepatic encephalopathy as exclusively an astrocytic disease needs re-evaluation.

From a clinical and prognostic point of view some considerations must be taken into consideration. Strategies focussed on avoiding or minimizing the occurrence of hepatic encephalopathy may prevent cognitive decline, especially in those patients with other neurological comorbidities and in those at risk of cognitive damage as liver transplant candidates.

2. Introduction

Implementation of new treatments is desirable since it reflects a new hope in the fight against a disease.

Advanced liver failure is a common scenario mostly due to the progression of chronic liver diseases. Despite a better knowledge of the pathophysiology and new drugs development, end-stage liver disease still carriers high rates of morbidity and mortality. The development of liver transplantation (LT) together with immunosuppressive therapies led to a great improvement in the prognosis of patients with advanced liver disease. Many beneficial effects were reported after successful LT as well as several complications related to the perioperative management and to the mandatory posttransplant immunosuppression.

A limiting factor is the organ availability, so the possibility of transplants is dependent upon the generosity of society. Taking these considerations into account, it is necessary to select patients carefully to ensure there are both individual and global benefits.

2.1. Cirrhosis and liver failure

Liver cirrhosis is a common feature of chronic liver diseases which is frequently diagnosed in developed and developing countries.

The last report from World Health Organization (in 2004) claimed that cirrhosis was the ninth cause of burden of disease in the European region and mortality from cirrhosis was $8x10^5$ for all ages which represented 1.3% of total deaths in the World [2].

The only curative treatment of end-stage liver diseases is liver transplantation. However, only 20% of patients with advanced cirrhosis can finally receive a new liver owing to the great imbalance between donation and potential recipients[3].

Chronic liver disease may be due to a number of factors including chronic viral hepatitis, alcohol abuse, metabolic syndromes and auto-immune disorders among other causes. In many cases, cirrhosis results from a combination of factors rather than a single cause. Regardless of the aetiology, the persistent insult results in hepatocyte necrosis, chronic inflammation and fibrosis in different degrees[145]. The progressive damage is associated with the distortion of the vascular bed, nodular regeneration of the remaining liver parenchyma and loss of normal cellular functions (figure 1).

Liver synthetic dysfunction results in the decrease in a range of proteins such as coagulation proteins, glycoproteins, lipoproteins and albumin.

In parallel, there is an accumulation of substances usually metabolized and excreted by liver cells as bilirubin and ammonia.

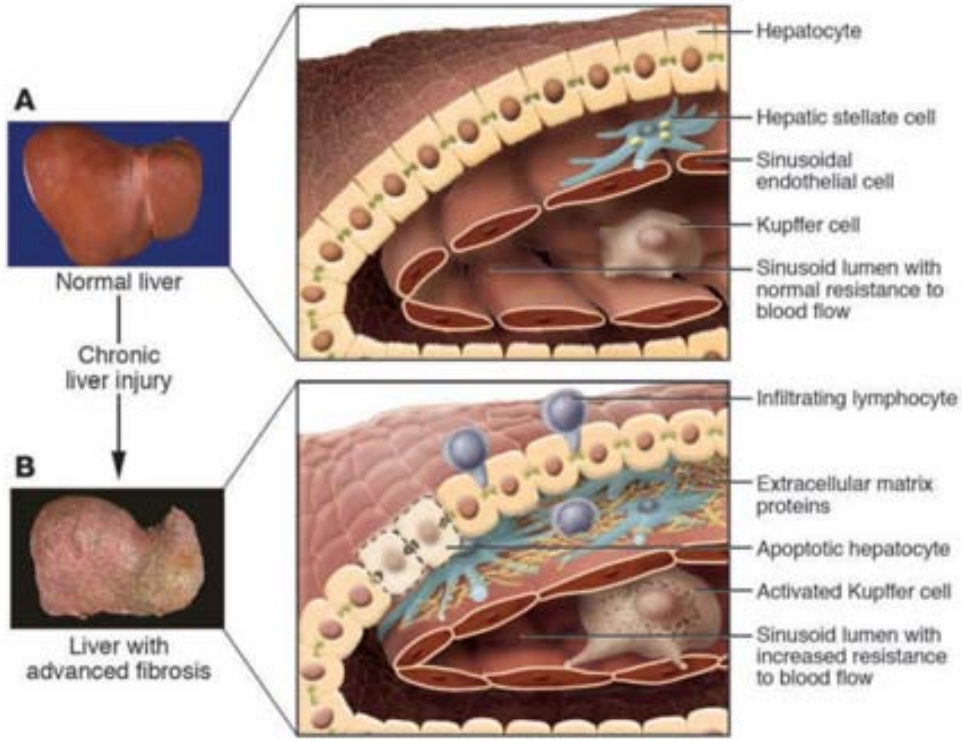


Figure 1
Changes in the hepatic architecture (A) with hepatic fibrosis (B). Following chronic liver injury inflammatory cells infiltrate the hepatic parenchyma, some hepatocytes undergo apoptosis, fibrosis is activated and changes in vascular bed causes increase resistance to blood flow.[16]

Besides, liver is a crucial organ in the metabolism of biological substance such as cholesterol, steroids, and phospholipids and also plays an important role in the metabolism of exogenous substances such as drugs. In addition, alteration in the intrahepatic vascular bed in liver diseases causes an increase in the portal pressure which results in splanchnic arterial vasodilatation and consequently leads to a high cardiac output, a reduced peripheral vascular resistance and arterial pressure. Compensatory mechanisms to recover the underfilling of the arterial circulation subsequently become activated such as sympathetic nervous system, renin-angiotensin-aldosterone system and, in late stages, hypersecretion of arginine vasopressin. Recent research has shown that bacterial translocation from

intestinal lumen to the mesenteric lymph nodes[171] and the systemic inflammatory response syndrome (SIRS)[149,171] have an important role in the pathogenesis of multiorgan involvement in cirrhosis.

All these abnormalities enhance the progression of liver decompensation as well as impairing the normal function of other organs[56] (figure 2).

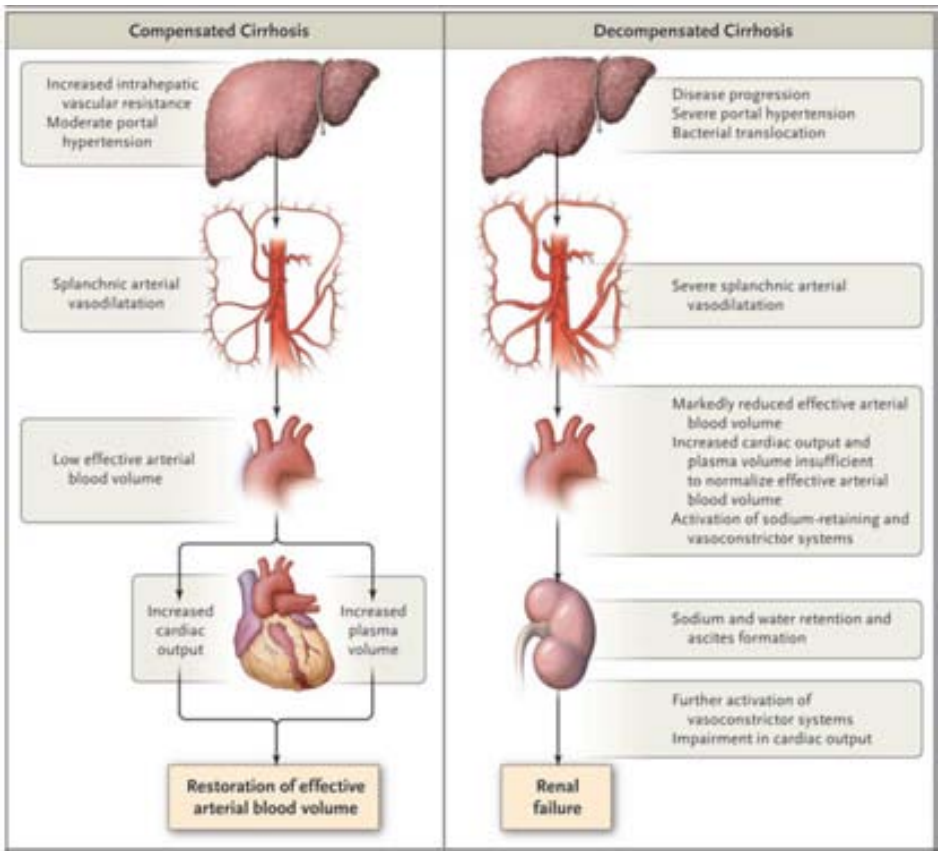


Figure 2. Splanchnic and systemic vascular abnormalities in cirrhosis and the association with multiorgan failure[56]

Major advances have been made in recent years to both prevent and treat the common complications of cirrhosis such as variceal bleeding, ascites, spontaneous bacterial peritonitis and encephalopathy. However, in advance liver failure morbidity and mortality still remain high without LT[145].

2.2. Hepatic encephalopathy

Hepatic encephalopathy (HE) is characterized by a wide spectrum of neurological manifestations that can be classified according to the underlying liver disease[50]. HE can be seen in acute liver failure, where it constitutes the clinical hallmark of this disorder[172]. In rare cases, HE develops in the absence of any sign of parenchymal liver disease and is solely caused by portal-systemic shunting either congenital malformation or by surgical induction. The most frequent situation is in the setting of liver cirrhosis, usually accompanied by extrahepatic portal-systemic shunts (spontaneous or surgical). In fact, HE is a common complication that occurs in 30-45% of cirrhotic patients[8,136] whereas minimal hepatic encephalopathy, characterized by subclinical deficits, affects approximately 20-60% of patients with liver disease[47,61,66,88,136]. According to the clinical evolution[50] HE can be episodic, persistent or minimal (table 1).

Table 1. Classification of hepatic encephalopathy in cirrhosis

Adapted from Ferenci P et al[50]

TYPES	SUBTYPES	FEATURES
Episodic¶	Precipitated	Acute change in mental state induced by: gastrointestinal haemorrhage, constipation, excessive protein intake, infection, renal failure, dehydration, electrolyte disturbance
	Spontaneous	Without recognized precipitating factors. Usually associated with large portosystemic shunts (spontaneous, surgical, TIPS)
Persistent	Mild	Chronic cognitive or motor manifestations that impact negatively on social and occupational activities but do not cause dependency
	Severe	Chronic symptoms that cause dependency
Minimal		Cognitive disturbances detected by neuropsychological or neurophysiological test that are not evident in the standard neurological examination

Patients with HE in the setting of acute liver failure, portosystemic shunting without intrinsic liver disease and acute-on-chronic liver failure are classified separately.
¶ Recurrent HE means 2 or more episodes of HE within the last six months.

Historical approach

The association between liver and brain function has been known for a long time. Hippocrates (460-370 B.C.) recognized paroxysms of mania complicating acute jaundice. A large variety of symptoms were described over centuries but it was in the 1950's when Adams and Foley[5] (1953) first give a comprehensive clinical and pathological description of hepatic coma and Sherlock et al (1956) had a in-depth analysis of the neuropsychiatric syndrome associated to cirrhosis, portosystemic vascular alterations and intolerance of nitrogenous substance[158]. In 1952, Phillips et al demonstrated deranged nitrogen metabolism in HE inducing the same clinical and electroencephalographic alterations in a group of patients after the administration of large amounts of dietary proteins, ammonium salt or urea[129]. Successive years of twentieth century led to a better knowledge of anatomy and physiology of liver and to a better understanding of the neuropsychiatric syndrome associated to liver disease and its neuropathological substrate. The first description of the brain changes in liver disease was given by von Hösslin & Alzheimer[72] in 1912. They depicted thinning of the cerebral cortex, enlargement of the lateral ventricles, lenticular degeneration and presence of astrocytes with enlarged, pale nuclei with peripheral

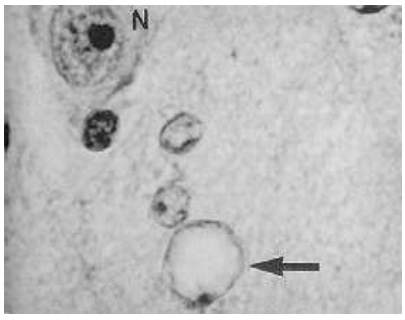
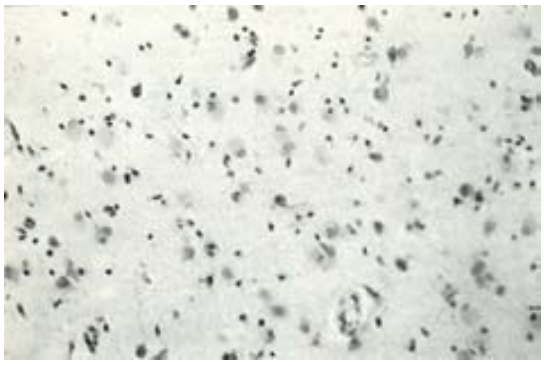


Figure 3.
Alzheimer type II astrocyte showing nuclear enlargement (arrow) with chromatin displaced to the periphery [70]

margination of chromatin and often prominent nucleoli commonly, known as Alzheimer type II astrocytes (figure 3).

Since then, numerous reports with concordant findings have been published. In contrast to these well-established astrocytic alterations, neuronal changes were seldom reported. In routine histopathological studies neurons appear to be normal in shape and number. However, several reports illustrated neuronal alterations in both human and animal models of cirrhosis. Adams and Foley[5] observed in a cohort of patients who developed hepatic coma nerve cells degenerated in the cerebral cortex, thalamus, lenticular, red and dentate nuclei in the most severe cases of the neurologic syndrome but not in the less critical. These findings were confirmed in a systematic description of the neurological syndrome afterwards labelled as acquired hepatocerebral degeneration (AHCD)[167] (figure 4) and confirmed by other authors[52] several years later. Neuronal cell loss was also observed in a systematic neuropathological study of cerebellar sections of 36 patients who died in hepatic coma[91]. Interestingly, loss of Purkinje cells was observed in 50% of patients without prior history of alcohol abuse. Reproducible data was obtained in different animal models of HE such as carbon tetrachloride induced liver disease or porto-caval shunt[33,48].

Figure 4.
Severe neuronal loss in deep layer of cerebral cortex and replacement by astrocytes and microgliacytes[167]



Pathogenesis

The mechanisms involved in the pathogenesis of HE remain unknown and many factors could be implicated in the development of this syndrome. Liver failure causes an increase in the exposure of the brain to several substances that under normal circumstances are efficiently metabolized by the liver; those that have a high first pass metabolism across liver are the most important, as shown by the major role of portal-systemic shunting in the development of HE. Other factors that are commonly present in patients with liver failure and can cause deterioration in neurological function are inflammation, circulatory derangements, nutritional deficits, comorbidities and other organ failures.

I. Ammonia toxicity. Ammonia has been historically considered the most important factor in the genesis of HE (Figure 5). Under

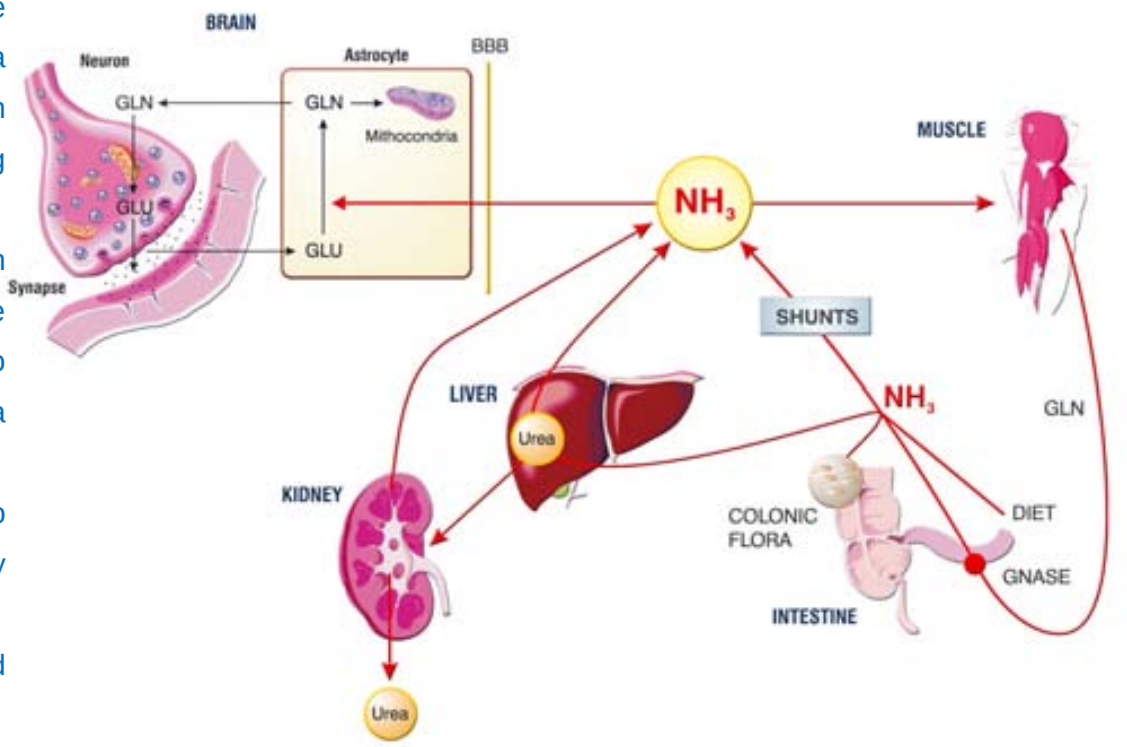


Figure 5. Interorgan ammonia trafficking and metabolism. Portal-systemic shunts and liver failure cause a rise in blood ammonia that affects brain function inducing several disturbances in astrocytes. Muscle is capable to decrease ammonia metabolizing ammonia to glutamine. Kidney participate excreting urea and generating ammonia[43]

normal circunstances, ammonia is produced by the gut with an important contribution from colonic bacteria. Its concentration in portal blood is high, and a high extraction occurs in the liver[119]. In addition to the intestine and the liver, kidney and muscle also contribute to the regulation of the arterial ammonia level[123]. In muscle, ammonia is converted into glutamine through the action of the enzyme glutamine synthetase. The ability of the muscle to "neutralize" appreciable amount of blood-borne ammonia becomes important to regulate arterial ammonia in context of liver failure and highlights the importance of maintaining an adequate muscle mass. Patients with HE have an increased diffusion of ammonia into the brain in relation to an increase in arterial ammonia[87]. In the brain, ammonia is metabolized to glutamine in astrocytes, where ammonia or glutamine exerts their toxic effects. Recent data provide more information on the mechanisms by which ammonia causes neuronal disturbances, but a complete explanation is still lacking[71]. Signs of oxidative stress, such as protein tyrosin nitration, have been found in experimental preparations. In addition to protein damage, ammonia induces RNA oxidation, which may have multiple effects in neurotransmission and postsynaptic protein synthesis[144].

II. Inflammation. The presence of SIRS has been linked to the development of HE in fulminant hepatic failure[165] and cirrhosis[148]. The activation of inflammatory mediators, such as

cytokines, may modulate the effect of neurotoxins on the brain. Peripheral inflammation may signal the brain through the activation of vagal afferents[103]. Other mechanisms of transduction of signals into brain are binding of cytokines to receptors in cerebral endothelial cells or direct access of cytokines into brain tissue at sites lacking blood-brain barrier[20]. Inflammation may also be localized in the brain. Microglial activation and proinflammatory cytokines synthesis has been shown in experimental models of acute liver failure[81]. Neuroinflammation has an important role in many neurological diseases. In HE, activation of inflammation in brain tissue may increase blood-brain barrier permeability, resulting in generation of intracerebral mediators of inflammation and cause astrocytic swelling[70,77].

III. Circulatory Dysfunction. There is a close relationship between renal and cerebral circulation in liver failure. In advanced liver failure, both organs lose their property of vascular autoregulation[155]. In patients with cirrhosis and ascites, there is renal and cerebral vasoconstriction, which is probably related to arterial hypotension and to the overactivity of vasoconstrictor systems[63]. The clinical experience also links renal failure to HE. In patients with advanced cirrhosis, an increase in serum creatinine and a decrease in serum sodium are the two most important factors involved in the recurrence of HE[62].

Abnormalities on the brain function

I. Astrocytes. The distinctive neuropathological alteration in HE is Alzheimer type II astrocyte. Glutamine is generated in the astrocytes during the detoxification of ammonia through the amidation of glutamate. The accumulation of glutamine causes an increase in intracellular osmolality[40] or induce mitochondrial injury by the activation of the mitochondrial permeability transition[7]. The change in the state of cellular hydration causes impairment of several metabolic pathways and has been proposed to be responsible for brain edema and for neurological manifestation of HE[70]. Factors that precipitate HE such as inflammation, hyponatremia and benzodiazepines can exacerbate swelling[57]. Mechanisms by which abnormal glial cells can influence neuronal function include interaction with glutamate reuptake[25] and activation of peripheral-type benzodiazepine receptors, causing increased synthesis of neurosteroids that are powerful ligands of the $GABA_A$ receptor (an inhibitory neurotransmitter)[6].

II. Neurotransmission. Multiple abnormalities of neurotransmitter systems have been described in animal models of HE, including disturbances in the excitatory glutamatergic[111] and inhibitory GABAergic[117] neurotransmitter systems. Some supportive data are provided by studies on human brains[97] and by neuroimaging techniques. However, it is difficult to relate the complexity of neurotransmission disturbances to the neurological

manifestations. Several therapeutic attempts have been conducted to restore disturbances in neurotransmission with specific drugs, but the results have not been remarkable[14].

III. Energy impairment. The brain is the tissue with the highest energy requirements in human body and depends entirely on the process of glycolysis and respiration within its own cells to meet its energy demands. In HE, a decrease in consumption of oxygen and glucose is accompanied by a decrease in cerebral blood flow[74]. It is not possible to distinguish whether the decrease in oxygen consumption is a cause or consequence to the encephalopathy. The current interpretation is that energy impairment is secondary to decrease in neuronal function, as in other metabolic encephalopathies. However, another explanation is the direct effect of ammonia on energy metabolism causing neuronal disturbances[174]. In fulminant hepatic failure, and possibly in acute-on-chronic liver failure, disturbances in energy metabolism may have an important participation on the clinical picture. In patients with acute liver failure increase in brain lactate, indentified by brain microdialysis is followed by surges of high intracranial pressure[161]. Furthermore, an increase in plasma lactate is a well-recognized prognostic factor in fulminant as well as acute-on-chronic liver failure[35]. Experimental models have shown that the increase in newly lactate synthesized in the brain rise in parallel of brain water in the intracellular compartment[34]. Ammonia may impair

glycolysis, because it inhibits α-ketoglutarate dehydrogenase, the rate-limiting enzyme of the tricarboxylic acid cycle[24], and may have a direct toxic effect on the mitochondria[7]. An alternative explanation is that lactate is generated aerobically by excess glutamatergic activation[127], induced by excessive amidation of glutamate, secondarily to an increase in brain ammonia. Irrespective of the mechanism involved, a drop of brain pH can cause injury at multiple levels, including astrocyte swelling[156].

IV. Brain edema. Brain edema is a recognized event in acute and chronic liver failure and can be identified by direct[146] and indirect[138] techniques of magnetic resonance (MR). The major factor involved in the development of brain swelling is the increase in plasma ammonia[38]. Others factors such as hyponatremia may enhance the effects of ammonia[41]. An intriguing finding is the different distribution of water in acute and in chronic liver failure, suggesting different pathogenic mechanisms. In acute liver failure, brain water is mainly located in the intracellular space[34], meanwhile in chronic liver failure is mostly extracellular[104]. Brain edema has been proposed to have major consequences on neuronal function[70], but good evidence is lacking. The increase in volume of the brain inside a rigid skull can cause intracranial hypertension and finally brain herniation, which is responsible for a significant number of deaths in fulminant hepatic failure. Compensatory mechanisms that require chronic induction, a lower plasma ammonia levels and a

smaller brain volume explain why intracranial hypertension is seldom seen in cirrhosis[49]. In fulminant hepatic failure and possibly in acute-on-chronic liver failure, cerebral vasodilatation and loss of autoregulation may worsen brain swelling[93]. Measures that decrease cerebral vasodilatation also decrease intracranial pressure[37].

V. Brain atrophy. Different neuroimaging techniques[101,173] as well as neuropathological studies[65] have proven brain atrophy in patients with chronic HE. The prevalence and the degree of atrophy were higher among the alcoholic patients. This feature could be explained by the fact that alcohol causes a dose-related brain atrophy which is partially reversible with abstinence[128]. On the other hand, this could be underestimated due to the coexistence of brain edema. A plausible explanation could be that exposure to toxins involved in the pathogenesis of HE could also cause loss of brain parenchyma. This hypothesis is supported by the neuropathological demonstration of neuronal loss in severe neurological manifestations of liver failure[52,167].

Assessment of HE

Hepatic encephalopathy is a syndrome with a wide spectrum of clinical manifestations that typically fluctuates during the course of the disease. Quantification of these clinical manifestations is useful for monitoring the clinical course and assessing the effect of therapeutic interventions. In contrast to the

traditional view that considered HE a categorical disease which progress from normal cognition (normal clinical exam and psychometric tests) to minimal HE (normal clinical exam and abnormal psychometric test) and to grade I-II (abnormal clinical exam and psychometric test), the current interpretation is that HE is a continuum cognitive impairment[13]. Those patients with cognitive disturbances (even if not obvious on clinical exam) are considered to have low-grade HE if the deficit is not severe enough to qualify as confusional syndrome (acute episodic HE) or dementia[69]. The available tools to assess HE have several limitations because some of them require patient collaboration and are limited by the consciousness state. So, the approach is different in acute episodic HE (were confusion is common and the clinical scales to grading HE are more useful) and in low-grade HE (were confusion was ruled out and the neuropsychological assessment is more valuable).

I. Clinical scales for grading episodic HE. These scales evaluate the presence of neurological manifestations. Although their standardization is still in initial phases[116], their expert use provides worthwhile assessment.

   a. West Haven Scale: Arbitrary method that establishes four stages of HE according to the existence of multiple manifestations for each stage. The lack of specific definition for each stage leads to an intuitive and subjective use of this classification. This limitation does not invalidate its

application to individual cases but diminishes its usefulness in clinical trials[42,64].

b. Hepatic Encephalopathy Scaling Algorithm (HESA). HESA combines clinical indicators with validated neuropsychological tools and well defined criteria for each stage[67] but requires staff training.

c. Clinical Hepatic Encephalopathy Staging Scale (CHESS). In contrast to other scales, CHESS was developed without a previous arbitrary definition of severity of HE[124]. Forty-eight items were evaluated in 36 patients. Nine items were finally selected by a group of experts applying principal component analysis. This scale shows good index of internal consistency, reproducibility, criterion-related validity and external responsiveness but requires further validation.

d. Glasgow coma Scale. Initially developed to assess posttraumatic coma, it is recommended to complete the assessment in severe cases of EH[50] in combination with other scales.

II. Neuropsychological tests. These tools measure cognitive functions (for instance memory, attention, motor or visuospatial skills) relevant in daily life. For this reason they are better than neurophysiologic tests and more appropriate for patients without impaired consciousness but confusion should be excluded before their application[133].

a. Expert neuropsychological assessment: This is considered as the best method to demonstrate cognitive deficits. Patients are requested to complete a series of tests which ideally are adjusted by confounding factors such age, gender and education and them compared to normative standards and interpreted by neuropsychologist. The result is suggestive but not specific for the disease.

b. Short neuropsychological batteries: These assessments[105,114,169] include a limited number of tests conducted by a trained technician and typically take less than 30 minutes. They are weighted to detect those deficits that characterize low-grade HE (attention, motor and executive function) and represent an alternative to expert examination. These batteries do not differentiate minimal from persistent low-grade HE and also have the limitation of learning effect in case of repeated testing.

III. Neurophysiological test. These techniques examine the electrical activity of the brain. They have the advantage of being objective and useful for repeat testing due to lack of learning effect but require equipment.

a. Electroencephalogram. This technique has been used in varying degrees in HE and five grades of severity have been established (from normal to coma).

b. Evoked potentials. These measures are not suitable for severe cases of HE as they require patient's cooperation.

c. Critical Flicker Frequency. This technique requires patient's collaboration and have been purposed as a diagnostic method for minimal HE[88].

IV. Neuroimaging. In patients with cirrhosis, MR is useful in exclusion of alternative diagnosis such us Wernicke's encephalopathy, viral encephalitis or stroke and is capable of detecting abnormalities that are characteristically present in the brain of patients with cirrhosis that develop HE[138]:

a. Paramagnetic substances in basal ganglia. A high signal intensity on T1-weigthed images typically in globus pallidus[92] is a common feature and probably caused by deposition of Manganese. The intensity of the signal is not related to the severity of HE[154] but its absence in cirrhotic patients with neurological symptoms suggests an alternative diagnostic.

b. Brain atrophy. The decrease in brain size has been associated with cirrhosis, more often in cases of alcohol aetiology[101,173]. The brain atrophy related to alcohol abuse is partially reversible after abstinence. However, the mechanism which induces brain atrophy in cirrhosis remains unknown.

c. Brain edema. Increase in brain water in liver failure has been shown using MR techniques in patients with chronic liver failure[146] and in experimental models with laboratory methods[34]. The location and severity of brain water appear

to be different according to the duration and degree of liver failure. Acute liver failure causes intracellular edema that can be severe and leads to brain herniation[132] while chronic liver failure induces low-grade interstitial edema[84]. Magnetization transfer (MT) imaging[39,75,141], fast fluid-attenuated inversion recovery (FLAIR) imaging[140] and diffusion-weighted imaging (DWI)[84,104] are sophisticated MR techniques more sensitive than the conventional sequences to show changes in the brain tissue water content. In chronic liver failure, the decrease in the MT ratio, the high signal in the corticospinal tract in FLAIR sequences and increase in brain water diffusivity are consistent with brain edema[138] (figure 6).

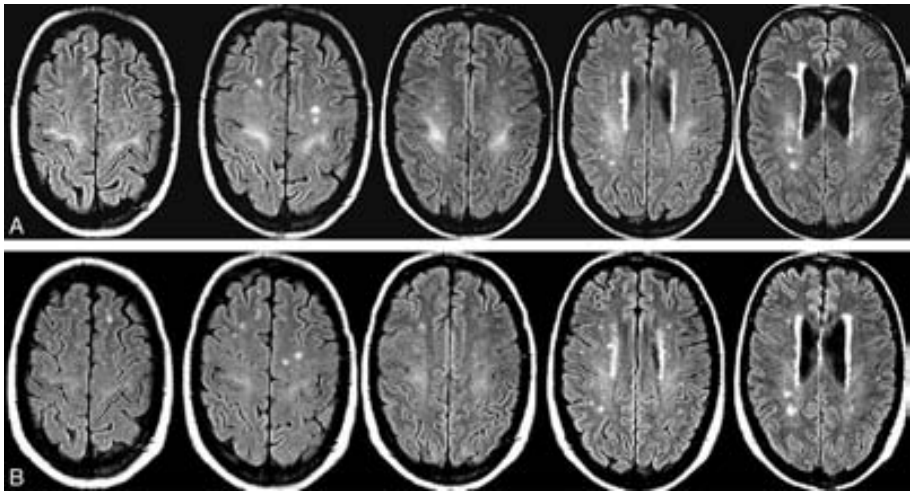


Figure 6.
T2-weighted fast FLAIR images in a patient (A) during an acute episode of HE (B) few months later without signs of HE. Increased signal intensity along corticospinal tract almost reverses on the follow-up. The most plausible explanation for this finding is mild brain edema[138]

d. Changes in organic osmolytes/ammonia related-metabolites. MR-spectroscopy reveals a typical pattern in HE: an increase in glutamine and a decrease in the choline-containing and myo-inositol peaks[90]. These features are related to the metabolism of ammonia in astrocytes which

incorporates glutamate and ammonia to glutamine. This leads to astrocyte swelling and to a compensatory osmotic response: decrease in myo-inositol and choline containing compounds[96]. The intensity of these alterations has been linked to the severity of HE[99] (figure 7).

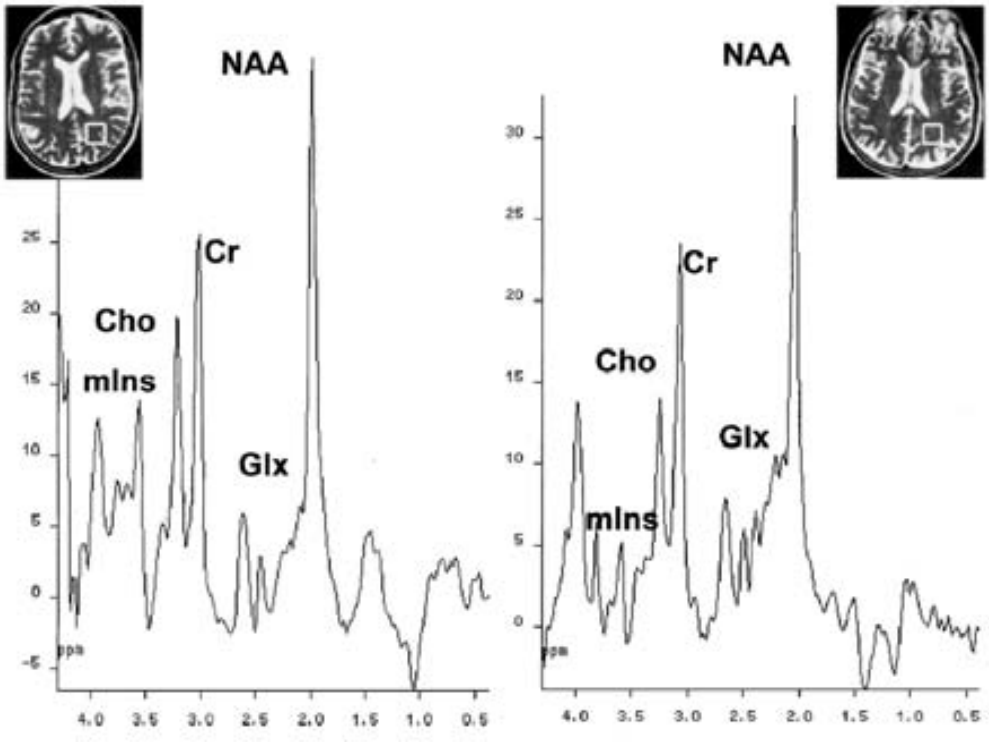


Figure 7.
1HMR spectroscopy in normal appearing white matter region in healthy control and cirrhotic patients. Comparison of the spectra shows a decrease in choline and myo-inositol with an increase in the glutamic/glutamine region in the cirrhotic patient[44].

Prognosis

HE is traditionally considered a symptom of liver failure with worrying considerations. However, the prognosis is not uniform and is dependent on the severity of the underlying liver condition and the patient's neurologic function.

In acute liver failure is a marker of the disease[130] and often associated with high morbidity and mortality without liver transplant[73].

In cirrhosis, the development of HE have been associated with a poor prognosis[23,36,143]. However, the outcome is

heterogeneous and dependent on the severity of liver insufficiency, neurological manifestations and the precipitating factor[19,157]. In agreement, the prognostic MELD score (Model for End Stage Liver Disease)[85] initially developed to predict mortality within three months of cirrhotic patients who undergo to transjugular intrahepatic portosystemic shunt (TIPS) did not include this variable. The prognosis after HE is clearly related to the severity of liver failure[23] and for this reason patients who develop HE should be evaluated for LT.

2.3. Liver transplantation

Liver transplantation advanced from an experimental therapy in the 1960s to a conventional treatment in the 1980s for a wide range of acute and chronic liver diseases[109]. The cornerstone in this challenge was the improvement in technical procedure together with the development of immunosuppressive drugs which prevented allograft rejection. Since 1990s, the number of LT and the survival rates have significantly risen in Europe (figure 8A) and all around the world (figure 8B). Thus, the main objectives of LT have been achieved as improved life expectancy[122] and better quality of life[51].

In parallel, there has been an increase in identification of subjects requiring LT. The true number of patients who might benefit from liver replacement is not known. From the diagnosis of liver failure towards gain a LT (referral to a transplant centre, evaluation and acceptation for liver transplant and reach the replacement) only less

than 20% of patients finally gain access to an organ[109]. This is partly related to the severe donor shortage which clearly affects every patient with liver failure and leading to the current LT practice.

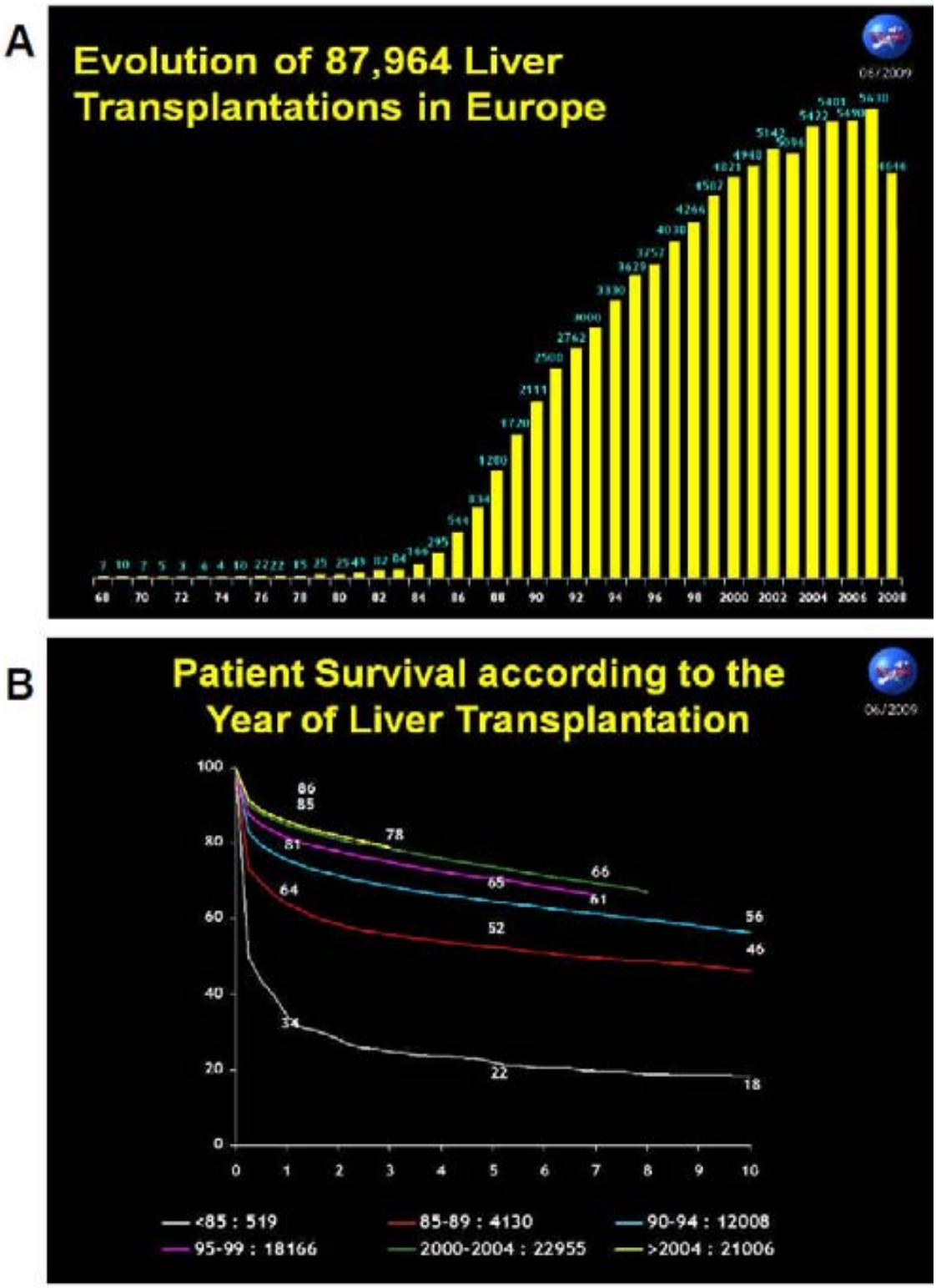


Figure 8.
A: Evolution of number of LT in Europe.
B: Survival of recipients in Europe according with the year of LT[1]

2.4. Hepatic encephalopathy and liver transplantation

Many of the liver transplant candidates exhibit cognitive disturbances[110]. These abnormalities can be related to the etiology of cirrhosis (alcohol abuse), complications of liver failure (hepatic encephalopathy, hyponatremia), or extrahepatic comorbidities (cerebrovascular disease, age, Alzheimer disease)[22].

The benefits of successful LT include the improvement in clinical symptoms of HE[9,102,107,115,121,125], even in those patients with severe manifestations[95] such as hepatic myelopathy[11,29,89,118,162,170] and the neuroradiological alterations associated with liver failure[39,139,142].

However, studies that addressed cognitive function post-LT found heterogeneous outcome with persistence of some neurologic deficits[108,153,159], even at long-term[102]. Tarter[159] and Mechtcheriakov[108] described an improvement in the majority of the explored cognitive domains after LT but also reported a lack of normalization of some of them when these subjects were compared to healthy controls. However, these authors failed to find any association with clinical conditions and the cause of these abnormalities could not be established. In a post-LT cross-sectional study Sotil et al[153] described a relationship between the development of HE prior to LT and a more pronounced cognitive dysfunction after LT. Nevertheless this study had several limitations such as lack of neurological structural data, the relatively small sample size and lack of longitudinal assessment. These factors restricted the interpretation of HE as cause of the post-LT deficits.

In the few cases of reported hepatic myelopathy, the outcome was heterogeneous[29,45,118] with lack of any improvement 6 months after LT in some of them[118].

Despite the limitations, these investigations support the notion that HE is associated with permanent sequels[10,137] challenging the classical view of HE as a fully reversible condition. In addition, this hypothesis is supported by the recent observations revealed by longitudinal studies performed in cohorts of patients who developed HE. A recent study conducted by Bajaj et al, showed that the patients who suffered episodic HE did not improve psychometric parameters despite repeated testing which implicates “lack of learning”[12].The same feature was observed with different psychometric test in other populations[100,134] confirming the persistence of neurological deficits after HE.

The existence of sequels due to HE is not fully clarified and this question is an important one. From a pathophysiological point of view the axiom of HE as a pure gliopathy requires further investigation. From a clinical and prognostic perspective this could have implications in the setting of LT, where the demonstration of sequels could lead to review the priority criteria in LT candidates. On the other hand, in patients who are not LT candidates, implementation of preventive therapies could have beneficial effects on cognitive decline. For these explained reasons additional longitudinal studies with new tools to evaluate potential permanent neurological damage caused by HE are need. Ideally, these studies

should control all the other factors that could affect the cognitive function.

2.5. Comorbidities with potential neurological damage

Liver transplant represents an ideal scenario to explore cognitive function once the liver function has been restored. For this reason, liver transplant recipients with normal liver function are the best population to analyse the possible sequels of HE. However, this issue is much more complex due to the following reasons:

a) Many factors can impact postransplant cognitive function of a patient apart from prior HE. These issues include pretransplant conditions (alcohol abuse, liver failure complications such as hyponatremia, extrahepatic neurological diseases such as cerebrovascular or Alzheimer disease), peritransplant factors (eg: ischemia associated to cerebral hypoperfusion), and postransplant events as direct effects of immunosuppressor drugs, cerebrovascular disease, or persistence of minimal HE due to portosystemic shunts that remain after LT[10].

b) Neurological complications after LT such as metabolic encephalopathies, seizures or infections of central nervous system are very common[30] affecting as many as 13%-43% of patients[4] and may be responsible of sequels.

All these factors can act in parallel or synergistically with HE and should be taken into consideration in the studies addressed to evaluate the effects of HE on the postransplant cognitive function.

3. Hypothesis

I. Hepatic encephalopathy is a metabolic syndrome with a reversible behaviour associated to the correction of the precipitating factors. However, brain's exposure to these pathogenic mechanisms could lead to an irreversible neurological damage.

II. The irreversible neurological damage may be revealed in LT recipients with good liver function once the metabolic alterations associated with the liver failure are fully resolved.

III. Permanent neurological damage is manifested as loss of normal neurological function and can be demonstrated by neuropsychological studies.

IV. The structural basis for this permanent damage can be related to the loss of brain tissue that includes neuronal loss. This loss of brain tissue could be determined with accurate neurological imaging as non-invasive methods.

V. Other factors could impair the neurological function, these may act synergistically or additively with HE.

4. Objectives

I. To describe the neuropsychological outcome in cirrhotic patients after liver transplantation at short and long-term.

II. To describe the brain structure and volume in cirrhotic patients after liver transplantation at short and long-term.

III. To determine the brain metabolites in cirrhotic patients following liver transplantation in order to account for the changes induced by ammonia exposure and relative concentrations of neuronal metabolites.

IV. To analyze the influence of prior hepatic encephalopathy in neuropsychological and neuroradiological outcome.

V. To estimate the effect of other factors that could have an impact in the postransplant neuropsychological and neuroradiological evolution.

5. Patients methods and results

5.1. Study I

García-Martínez R, Rovira A, Alonso J, Aymerich FX, Huerga E, Jacas C, Simón-Talero M, Vargas V, Córdoba J.

A Long-Term Study of Changes in the Volume of Brain Ventricles and White Matter Lesions After Successful Liver Transplantation

Transplantation • Volume 89, Number 5, March 15, 2010

Comment in:

Nat Rev Gastroenterol Hepatol. 2010 May;7(5):239.

CLINICAL AND TRANSLATIONAL RESEARCH

A Long-Term Study of Changes in the Volume of Brain Ventricles and White Matter Lesions After Successful Liver Transplantation

Rita García Martínez,[1,2] Alex Rovira,[3] Juli Alonso,[3,4] F. Xavier Aymerich,[3] Elena Huerga,[3] Carlos Jacas,[2,4,5] Macarena Simón-Talero,[1,2] Víctor Vargas,[1,2,4] and Juan Córdoba[1,2,4,6]

Background. A prolonged survival in liver transplant recipients due to a better management exposes them to multiple factors that can impair neurologic function in the long term.

Methods. Twenty-two patients were studied by brain magnetic resonance and completed a neuropsychologic assessment shortly before liver transplant, 6 to 12 months after (short term), and 6 to 9 years (long term) after liver transplant. Thirteen healthy controls matched by age were studied in parallel.

Results. An enlargement in the ventricular size (an indirect measure of brain volume) was observed in the short term (+8%) and in the long term after liver transplant (+22%); the size of ventricles was larger than in healthy controls. In addition, a progression in the volume of focal T2 white matter lesions (an index of small vessel cerebrovascular disease) was detected in the long term (+49%) and was related to vascular risk factors in those with larger increases (>12.5% per year). Neuropsychologic function showed a significant improvement after liver transplant and remained stable in the long term, except for memory loss in those patients with larger increases in white matter lesions.

Conclusions. Improvement in neuropsychologic function after successful liver transplant can be demonstrated up to 9 years. However, these patients experience a progressive accumulation of focal T2 brain lesions and show a smaller brain volume than controls, which can be related to their previous cirrhosis. A good management to minimize brain injury before transplantation and an accurate treatment of vascular risk factors may be important to prevent consequences on cognitive function.

Keywords: Hepatic encephalopathy, Brain size, Magnetic resonance imaging, Cognitive function, Liver transplantation, Human.

(*Transplantation* 2010;89: 589–594)

The overall goals of liver transplantation (LT) are to prolong survival (*1*) and improve quality of life (*2*). This can be achieved with the recovery of those functions that were impaired by liver failure and by avoiding complications that are caused by transplantation and immunosuppressive therapy. Magnetic resonance (MR) has shown the reversibility of several abnormalities that are present before LT and have been linked to hepatic encephalopathy (HE): T1 hyperintensity at basal ganglia (*3*), T2 hyperintensity along the corticospinal tract (*4*), a decrease in magnetization transfer ratio (*5*), and changes in brain metabolites (*6*). The normalization of these disturbances has been associated with an improvement in cognitive function. However, neuropsychologic defects may persist to some degree (*7, 8*).

Multiple factors may account for neuropsychologic impairment after LT. They include perioperative complications, the effect of immunosuppression, and possible sequeale of HE. A high incidence of severe neurologic complications (13%–43%) has been described (*9*), but seems to have decreased probably due to a better postoperative management. However, the increasing age of subjects who have been trans-

This work was supported by grant FIS 05/0226 and FIS grant CM07/00109 (R.G.-M.). CIBEREHD was supported by Instituto de Salud Carlos III.
The authors declare no conflict of interest.

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R.G.-M. participated in data analysis and writing of the manuscript; A.R. participated in research design; J.A. participated in research design and data analysis; F.X.A., E.H., and C.J. participated in performance of research; M.S.-T. participated in data analysis; V.V. participated in research design; and J.C. participated in research design, data analysis, and writing of the manuscript.
Received 8 August 2009. Revision requested 28 August 2009.
Accepted 28 September 2009.

ISSN 0041-1337/10/8905-589
DOI: 10.1097/TP.0b013e3181ca7bb3

planted may pose them at risk for long-term neurologic disturbances. Vascular risk factors are frequent in these subjects and may have a major impact on neurologic function. Arterial hypertension is present in up to 75% (*10*), diabetes mellitus in 10%–30% (*11*), and hyperlipidemia in 40%–66% (*12*). In addition, some degree of renal impairment is found in approximately 80% of recipients (*13*). It is plausible that vascular risk factors may cause cerebral small vessel disease that can lead to neuronal loss and cognitive decline. The hallmark of small vessel disease is the presence of white matter lesions (WMLs) that may be combined with some degree of brain atrophy (*14*).

We postulated that the liver failure before transplant plus neurologic complications during the early postoperative period and small vessel cerebral disease during the late postoperative period will cause neurologic injury and could result in cognitive impairment in the long term after liver transplant. For this purpose, we assessed neuropsychologic tests, and we applied MR imaging to determine the volume of lateral ventricles (a reverse estimate of brain size) (*15*) and the volume of WMLs (an index of small vessel disease) before LT, during the first year after LT, and several years after LT. Our goal was to assess the different contribution of each period on the neurologic outcome.

PATIENTS AND METHODS

Design

The study consisted in the performance of MR imaging and a neuropsychologic assessment in 22 patients who underwent LT between April 1998 and December 2001. The evaluation was performed shortly before LT (1–2 months), shortly after LT (6–12 months), and in the long term after LT (6–9 years). This cohort came from a group of 35 patients previously included in two prospective studies with different objectives, for which results have been formerly published (*16*, *17*). The remaining 13 patients could not be included: 10 died in the follow-up and 3 rejected to participate. Thirteen healthy controls matched by age and gender were studied by MR imaging in parallel with the patients.

Performance of the study was approved by the Institutional Review Board of Hospital Universitari Vall d'Hebron, and all subjects gave written consent for participation.

Patients

All the participants had cirrhosis without clinical evidence of overt HE and were evaluated for LT at our institution following standard procedures. Patients were informed that the study was designed to evaluate neurologic manifestations related to cirrhosis and liver transplant and that they would complete psychometric tests and undergo brain MR imaging. Patients with a history of drug abuse, those affected by neurologic or psychiatric diseases, and those receiving medication known to have significant effects on the central nervous system were excluded. The study included 22 patients (Table 1). The most common indication for LT was liver failure (17 patients); hepatocellular carcinoma was the indication in five patients. Four patients were on Child-Pugh stage A, 10 were on Child-Pugh stage B, and 8 were on Child-Pugh stage C. Nine patients had suffered at least one episode of HE before LT. At the time of the long-term evaluation, all patients exhibited good liver function. The immunosuppression regime was based on tacrolimus therapy in monotheraphy (n=15) or in association with mycophenolate mofetil (n=6) or with sirolimus (n=1). Before transplantation, five patients had diabetes mellitus, and three patients had arterial hypertension that persisted after LT. In addition, after LT, six patients developed de novo diabetes mellitus and 11 patients had arterial hypertension (Table 1). None of the patients have experienced neurologic complications (e.g., encephalopathy, stroke, and seizures) during the postoperative period, neither before the short-term nor the long-term assessment.

TABLE 1. Clinical and biochemical characteristics of patients at the moment of long-term assessment after liver transplantation

	Long-term post-LT
Age (yr)	62±11
Gender	
Male/female	19/3
Etiology	
Viral hepatitis	10
Alcohol	6
Viral hepatitis+alcohol	5
Autoimmune hepatitis	1
Laboratory test	
Creatinine (mg/dL)	1.27±0.2
GFR (mL/min)[a]	60±13
ALT (IU/L)	29 (39)
Bilirubin (mg/dL)	0.7 (0.5)
Prothrombin activity (%)	103 (15)
Vascular risk factors (%)	
Diabetes mellitus	11 (50)
Arterial hypertension	14 (64)
Hyperlipidemia	5 (23)
Smoking	11 (50)

Measurements are expressed as mean±SD or median (IQR).

[a] GFR: GFR estimated with the Crockcoft-Gault method using body weight, height, age, gender, and creatinine.

LT, liver transplantation; GFR, glomerular filtration rate; ALT, alanine aminotransferase.

MR Imaging

Brain MR imaging was obtained with a 1.5-T Magneton Vision-plus superconductive magnet (Siemens, Erlangen, Germany) using a quadrature transmit/receive head coil (Fig. 1). The MR protocol included the following pulse sequences: transverse T2-weighted fast spin-echo (3000/85/2=repetition time/echo time/acquisitions), fast fluid-attenuated inversion recovery (FLAIR) (9900/110/2500/1=repetition time/echo time/inversion time/acquisitions), and T1-weighted spin-echo (600/15/2). The sequences were registered using the following parameters: section thickness 5 mm, interleaved imaging mode, intersection gap 1.5 mm, pixel size of approximately 1×1 mm, and acquisition matrix 256×256 mm.

All fast-FLAIR images obtained in the baseline and follow-up scans were transferred to a Sun Ultra 10 Workstation (Sun Microsystems Inc., Palo Alto, CA) to calculate T2 WML volume. Lesions were marked on the fast-FLAIR hard copies

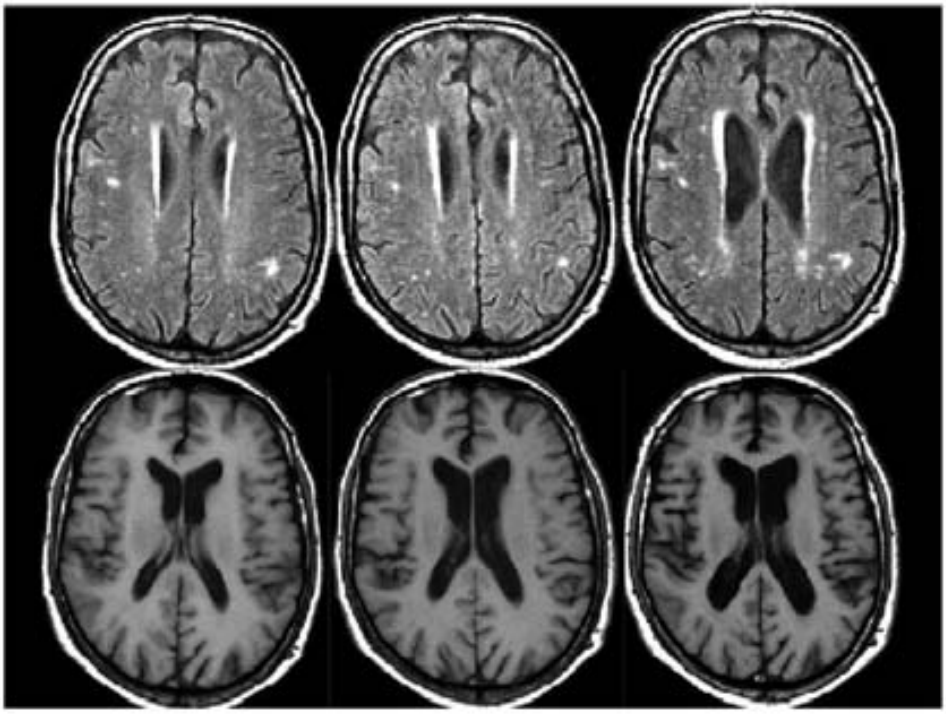

FIGURE 1. Brain fast fluid-attenuated inversion recovery (*top*) and T1-weighted (*bottom*) transversal images of a patient before liver transplantation (LT; *left*), at short term (*center*), and at long term (*right*) after LT. The image shows changes in the volume of white matter lesions (*top*): a decrease in the short term and an increase in the long term after LT and an enlargement of ventricles (*bottom*) in the consecutives assessments.

by the same neuroradiologist. Only focal WMLs located in the brain hemispheres and with a minimum size of 3 mm were considered for the volume measurement. A single rater (E.H.) previously trained to ensure a high level of reproducibility performed the T2 WML analysis as follows: (1) the hard copies with all lesions outlined were placed alongside the identical computer-generated image; (2) all lesions marked on the hard copies were outlined on the computer image using a semiautomatic local thresholding contour technique (DISPImage program, Dave Plummer, London, UK), or in case, if the lesion could not be outlined satisfactorily with this approach, a manual outlining was performed; (3) then, a computer program added up the individual lesion volumes, and a final T2 WML volume was obtained. Follow-up scans were assessed by the same rater who calculated the baseline T2 WML. T1-weighted spin-echo images were used to calculate the volume of lateral ventricles with the procedure described for WML volume determination by the same trained rater. The rater was not blinded for the date of examination, but she was not aware of the aim of the study and of the clinical and neuropsychologic conditions of the patients.

Neurologic Assessment

At the time of assessment, patients were perfectly alert, without flapping tremor, and were oriented in space, person, and time. In all of them, the neurologic examination was considered normal. The patients completed a neuropsychologic test battery that included: Trail Making test (part A), Symbol Digits (oral version), Grooved Pegboard test (dominant and nondominant hand), Auditory Verbal Learning test, Judgment of Line Orientation test, Hooper test of visual organization, and Controlled Oral Word Association test. The scores of each test were transformed into T-values with the aid of metanorms adjusted by age, gender, and ages of education. T-values were calculated according to the formula $T=50+10\,([x-x_n]/SD_n)$, where x represents the raw result of the test, x_n represents the mean value, and SD_n represents the standard deviation value for the test in the normal population. The scores of the different tests were grouped into indexes of memory (Auditory Verbal Learning), attention (Trail A, Symbol Digit), executive function (Controlled Oral Word Association test), psychomotor function (Grooved Pegboard), and visuoperceptive function (Judgment of Line Orientation test and Hooper Test). An overall score was calculated as the average of all the tests. Impairment was defined as a T-value 30 to 40 (mild), 20 to 29 (moderate), and less than 20 (severe) according to standard proceedings.

Statistical Analysis

Results are expressed as mean ± standard deviation or median (interquartile range), if not expressed otherwise. Depending on the behavior of the variables, parametric or nonparametric tests were applied to study the differences between groups of patients (student's *t* test or the Mann-Whitney Rank Sum test) and to study the intrasubject differences (paired student's *t* test or Wilcoxon signed-rank test). A chi-square test or the Fisher's exact test was used to study the existence of significant differences between nominal variables. Finally, Pearson's or Spearman's correlation coefficient test was applied to study correlations among variables. A *P* value less than 0.05 was considered statistically significant. The statistical calculations were performed with SPSS 15.0 software (SPSS, Chicago, IL).

RESULTS

Magnetic Resonance

Baseline Assessment

The volume of ventricles (22.3 [20.9] mL) showed high variability with large range of measures (minimal: 11.5 mL,

maximal: 54.7 mL) and was related to the age of patients (r=0.673, P=0.001). WMLs were present in 16 patients (76%), who were significantly older than those without WML (58±9 vs. 45±12 years, P=0.012). There were no associations between clinical parameters (gender, etiology of cirrhosis, Child-Pugh stage, and prior HE) with the volume of ventricles or the presence of WML.

Short-Term Assessment

The median volume of ventricles increased to 25.4 (24.5) mL (+8%), and the median volume of WML decreased to 0.7 (0.9) mL (−19%) (Fig. 2). Changes in the volume of ventricles did not relate to clinical parameters. However, the decrease in WML was more pronounced in those with HE before LT (−37.8% vs. −2.5%, P=0.05; Fig. 3).

Long-Term Assessment

The median volume of ventricles increased to 30.4 (30.9) mL (+22% compared with short term) and the median volume of WML to 1.4 (1.9) ml (+49% compared to short term) (Fig. 2). None of the patients without WML before LT developed new WML after LT. The increase in the volume of WML from short-term to long-term assessment was not directly related to the presence of arterial hypertension, diabetes mellitus, or creatinine levels. However, three of those four patients with an increase of WML more than 12.5% per year (above percentile 75) had arterial hypertension, diabetes mellitus, and renal impairment (creatinine >1.4 mg/dL), whereas the coexistence of these three factors was only present in 2 of those 12 patients with an increase in WML lower than 12.5% per year (P=0.06; Fig. 3).

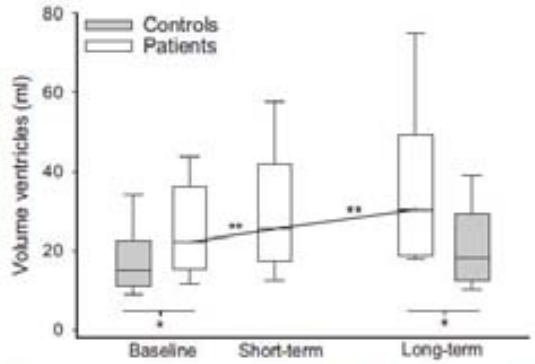

FIGURE 2. Change in the volume of ventricles. An enlargement in the volume of ventricles was documented in the short and long term after liver transplant (**P<0.01). The volume of ventricles was higher in patients than in controls (*P<0.05).

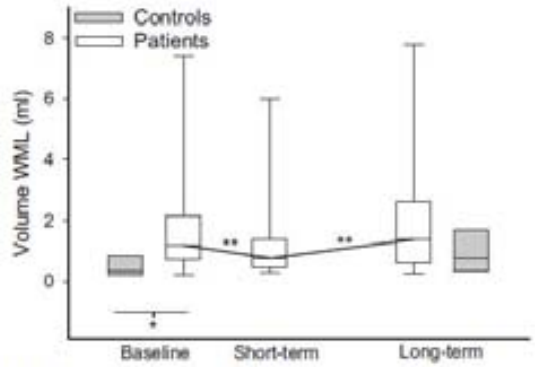

FIGURE 3. Changes in the volume of white matter lesions (WML). At baseline: WML were higher in patients than in controls (*P<0.05). Short term after liver transplantation (LT): WML decreased compared with baseline (−19%, **P<0.01) but was higher than controls at baseline (P=0.03). Long term after LT: WML increased compared to short term (+49%, **P<0.01). Healthy controls did not show significant changes between long term and baseline.

Healthy Controls

The volume of ventricles was lower in controls compared with patients in the baseline assessment (15.1 [11.8] mL) and experienced an increase to 18.2 (17.1) mL (+15%) in the long term, that was still lower than patients (Fig. 2). WML were present in seven subjects (54%) and had a median volume of 0.3 (0.6) mL. This volume was lower than in patients assessed before LT (P=0.018) and in the short term after LT (P=0.03). The median volume of WML in the long term (0.7 [1.3] mL) did not experience significant statistical changes (Fig. 3). Those healthy controls without baseline WML did not develop new lesions (Figs. 2 and 3).

Neuropsychologic Tests

Before LT

Several cognitive domains (Fig. 4) were impaired (T-values <40). Global cognitive function (an average of all domains) was worse in the nine patients that had experienced HE before LT (37±6 vs. 44±7, P=0.041).

After LT

Cognitive function experienced a significant improvement (Fig. 4). The raw results of the neuropsychologic tests were worst in the long-term assessment (data not shown). However, these differences can be attributable to age, because there were no differences in the T-values (age-adjusted results) of the neuropsychologic tests between the short-term and the long-term assessment. Neuropsychologic tests after LT

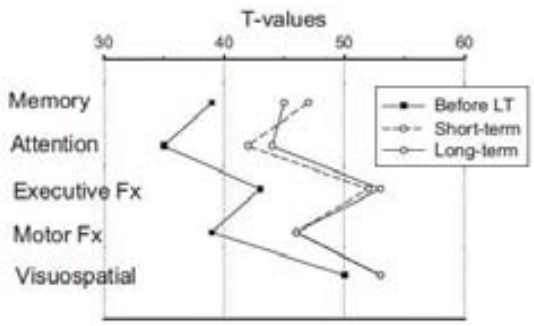

FIGURE 4. Changes in neuropsychologic tests grouped by domains. Before liver transplant, the mean T-values were mildly impaired (between 30 and 40) for memory, attention, and motor function. T-values after liver transplant were significantly different from pretransplant assessment for all domains (P<0.05). There were no significant differences between short-term and long-term assessment.

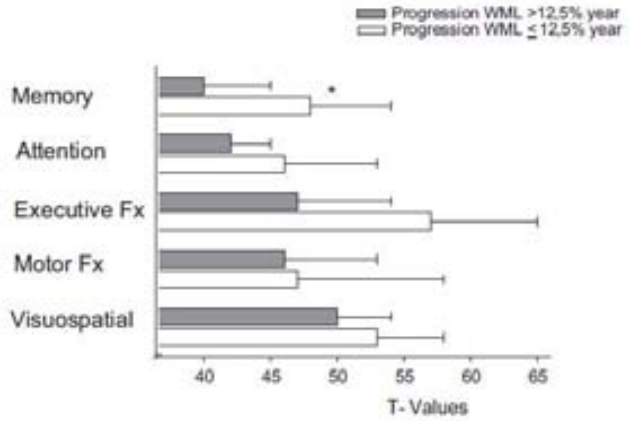

FIGURE 5. Cognitive function in the long-term evaluation in relation to the progression of white matter lesion (WML). A significant decrease in the memory index was observed in the group of patients with larger increase in WML (*$P<0.05$).

did not relate to clinical parameters before LT (Child-Pugh, HE) or after LT (laboratory tests, dose of immunosupressors).

Relationship Between MR and Neuropsychologic Tests

The volume of ventricles did not relate to neuropsychologic results at baseline, short-term, or long-term assessment. Patients with or without WML did not differ in the results of the neuropsychologic tests at baseline or after LT. In those with WML, there was an association between the decrease in the volume of WML and the improvement of several neuropsychologic parameters in the short-term assessment: attention ($r=-0.57$, $P=0.005$), executive function ($r=-0.56$, $P=0.007$), motor function ($r=-0.61$, $P=0.03$), and global cognitive function ($r=-0.60$, $P=0.005$). In the long-term assessment, the subgroup of patients with a larger degree of progression of WML (>12.5% per year) showed a worse memory index (40 ± 5 vs. 48 ± 6, $P=0.015$) than those with a smaller degree of progression (Fig. 5).

DISCUSSION

This study shows that in the long term after successful LT, despite an increase in the volume of ventricles and in WMLs, patients exhibit a good cognitive outcome. LT improved neuropsychologic function, which remained stable up to 9 years after the intervention. However, the progressive increase in the volume of ventricles and in WMLs can lead to cognitive disturbances that may become more evident with an extended follow-up or in larger cohorts.

Two-dimensional measurement of ventricular size could be used in the absence of hydrocephalus as an indirect measure of brain volume (*15*). T2 WML likely represents areas of demyelination, gliosis, and loss of axons secondary to damage of small vessels (*18*). Aging is associated with an increase in the volume of ventricles and WML. Larger increases in both, volume of ventricles (*19*) and in WML (*20, 21*), indicate neurologic damage and are closely associated with the development of mild cognitive impairment and dementia (*22, 23*). Vascular risk factors aggravate the progression of WML that could be stabilized with appropriate treatment (*24*). Our results indicate that the most important factors that determine the outcome beyond the first year are age and probably, vascular-induced injury. We observed that those four patients with the most significant progression of WML presented several vascular risk factors and a deterioration of memory in the long term. These results highlight the importance of controlling vascular risk factors, which are responsible for an important burden of morbidity in the long term for transplanted patients.

Several studies have shown an improvement of cognitive function during the first year after liver transplant (*25, 26*), but few studies have assessed the outcome in the long term. The available data suggest that the improvement achieved during the first year may not be complete and may persist beyond this period (*27*). One study performed 10 years after liver transplant found significant cognitive dysfunction and worse than normal health-related quality of life in 12 subjects (*28*), with a pattern of dysfunction that was similar to what has been described shortly after transplant. Our data are in accordance with the notion that cognitive function does not improve and can worsen beyond the first year of follow-up. We observed (using T-values which adjust results by age) the same profile of neuropsychologic function in the short and in the long term after transplant. On average, the neuropsychologic performance was within the range of normal values, but two patients who did not return to normal persisted mildly impaired at long term (T-values of global cognitive function between 35 and 40).

Short-term cognitive function outcomes depend on the correction of factors that are present before liver transplant. We observed an increase in the volume of ventricles and a decrease in WML shortly after liver transplant. These changes were related to an improvement of neuropsychologic function and were more pronounced in patients with prior episodes of HE. The better explanation is that the improvement of liver function reverses low-grade brain edema (*29*). We have previously shown a decrease in the volume of WML in association with an improvement of cognitive function after episodic HE and after LT (*30, 31*). The increase in the volume of ventricles found in this study gives further support to this hypothesis. Similar changes in ventricular volume using MR have been described in relation to the evolution of HE in a group of nine patients (*32*).

Brain size and cognitive function shortly after transplant can be considered as the neurologic status in cirrhosis after correction of the metabolic disturbances caused by minimal HE. We observed that at such time, the volume of ventricles was approximately 68% larger in cirrhotic patients than in controls. These results, which are in accordance with prior studies using computed tomography (*33, 34*) and MRI (*35*), indicate that cirrhosis is associated with loss of brain parenchyma, which indeed may be difficult to diagnose due to low-grade brain edema.

The mechanisms that are responsible for the loss of brain parenchyma in cirrhosis are not clear. Alcohol consumption causes a dose-related decrease in brain size that is aggravated by nutritional status and can at least partially reverse with abstinence (*36*). Neuropathology studies in alcoholics have shown further degrees of brain atrophy in those with cirrhosis (*37*), indicating that neuronal loss may be directly related to liver failure. The decrease in brain size may be

caused by substances that are involved in the pathogenesis of HE (*38*). Brain atrophy is common in patients with persistent HE (*39*) and may cause posttransplant cognitive impairment. We could not find an association between brain size, alcohol consumption, and prior HE. However, our cohort (which was relatively small) presented a wide range of ages, which was the most important factor determining brain size.

In summary, brain size decreases after liver transplant due to the resolution of low-grade brain edema and is lower than normal due to brain atrophy associated with cirrhosis. The cognitive function can be influenced by pretransplant factors, such as alcohol or encephalopathy, but in our study, the posttransplant outcome was good. The decrease in brain size persists in the long term and may be aggravated by aging-related factors that induce WML. A major goal after successful liver transplant is the prevention of these neurologic disturbances, which may be achieved with adequate control of vascular risk factors.

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TRANSPLANTATION

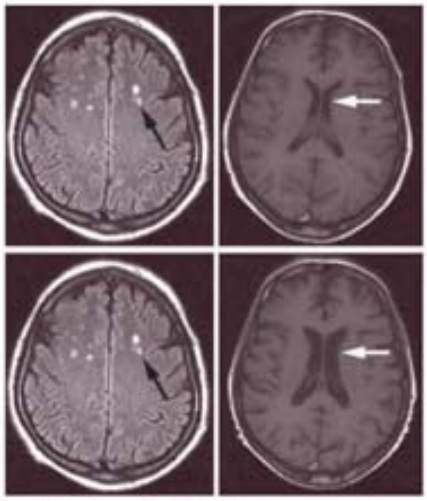
Long-term changes in brain volume and cognitive function after liver transplantation

Liver transplantation has a beneficial effect on cognitive function in patients with cirrhosis, according to the results of a recent study investigating the long-term effects of liver transplantation on brain volume and neurologic function.

It is well established that liver transplantation improves survival and quality of life in patients with end-stage liver disease. Previous studies have demonstrated that low-grade brain edema and other abnormalities linked with hepatic encephalopathy normalize after liver transplantation, with an associated improvement in cognitive function in the short term. "However, persistent cognitive deficits have been documented in patients with cirrhosis after liver transplantation," notes Juan Cordoba, one of the authors of the study. "On the other hand, some degree of brain atrophy has been reported in neuroradiological studies in patients with cirrhosis—even in those without a history of alcohol abuse."

These data led Cordoba and colleagues to wonder whether cirrhosis and hepatic encephalopathy could lead to permanent sequelae after liver transplantation. The authors also hypothesized that vascular risk factors (including hypertension, diabetes mellitus and hyperlipidemia) could cause small vessel cerebrovascular disease, potentially leading to neurologic injury in the long term. "We wanted to determine short-term and long-term neurological outcomes in a cohort of liver transplant recipients," explains Cordoba. As such, the authors believe that their study is the first long-term neuroradiological study in a group of liver transplant patients.

All 22 patients enrolled in the study had cirrhosis and underwent liver transplantation between 1998 and 2001. MRI was used to determine the volume of lateral ventricles (an indirect and inverse measure of brain volume) and the volume of white matter lesions (an indirect index of small vessel cerebrovascular disease). In addition, patients underwent a neuropsychological assessment to test memory, attention, executive function (that is, cognitive control), psychomotor function and visuoperceptive function.



Fast-FLAIR (left) and T1-weighted (right) transversal images in a patient before (top) and 1 year after (bottom) liver transplantation. The image shows a decrease in the volume of white matter lesions (black arrow) and an enlargement of ventricles (white arrow). Courtesy of J. Cordoba.

Patients were evaluated at baseline (1–2 months before transplantation), shortly after transplantation (6–12 months) and in the long term after transplantation (6–9 years). 13 healthy controls matched by age and gender were also included in the study.

An increase in ventricular volume was observed in the short term and long term after liver transplantation, and the size of ventricles was larger in patients who underwent transplantation than in healthy controls. The volume of white matter lesions decreased in the short tem but increased in the long term in patients who underwent liver transplantation. Cognitive function improved in the long term after liver transplantation. There was no relationship between the volume of ventricles or presence of white matter lesions and neuropsychological assessment.

"Liver transplantation has a beneficial effect on cognitive function in patients with cirrhosis, an effect that persists for many years," the authors conclude. "However, cirrhosis is associated with some degree of brain atrophy that persists after liver transplantation and that may be indicative of an irreversible deterioration secondary to liver failure."

According to Cordoba, another important finding is that "the brain ages more rapidly after liver transplantation, probably in proportion to vascular risk factors in this population."

The authors plan to conduct further studies to investigate the long-term effects of hepatic encephalopathy and confirm the presence of brain atrophy in patients with cirrhosis. "The relationship between episodes of hepatic encephalopathy and persistent neurological damage (brain atrophy and loss of neurological abilities) may have important implications, especially in patients with other neurological comorbidities and patients awaiting liver transplantation," says Cordoba. The authors suggest that attempts to minimize brain injury and vascular risk factors before transplantation could help to prevent neurologic impairment after transplantation.

Isobel Franks

Original article Garcia Martinez, R. *et al.* A long-term study of changes in the volume of brain ventricles and white matter lesions after successful liver transplantation. *Transplantation* 89, 589–594 (2010)

5. Patients methods and results

5.2. Study II

García-Martínez R, Rovira A, Alonso J, Jacas C, Simón-Talero M, Chavarria L, Vargas V, Córdoba J.

Hepatic Encephalopathy Is Associated With Posttrasplant Cognitive Function and Brain Volume

Liver Transplantation 17:38-46, 2011

LIVER TRANSPLANTATION 17:38-46, 2011

ORIGINAL ARTICLE

Hepatic Encephalopathy Is Associated With Posttransplant Cognitive Function and Brain Volume

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Hepatic encephalopathy (HE) is a common complication of cirrhosis that is associated with brain atrophy and may participate in impaired cognitive function after liver transplantation. This study analyzes the relationship of HE with cognitive function and brain volume after transplantation. A total of 52 consecutive patients with cirrhosis (24 alcohol abuse, 24 prior HE, 14 diabetes mellitus) completed a neuropsychological assessment before liver transplantation and again, 6 to 12 months after transplantation. In 24 patients who underwent the posttransplant assessment, magnetic resonance imaging was performed in addition, with measurement of brain volume and relative concentration of *N*-acetylaspartate (NAA) and creatine/phosphocreatine (Cr), a neuronal marker, by magnetic resonance spectroscopy. Neuropsychological assessment prior to transplantation identified minimal HE in 28 patients. All cognitive indexes improved after liver transplantation, but 7 patients (13%) showed persistent mild cognitive impairment. Global cognitive function after transplantation was poorer in patients with the following variables before liver transplantation: alcohol etiology, diabetes mellitus, and HE. Brain volume after transplantation was smaller in patients with prior HE. Brain volume correlated to NAA/Cr values ($r = 0.498$, $P = 0.013$) and poor motor function ($r = 0.41$, $P = 0.049$). In conclusion, the association of HE with cognitive function and brain volume suggests that having experienced HE before liver transplantation impairs the posttransplantation neurological outcome. ***Liver Transpl 17:38-46, 2011.*** **© 2011 AASLD.**

Received May 31, 2010; accepted September 23, 2010.

Liver transplant candidates often exhibit cognitive disturbances, which are related to the effects of liver failure, the etiology of liver disease (eg, alcoholism), complications of cirrhosis (eg, hyponatremia), and extrahepatic comorbidities (eg, cerebrovascular disease).[1] The most common cause of cognitive impairment appears to be minimal hepatic encephalopathy (HE), which is secondary to liver failure and shares the same pathogenic mechanism as overt HE. Liver transplantation (LT) restores liver function and results in an improvement of minimal HE.[2] However, it is unclear whether minimal HE is fully reversible or

Abbreviations: AVL, auditory verbal learning test; Cho, choline-containing compounds; COWAT, controlled oral word association test; Cr, creatine/phosphocreatine; Glu, glutamate; Gln, glutamine; HE, hepatic encephalopathy; JLO, judgment of line orientation test; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; mIns, myo-inositol; MR, magnetic resonance; NAA, *N*-acetylaspartate; NPS, neuropsychological; NS, not significant; SD, standard deviation; TE, echo time; TR, repetition time.

This project was funded by grants FIS 05/0226 and 07/0641. CIBERehd is supported by Instituto de Salud Carlos III. R.G.-M. is the recipient of grant CM07/00109.

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DOI 10.1002/lt.22197
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

persists to some degree after LT.[3] Most studies that have specifically investigated the persistence of cognitive deficits in this population include a small group of patients[4] or assess neuropsychological performance only in the post-LT period.[5]

Neuroimaging studies in diseases characterized by cognitive decline have demonstrated structural damage at different levels, thereby providing a structural basis for neuropsychological disturbances. In transplant recipients, prospective studies have shown resolution of neuroimaging abnormalities related to HE. Magnetic resonance (MR) imaging in patients with cirrhosis commonly depicts a high signal intensity on T_1-weighted images in the basal ganglia and a high signal intensity on T_2-weighted images along the corticospinal tract; both these abnormalities normalize within 1 year after LT.[6,7] This outcome relates to the improvement in cognitive function, suggesting a common pathogenic mechanism. Neuropathological studies have documented brain atrophy in cirrhosis,[8] which has been corroborated *in vivo* using computed tomography.[9] However, the outcome of brain volume after LT has not been studied in depth, in part because of methodological difficulties. In a previous study, we observed that the size of brain ventricles in patients with cirrhosis after LT was larger than in controls, supporting the notion that cirrhosis may cause a decrease in the size of the brain that persists after LT.[10]

A recent study in patients with cirrhosis who underwent prospective assessment of cognitive function showed a decline in neuropsychological function following episodes of HE.[11] This finding is consistent with demonstration of poorer neuropsychological scores in LT recipients who had previous episodes of HE.[5] These observations are the basis for the proposal that the lesion caused by HE has a metabolic component that reverses with improvement of liver failure and a structural component that persists regardless of the outcome of liver function. For this reason, we designed a prospective study to investigate the effect of previous HE episodes on the neuropsychological outcome following LT. In addition, we determined whether HE might have caused a loss of neuronal tissue in a subgroup of these patients by measuring the following after LT: brain volume by MR imaging using the SIENAX (structural image evaluation using normalization of atrophy) method, and the relative concentration of *N*-acetylaspartate (NAA), considered to be a neuronal marker, by MR spectroscopy.

PATIENTS AND METHODS

Design

This study consisted of prospective assessment of neuropsychological function before and after LT. Consecutive outpatients on the transplant waiting list between March 2004 and October 2007 were assessed within 2 months of LT and again 6 to 12 months after LT. After March 2006, patients who underwent post-LT neuropsychological assessment were examined by MR of the brain. The study was approved by the Institutional Review Board of Hospital Universitari Vall d'Hebron, and all patients gave written consent for participation.

Patients

All participants had cirrhosis and were evaluated for LT at our institution following standard procedures. At the time of the first assessment, they were on the waiting list and had no evidence of overt HE. A total of 70 patients were initially selected, of which 11 died during the first year after LT and 7 declined to participate. Thus, 52 patients completed the neuropsychological assessment (pre-LT and post-LT) and 24 of them underwent MR examination (post-LT).

In the clinical protocol, the history and complications of cirrhosis were recorded before LT, and the clinical outcome after LT. To assess the effect of previous HE on the post-LT cognitive function, the following variables were recorded: number of episodes of overt HE, time to LT since first episode of overt HE, and maximal severity of HE (West Haven I-II or III-IV). These data were obtained from the patients' medical records, which are systematically incorporated in the liver transplant unit database. Most patients included in the study had been regularly seen in our hospital since the first manifestation of liver disease. In the few cases of patients who went to other centers for episodes of decompensation, the information was obtained from their clinical documents. In all cases, the accuracy of the data was confirmed by the patients and their relatives. The clinical characteristics of the patients are summarized in Table 1. The majority experienced complications of cirrhosis before LT (79% ascites, 31% bleeding secondary to portal hypertension); approximately one-fourth (27%) had diabetes mellitus before LT. The indication for LT was end-stage liver disease in 39 patients and hepatocellular carcinoma associated with cirrhosis in 13 patients. During the postoperative period, 4 patients experienced major neurological complications. Two of them died (1 cerebrovascular hemorrhage, 1 ischemic-hypoxic encephalopathy); the other 2 patients (1 seizure, 1 gaseous embolism) recovered well and were reassessed in the second study. Patients who completed the post-LT evaluation had good liver function (alanine aminotransferase = 41 ± 50 IU/L, bilirubin = 0.6 ± 0.4 mg/dL, albumin = 4.01 ± 0.5 mg/dL) and normal creatinine (1.3 ± 0.4 mg/dL). Immunosuppression at the time of the second study consisted of tacrolimus given as monotherapy (n = 25) or in association with mofetil mycophenolate (n = 20) or inhibitors of mammalian target of rapamycin (n = 6); 1 patient was treated with cyclosporine associated with mofetil mycophenolate.

Neuropsychological Assessment

At the time of the neuropsychological evaluation, patients were perfectly alert, showed no flapping

TABLE 1. Clinical and Biochemical Characteristics of Patients

Characteristics	NPS Study	MR Study
Demographics		
Number of subjects	52	24
Age (years)	54 ± 10	54 ± 9
Male/female	81%/19%	75%/25%
Etiology		
Viral	25 (48%)	13 (54%)
Alcohol	24* (46%)	8 (33%)
Others	3 (6%)	3 (13%)
Abstinence prior to LT (months [range])	24 (6-192)	12 (6-51)
HE		
Prior to LT (episodic HE)	24 (46%)	12 (50%)
Number of episodes	1.1 ± 1.7	1.4 ± 2
Time from first episode to LT (months)	22 ± 33	20 ± 29
Maximal severity grade I-II	20 (83%)	11 (92%)
Maximal severity grade III-IV	4 (17%)	1 (8%)
Minimal HE		
(pre-LT neuropsychological assessment)	28 (54%)	11 (46%)
Biochemical parameters before LT		
Bilirubin (mg/dL)	3.5 ± 3	3.7 ± 4
Albumin (mg/dL)	2.8 ± 0.6	3.1 ± 0.5
Creatinine (mg/dL)	1 ± 0.4	0.9 ± 0.3
Prothrombin activity (%)	56 ± 18	58 ± 19
Sodium (mg/dL)	135 ± 5	136 ± 4
MELD	17 ± 6	17 ± 6

Values in mean ± SD or n (%).
*In 8 of them coexisting with hepatitis C.

tremor, and were oriented in space, person, and time. That is, none of them exhibited signs of overt HE. The battery of neuropsychological tests used included the following[12]:

Auditory Verbal Learning (AVL):

A memory test consisting of 5 presentations of a 15-word list, one presentation of a second 15-word list, a sixth recall trial after interference, and a 20-minute delayed recall of the first list. The score for each trial is the number of words correctly recalled. The test generates several indexes that are averaged in a memory index.

Trail-Making Test (Part A):

An attention test, in which the subject must draw lines to consecutively connect 25 numbered circles. The score is the time in seconds required to finish the test.

Symbol Digit Modalities Test (Oral Version):

An attentional and speed-of-information-processing test that consists of a series of rows containing symbols that should be paired to assigned numbers from 1 to 9 according to a written code. The score is the number of correct assignations in 90 seconds.

Grooved Pegboard:

A motor test, consisting of a small board containing slotted holes angled in different directions. Each peg has a ridge along one side requiring it to be rotated into position for correct insertion. The score is the time to completion in seconds with the dominant and nondominant hand.

Controlled Oral Word Association Test (COWAT):

A language and executive function test consisting of three phonemic letter-naming trials. The examiner asks subjects to say as many words as they can in 1 minute beginning with a given letter (for example: F, A, S). The score is the sum of all acceptable words.

Hooper Visual Organization Test:

A visual and executive test of perceptual organization containing a series of more or less readily recognizable pictures of cut-up objects that should be identified by the subject. The total number of items is 30 (maximum score).

Judgment of Line Orientation (JLO):

A visuo-perceptual organization test that examines the ability to estimate angular relationships between line segments by visually matching angled line pairs to 11 numbered segments forming a semicircle. The score refers to the number of line pairs correctly matched.

The scores for each test were transformed into T values, calculated using the formula $T = 50 + 10([x - x_n]/SD_n)$, in which x is the raw result of the test, x_n is the mean value, and SD_n the standard deviation (SD) of the test in the normal population. Normal values were obtained from Spanish population data, adjusted by age, sex, and years of education (categorized into levels: 0 = ≤1 year; 1 = 2-3 years; 2 = 4-7 years; 3 = 8-9 years; 4 = 10-11 years; 5 = >11 years). The test scores were grouped into indexes of memory (AVL), attention (Trail A, Symbol Digit), executive function (COWAT), psychomotor function (Grooved Pegboard), and visuo-perceptive function (JLO, Hooper Test). An overall score (overall cognitive index) was calculated as the average of all the tests. The definition of impairment was based on the following T values: 30-40 (mild), 20-29 (moderate), and <20 (severe), according to standard procedures.

Minimal HE was diagnosed in patients with cirrhosis in the pre-LT examination. The diagnosis was defined by the presence of 2 or more impaired cognitive indexes (<40) in the absence of a known cause of impairment, with a normal neurological examination.[13] Overt episodic HE was defined as the development of confusional syndrome attributable to liver

failure in the absence of an alternative diagnosis.[14] Cognitive impairment after LT was based on an overall cognitive function score <40.

MR Imaging

MR studies were performed in 24 patients, 6 to 12 months after LT, on a 1.5-T Symphony Quantum Maestro Class system (Siemens, Erlangen, Germany) equipped with a circular polarized receiver head array coil, with the body coil acting as transmitter. The MR protocol included acquisition of proton density and T_2-weighted fast spin-echo (repetition time [TR]/echo time [TE]/echo train length/acquisitions = 3550 ms/14-86 ms/5/1) and T_1-weighted spin-echo (TR/TE/acquisitions = 650 ms/17 ms/1) images. A total of 46 contiguous transverse slices with a thickness of 3 mm, a 3/4 rectangular field of view of 250 mm, and an acquisition matrix of 256 mm × 256 mm were used to record images.

Brain tissue volume, normalized for subject head size, was estimated with SIENAX,[15] a part of the FSL (Oxford Centre for Functional MRI of the Brain [FMRIB] Software Library)[16] from T_1-weighted images. Normalized brain volume values are expressed in milliliters.

The [^{1}H]MR spectroscopy was performed from a volume of interest localized at the parieto-occipital region and defined by a cube of 2-cm side containing mainly white matter. A 90°-180°-180° spin-echo–based pulse sequence was used (1600/30 = TR/TE). For water suppression, a chemical shift selective gaussian pulse was applied. A total of 1024 data points were collected over a spectral width of 1000 Hz. Four dummy scans plus 128 acquisitions were accumulated for each spectrum. Spectrum analysis was performed using the LCModel, a user-independent fitting routine, which is based on a library of model spectra of individual metabolites.[17] Measurements were performed at the following resonances: *N*-acetylaspartate (NAA), glutamine (Gln), glutamate (Glu), creatine/phosphocreatine (Cr), choline-containing compounds (Cho), and myo-inositol (mIns). Results are expressed as metabolite ratios with respect to the Cr resonance. Images were assessed by two neuroradiologists (A.R., J.A.), who were blinded to the neuropsychological test results.

Statistical Analysis

Continuous variables are reported as the mean ± SD or median (interquartile range). The chi-square test or Fisher's exact tests were used to study the existence of significant differences between nominal variables. Normality of continuous variables was explored using the Shapiro-Wilk Test. Depending on the distribution of the variables, parametric or nonparametric tests were applied to study the between-group (Student *t* test or Mann-Whitney rank-sum test) or within-subject (*t* test for paired samples or Wilcoxon test) differences. Comparisons among 3 or more independent groups were performed with 1-way analysis of variance, and the Bonferroni correction was applied in the post hoc analysis of normally distributed continuous variables with equal variances. The Pearson or Spearman correlation coefficient test was applied to study correlations between variables. Linear trends were determined with the polynomial linear term or Jonckheere-Terpstra test when the independent variable was ordinal. Linear multiple regression analysis was used to identify those factors (ie, cognitive risk factors: those pretransplant factors that were associated with posttransplant cognition) that were independently associated with the quantitative variables of interest (ie, global cognitive function). The criterion used to select the final model was higher R^2 with lower Mallow's Cp.[18,19] The B coefficient for each variable that remains in the final model represents the standardized coefficient (beta). A P value <0.05 with correction for multiple testing, if applicable, was considered statistically significant. Collinearity between variables was examined by estimating tolerance and the variance inflation factor; variables with problems were excluded. Statistical calculations were performed with SPSS, version 15.0 (SPSS Inc., Chicago, IL).

RESULTS

Cognitive Function

Before LT, patients exhibited several disturbances in cognitive function, but these were not obvious on clinical examination. None of the patients showed chronic, overt HE. Three neuropsychological indexes (memory, attention, and motor function) showed mild impairment (Fig. 1), and 28 patients (52%) were diagnosed with minimal HE. Patients who had minimal HE had poorer liver function and were more likely to have experienced previous HE than those who did not (Table 2).

After LT, all the neuropsychological indexes showed a significant improvement, with an increase in the global cognitive function score from 42 ± 8 to 49 ± 6. The degree of improvement in global function was higher in patients who had episodes of HE and ascites before LT, and correlated with the alterations in bilirubin, albumin, prothrombin activity, and sodium, and the Model for End-Stage Liver Disease (MELD) score results (Table 3). There was no association between the cognitive test scores and the time since LT. The multivariate analysis—including the following variables: prior HE, ascites, bilirubin, albumin, prothrombin activity, sodium, and MELD score (Table 3)—found that the best explicative model for the improvement in global cognitive function ($R^2 = 0.376$, $Cp = 2.95$, $P < 0.001$) was associated with previous HE ($B = 0.28$), serum sodium concentration ($B = -0.32$), and prothrombin activity ($B = -0.29$).

In the post-LT evaluation, the average scores for the different indexes were within normal values (T values >40 and <60) (Fig. 1). However, 7 patients (13%) exhibited mild cognitive impairment (global cognitive function <40). The clinical and biochemical characteristics of this patient subgroup before and after LT are summarized in Table 4. Analysis of peritransplantation

conditions, post-LT infection, and effect of immunosuppressive drugs (including shifts in serum sodium or tacrolimus levels after transplantation) showed no impact of these factors on post-LT cognitive function. Global cognitive function was poorer in patients with a lower educational level, alcohol etiology, diabetes mellitus or HE prior to LT (Table 3). The best explicative model (R^2 = 0.231, Cp = 7.75, P = 0.005), which incorporated these 4 covariables (Table 3) in the multivariate analysis, showed that the posttransplant cognitive function was associated with alcohol etiology (B = −0.26), diabetes mellitus (B = −0.26), and prior HE (B = −0.22) These variables were considered cognitive risk factors based on these results. The number of pre-LT cognitive risk factors was associated with the overall post-LT cognitive function (Fig. 2), and the pattern of impairment was related to the risk factors. Transplant recipients with alcohol-induced cirrhosis showed a memory decline, those with diabetes mellitus exhibited attention impairment, and those with prior HE defects had impaired motor function (Fig. 3).

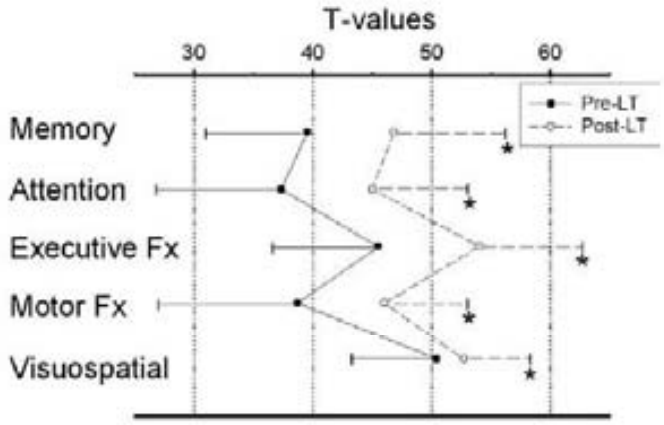

Figure 1. Cognitive function (Fx) before liver transplantation (pre-LT) and after liver transplantation (post-LT). The results are expressed as T values. The different neuropsychological domains showed a significant improvement after liver transplant (*P < 0.05). Please note that pre-LT, the average results for memory, attention, and motor function are impaired (values <40).

Magnetic Resonance

The characteristics of patients evaluated by MR were similar to those of the overall group (Table 1). The post-LT normalized brain volume in these patients was 1556 ± 96 mL. Brain volumes were lower with increasing age (R = −0.641, P = 0.001), alcohol etiology (1504 ± 69 mL versus 1582 ± 92 mL, P = 0.049), prior HE (1505± 96 mL versus 1607 ± 52 mL, P = 0.004) and increasing time from first HE (R = −0.641, P = 0.025). The multivariate analysis in which these 4 covariables were tested indicated that age and time since first episode of HE were independently associated with a significant decrease in brain volume (time from first episode of HE to LT: B = −0.64, age: B = −0.50; R^2 = 0.76; Cp = 2.44; P = 0.002). The age-related decrease in brain volume was more pronounced in patients with previous HE (Fig. 4).

Although cognitive function after LT was within normal values on average, patients with a smaller brain volume showed poorer function on motor tests (R = 0.41, P = 0.049).

MR spectroscopy results (Cho/Cr = 0.29 ± 0.06, mIns/Cr = 0.83 ± 0.29, Gln/Cr = 1.9 ± 0.51, NAA/Cr = 1.45 ± 0.18) were within the range of normality of our center. NAA/Cr, a neuronal marker, correlated significantly with brain volume (R = 0.50, P = 0.013) (Fig. 5).

DISCUSSION

The findings of this study indicate that HE, in addition to diabetes mellitus and prior alcohol consumption, has effects on cognitive function that persist after LT. LT candidates often experience episodes of

TABLE 2. Clinical and Biochemical Characteristics of Patients According to the Presence of Minimal HE

Characteristic	Minimal HE (n = 28)	Normal Neuropsychological Function (n = 24)	P
Age	56.5 (13)	54.5 (16)	ns
Sex (male/female)	82%/18%	79%/21%	ns
Education (level)	2 (3)	3 (1)	ns
Alcohol etiology	61%	29%	0.023
Diabetes mellitus	36%	17%	ns
Prior HE	64%	25%	0.005
Ascites	89%	67%	0.033
Prior variceal bleeding	32%	29%	ns
Bilirubin (mg/dL)	3.1 (2.8)	1.7 (2.5)	0.023
Albumin (mg/dL)	2.5 (0.8)	2.9 (0.6)	0.004
Creatinine (mg/dL)	0.9 (0.5)	0.9 (0.2)	ns
Prothrombin activity (%)	53(12)	64 (30)	0.009
Sodium (mg/dL)	135 (6)	137 (3)	0.029
MELD score	17 (6)	15 (7)	0.006

Values in median (interquartile range) or percent (%).
Mann-Whitney rank sum test, chi-square, or Fisher's tests.

TABLE 3. Factors Related to the Change in Global Cognitive Function With LT and the Final Cognitive Function After LT

Factor	Change in Global Cognitive Function With LT*		Global Cognitive Function After LT†	
Age	0.043	ns	−0.205	ns
Sex (male vs female)	7 ± 5 vs 6 ± 4	ns	48 ± 6 vs 50 ± 7	ns
Education (years)	0.117	ns	0.296	0.033
Alcohol etiology (yes / no)	7 ± 5 vs 6 ± 4	ns	46 ± 7 vs 50 ± 5	0.027
Diabetes mellitus (yes / no)	8 ± 6 vs 6 ± 4	ns	45 ± 6 vs 50 ± 6	0.022
Prior HE (yes / no)	9 ± 5 vs 5 ± 4	0.008	47 ± 6 vs 50 ± 6	0.023
Ascitis (yes / no)	8 ± 4 vs 3 ± 4	0.002	49 ± 6 vs 50 ± 5	ns
Bilirubin (mg/dL)	0.353	0.01	−0.172	ns
Albumin (mg/dL)	−0.342	0.013	0.238	ns
Creatinine (mg/dL)	−0.140	ns	−0.181	ns
Prothrombin activity (%)	−0.422	0.002	0.146	ns
Sodium (mg/dL)	−0.472	<0.001	0.096	ns
MELD score	0.377	0.006	−0.182	ns

Pearson's or Spearman's coefficient test or Student *t* test;
*T values post-LT minus pre-LT
†T values post-LT (mean ± SD).

TABLE 4. Clinical and Biochemical Characteristics Prior to LT in Relation to the Development of Mild Cognitive Impairment After LT

Characteristic	Mild Cognitive Impairment After LT (n = 7)	Normal Cognitive Function After LT (n = 45)
Age (years)	52 ± 14	54 ± 10
Alcohol etiology	5 (71%)	19 (42%)
Diabetes mellitus before LT*	5 (71%)	9 (20%)
Prior hepatic encephalopathy	5 (71%)	19 (42%)
Time since first episode of HE (months)	26 ± 38	21 ± 32
Severity of HE(0/I-II/III-IV)	1/3/2	0/17/ 2
Number of cognitive risk factors*	2 (2)	1 (2)
MELD	18 ± 4	16 ± 6
Na pre-LT (mg/dL)	139 ± 1	135 ± 6
Change in Na (7 days post-LT)†	0.4 ± 2	3 ± 6
Tacrolimus concentration post-LT (ng/mL)‡	6 ± 3	7 ± 4
Infections post-LT (number of episodes)§	1.8 ± 0.8	1.5 ± 1.7

*$P < 0.05$ (Mann-Whitney rank sum test or chi-square test).
†Maximum change in serum sodium seen 7 days posttransplant.
‡Serum tacrolimus levels at the time of the posttransplant neuropsychological assessment (± 1 week).
§Infection of central nervous system was not detected in any of the groups.

HE or exhibit minimal HE, as was seen in two-thirds of our patients. This population is considered at higher risk of developing neurological complications following LT.[20] Our data demonstrate that overall cognitive function may be impaired after LT in the absence of major neurological complications related to the surgical procedure or the postoperative management. The neuropsychological impairment after LT appears to have a distinct pattern in relation to the pre-LT cognitive risk factor, with psychomotor disturbances the most characteristic of HE. Fortunately, the number of patients with cognitive impairment is small and the severity of the persisting defect is mild.

HE has been classically considered a metabolic encephalopathy. This concept is supported by the results of several studies showing improved cognitive function after LT.[4,21] Reversibility has even been shown for the more extreme cases of chronic deterioration, commonly called acquired hepatocerebral degeneration.[22,23] However, there is evidence that HE causes some degree of permanent central nervous system damage.[3,11]

Cognitive impairment after LT has been documented in previous studies.[4] Nevertheless, the majority of transplant recipients exhibit normal cognitive function that remains stable for many years.[10] Only

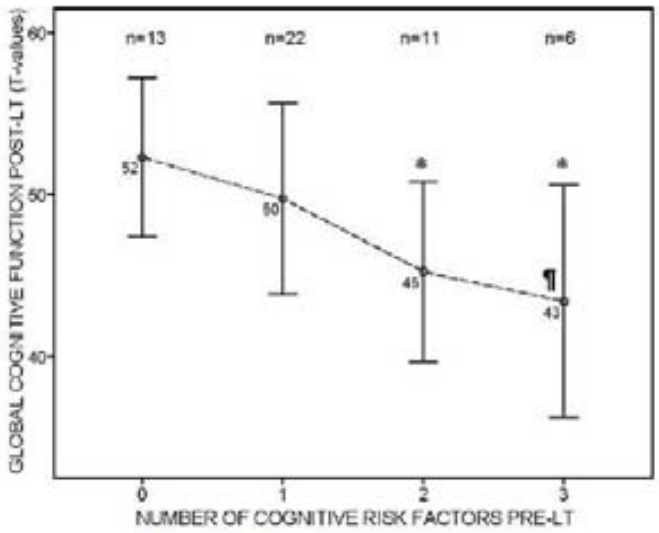

Figure 2. Global cognitive function after liver transplantation (T values) according to the number of cognitive risk factors present before liver transplantation (1-way analysis of variance). *Post hoc analysis with Bonferroni correction, compared to 0 risk factors ($P < 0.05$). ¶Linear tendency determined with polynomial contrast and Jonckheere-Terpstra test ($P < 0.01$).

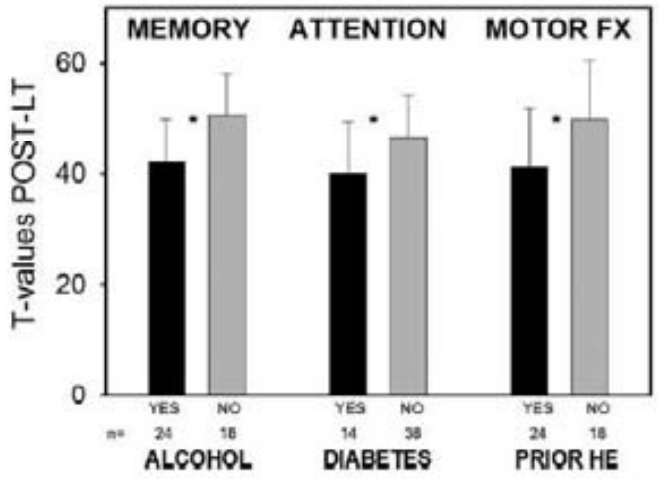

Figure 3. Cognitive indexes (T values) after liver transplantation in relation to pretransplant risk factors (factors associated with cognitive indexes in the multivariate analysis).

13% of our patients qualified for mild cognitive impairment. There are many possible causes of central nervous system deficits, such as preexisting events (eg, alcoholism, trauma, stroke), intraoperative brain injury (eg, hypotension, ischemia), persistent portosystemic shunts, and toxic effects of immunosuppression.[24] Although we cannot exclude participation of these factors, our data indicate that susceptibility to persistent cognitive deficits is higher among patients with alcohol-induced disease, diabetes mellitus, and prior episodes of HE. Two or more of these factors were present in 71% of patients with mild cognitive impairment following LT and in only 27% of those with normal neuropsychological function.

Our findings show that several pre-LT factors with known effects on neuropsychological performance

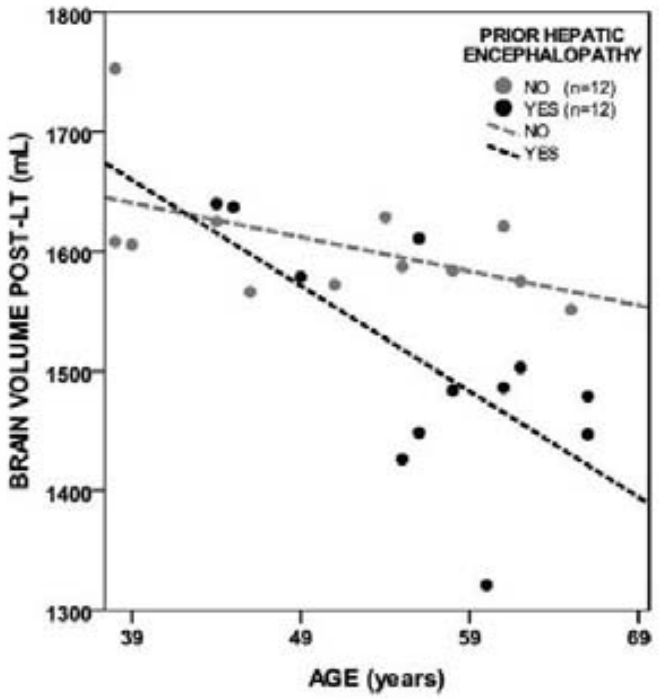

Figure 4. Brain volume after liver transplantation in relation to age and presence of HE before transplantation.

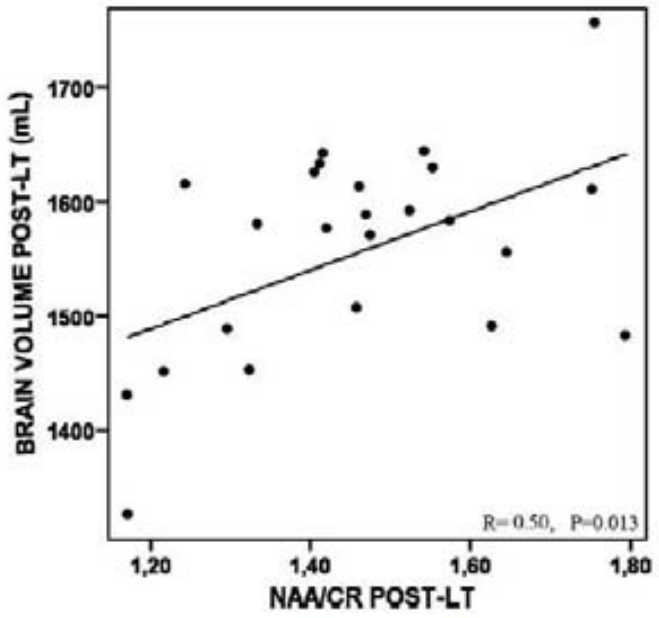

Figure 5. Relationship between brain volume and NAA/Cr.

may be responsible for sequelae when they act additively. Alcohol is neurotoxic and alcohol abuse causes cognitive disturbances that affect many domains.[25] Neuropsychological function improves with abstinence, but several defects may persist,[26] particularly with regard to memory, as was seen in our study. Diabetes mellitus type 2 has been associated with a decline in cognitive function that affects attention, executive function, and memory.[27] This decline is more marked in older patients and has been related to

white matter lesions and brain atrophy, probably indicating vascular-induced injury that can be enhanced in the posttransplant period by other vascular risk factors (eg, hypertension, hypercholesterolemia).[10]

HE may cause damage to brain tissue, including neuronal loss.[28] In neuroradiological studies, brain volume loss, a marker of brain atrophy and a consequence of neuronal loss, has been documented in 50% of nonalcoholic patients with chronic HE.[9] A confounding factor in these studies may be low-grade brain edema,[29] which resolves after LT. In the current study, which used an accurate volumetric method, brain volume was found to be smaller in relation to age (as was expected), and the development of episodes of HE. The association between HE and brain volume should be interpreted cautiously. The MR data proceed exclusively from the post-LT period; the findings should be corroborated in longitudinal studies of patients who experienced repeated bouts of HE. Nevertheless, a plausible explanation for the association is that HE induces a loss of neurons. This notion is supported by the association between brain volume and the relative content of NAA. Animal models provide convincing evidence that several neuronal cell death mechanisms are activated in HE.[30] In comparison to chronic HE, the loss of neurons and decrease in brain volume resulting in episodic HE is mild. In our study, the majority of patients with cirrhosis exhibited brain volumes and NAA/Cr peaks within the normal range. Nevertheless, brain tissue loss, even when it is mild, can participate in a decline in cognitive function, as was suggested by the correlation found between brain volume and psychometric test results.

The current study has several limitations that should be taken into consideration in the interpretation of the results. Cognitive function is influenced by several factors that are present before and after LT and some of these may not have been adequately recognized in the study. One weakness that is difficult to resolve is how to attribute neuropsychological impairment to a specific factor in patients with multiple comorbid conditions. The diagnosis of minimal HE is based on demonstration of cognitive disturbance in the absence of other causes, but several factors could have participated in the deterioration seen on pre-LT neuropsychological testing. Alcoholism is a well-recognized factor that was more common in patients with minimal HE, even after patients had been abstinent for more than 6 months. Alcohol impairs cognition and can cause brain atrophy, which affects most remarkably frontal lobes and cerebellum and may reverse in association with cognitive function with long-term sobriety.[31] To control for the confounding effects of alcoholism some studies have excluded these patients in the characterization of minimal HE, but the results have been similar[6] to those that included alcoholics on abstinence.[32] We did not exclude prior alcohol consumption because our aim was to keep the applicability of the results by investigating patients who are representative of clinical practice. The contributing effect of this factor was evaluated in the multivariable analysis. In addition, postoperative complications, especially those in the early post-LT period may also have affected cognitive function. Recent studies have shown that among older patients, hospitalization and intensive care unit admission are risk factors for developing dementia several years later.[33] Perioperative factors that could have worsened cognitive function were not identified, but may show up in studies with larger samples and longer follow-up periods.

Despite these limitations, our findings may have implications in the management of LT candidates. First, therapies that are effective in preventing HE (rifaximin and lactulose) are currently available.[34] Second, the consequences of HE on postoperative cognitive status may have an influence on prioritization policies.[35] This is especially important for LT candidates with recurrent and difficult to treat episodes of overt HE and low MELD scores. Additional factors that should be also considered are age, a history of alcoholism, and concurrent diabetes, because patients with these factors are the most vulnerable.

In conclusion, this prospective study shows that HE has an effect on cognitive function after LT, likely because it results in neuronal and brain volume loss. These findings suggest that implementation of measures to prevent HE may improve cognitive outcome after LT.

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5.3. Supplementary material I

Juan Córdoba and Rita García Martínez

Hepatic Encephalopathy and Alterations of Cerebral Function

Clinical Gastroenterology: Chronic Liver Failure

Edited by: P. Ginès et al

Hepatic Encephalopathy and Alterations of Cerebral Function

Juan Córdoba and Rita García-Martinez

CONTENTS

Key Words: Hepatic encephalopathy, Ammonia, Energy impairment, Brain edema, Hyponatremia, Acute-on-chronic liver failure

1. INTRODUCTION

Liver failure is characterized by the induction of a series of abnormalities of brain function which are included under the term hepatic encephalopathy (HE). The neurological manifestations are very variable and can be acute, chronic, or subclinical (minimal HE) (1). In addition, HE can be associated with cirrhosis, fulminant hepatitis, or portosystemic bypass without intrinsic liver disease. In patients with cirrhosis, the most common underlying disease, HE, may be precipitated by an extrahepatic factor (gastrointestinal bleeding, infection, TIPS, disturbances of electrolytes, constipation) or be secondary to an acute exacerbation of a liver disease (acute-on-chronic). Distinction

From: *Clinical Gastroenterology: Chronic Liver Failure*,
Edited by: P. Ginès et al., DOI 10.1007/978-1-60761-866-9_9,

between both situations is clinically and pathophysiologically relevant. Precipitating factors appear to have a major role in increasing the exposure of the brain to toxins, and HE resolves usually rapidly with the correction of the precipitating factor (2). In patients with acute-on-chronic liver failure, there are additional aspects of major pathophysiological importance: systemic inflammatory response, circulatory dysfunction, and failure of other organs that can cause directly disturbances of brain function (3).

2. EFFECTS OF LIVER FAILURE ON BRAIN FUNCTION

2.1. Disturbances of Neurotransmission

HE, as other forms of metabolic encephalopathy, results in abnormalities in neurotransmission (4). This hypothesis is supported by its potential reversibility and by the lack of neuronal damage. Multiple abnormalities of neurotransmitter systems have been described in animal models of HE, including disturbances in the excitatory glutamatergic (5) and inhibitory GABAergic (6) neurotransmitter systems. Some supportive data are provided by studies on autopsied material (7) and by neuroimaging techniques. However, it is very difficult to relate the complexity of the disturbances of neurotransmission to the neurological manifestations. Several therapeutic attempts have been conducted to restore disturbances in neurotransmission with specific drugs, but the results have not been remarkable (8).

2.2. Injury to Astrocytes

Astrocytes are the cells of the central nervous system that are affected in HE (9). The distinctive neuropathological alteration is the Alzheimer type II astrocytic change, probably a chronically degenerated astrocyte secondary to cellular swelling. Glutamine is generated in the astrocytes during the detoxification of ammonia through the amidation of glutamate. The accumulation of glutamine may cause an increase in intracellular osmolality (10) or induce mitochondrial injury by the activation of the mitochondrial permeability transition (11). Factors that precipitate HE, such as inflammation, hyponatremia, and benzodiacepines, can exacerbate swelling (12). The change in the state of cellular hydration causes impairment of several metabolic pathways and has been proposed to be responsible for brain edema and for the neurological manifestations of HE (13). Mechanisms by which abnormal glial cells can influence neuronal function include interaction with glutamate reuptake (14) and activation of peripheral-type benzodiacepine receptors, causing increased synthesis of neurosteroids that

are powerful ligands of the neuronal $GABA_A$ receptor (an inhibitory neurotransmitter) (15).

2.3. Energy Impairment

The brain is the tissue with the highest energy requirements of the body and depends entirely on the process of glycolysis and respiration within its own cells to synthesize its energy demands. In HE in humans, a decrease in consumption of oxygen and glucose is accompanied by a parallel decrease in cerebral blood flow (16). It is not possible to separate whether the decrease in oxygen consumption is the cause or the consequence of encephalopathy. The current interpretation is that, as in other metabolic encephalopathies, energy impairment is secondary to the decrease in neuronal function. However, a direct effect of ammonia on energy metabolism causing neuronal disturbances is also possible (17).

In fulminant hepatic failure, and possibly in acute-on-chronic liver failure, disturbances in energy metabolism may have an important participation in the clinical picture. In patients with acute liver failure increases in brain lactate, identified by brain microdialysis, are followed by surges of high intracranial pressure (18). Furthermore, an increase in plasma lactate is a well-recognized prognostic factor in fulminant and in acute-on-chronic liver failure (19). Experimental models have shown that the increase in brain lactate is of newly synthesized origin and parallels the increase of brain water in the intracellular compartment (20). Ammonia may impair glycolysis, because it inhibits α-ketoglutarate dehydrogenase, the rate-limiting enzyme of the tricarboxylic acid cycle (21), and may have a direct toxic effect on the mitochondria (11). An alternative explanation is that lactate is generated aerobically by excessive glutamatergic activation (22), which may be induced by excessive amidation of glutamate, secondarily to an increase in brain ammonia (Fig. 1). Irrespective of the mechanism being involved, a drop of brain pH can cause injury at multiple levels, including astrocyte swelling (23).

2.4. Brain Edema

Brain edema is now recognized as an element that is present in acute and in chronic liver failure and can be identified by indirect (24) and direct techniques of magnetic resonance (25). The main factor involved in the generation of brain swelling is the increase in plasma ammonia (26). Other factors, such as hyponatremia, may enhance the effects of ammonia on brain swelling (27). An intriguing finding is the different distribution of water in the brain in acute and in chronic liver

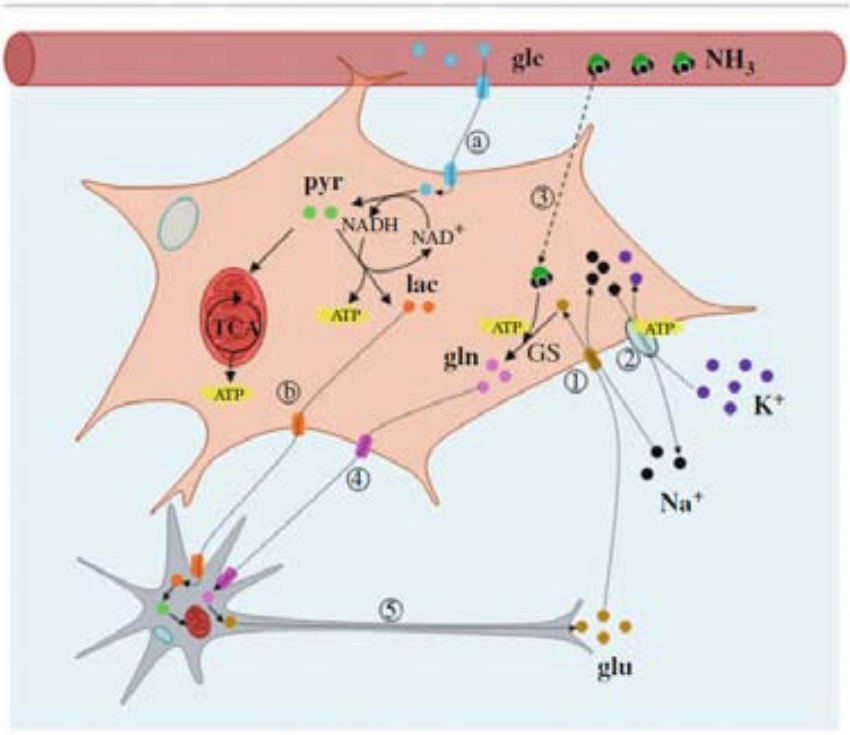

Fig. 1. Interaction between ammonia, the glutamate–glutamine cycle, and energy metabolism in astrocytes and neurons. Glutamate (Glu, a neurotransmitter) is reuptaken from the synaptic cleft by the astrocyte (1) in cotransport with Na^+ (2). The excess Na^+ is interchanged with K^+ through a Na/K pump (3). Ammonium (NH_3) enters the astrocyte from blood through passive diffusion and combines to glutamate to synthesize glutamine (gln), a reaction (4) that is catalyzed by glutamine synthetase (GS). Glutamine is transported to the neuron where it is transformed again to glutamate, a major neurotransmitter, and closes the glutamine–glutamate cycle. Glucose (glc) enters the astrocyte from blood (a) and is transformed into pyruvate (pyr) to produce ATP in mitochondria. A significant amount of pyruvate is transformed to lactate (lac) that is shuttled to the neuron (b). Lactate reaches the neuron where it is transformed into pyruvate to provide ATP through the tricarboxylic acid cycle (TCA).

failure, suggesting different pathogenetic mechanisms. In acute liver failure, brain water is mostly located in the intracellular space (28), while in chronic liver failure is mostly extracellular (29). This difference suggests a role for increased blood–brain barrier permeability in the chronic situation, which may be mediated through inflammatory mediators.

Brain edema has been proposed to have major consequences on neuronal function (13), but good evidences are lacking. The increase in the volume of the brain inside a rigid skull can cause intracranial hypertension, which is responsible for a significant number of deaths in fulminant hepatic failure. Compensatory mechanisms that

require chronic induction, a lower rise in plasma ammonia, and a smaller brain volume explain why intracranial hypertension is seldom seen in cirrhosis (30). In fulminant hepatic failure, and possibly in acute-on-chronic liver failure, cerebral vasodilatation and loss of autoregulation may worsen brain swelling (31); measures that inhibit cerebral vasodilatation decrease intracranial pressure (32).

2.5. Brain Atrophy

Different neuroimaging techniques have shown brain atrophy in more than half of the patients with cirrhosis and chronic HE (33). The prevalence is higher among alcoholic patients, because alcohol causes a dose-related decrease in brain size that is aggravated by a poor nutritional status but is partially reversible with abstinence (34). Similarly, the chronic exposure to neurotoxins involved in the pathogenesis of HE could lead to loss of brain tissue that can explain the persistence of neuropsychological deficits after liver transplant (35).

3. MECHANISMS BY WHICH LIVER FAILURE INDUCES HE

Liver failure causes an increase in the exposure of the brain to several substances that under normal circumstances are efficiently metabolized by the liver; those substances that have a high "first-pass" metabolism are the most important, as shown by the major role of portosystemic shunting in the development of HE. In addition, other factors that are commonly present in patients with liver failure and may worsen neurological function are inflammation, circulatory derangements, nutritional deficits, comorbidities, and failure of other organs.

3.1. Ammonia Toxicity

Ammonia has been historically viewed as the most important factor in the genesis of HE (Fig. 2). In normal conditions, ammonia is produced by the gut and an important amount is of bacterial origin (36). The concentration of ammonia in portal blood is high, and a high degree of extraction occurs in the liver (37). Ammonia levels are high in patients with HE (38), specially among those with large portosystemic shunts. Similarly, effects on blood ammonia and brain metabolites are seen in shunts secondary to portal vein thrombosis (39) or of congenital origin.

In addition to the intestine and the liver, kidney and muscle contribute to regulate the arterial ammonia level (40). In muscle, ammonia is transformed into glutamine through the action of glutamine synthetase.

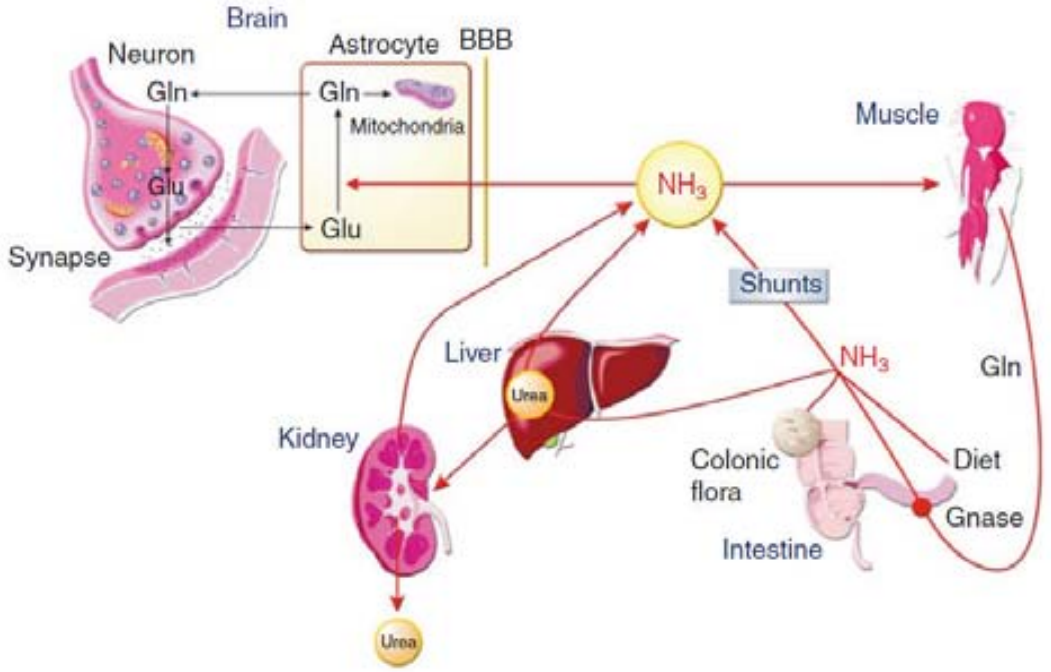

Fig. 2. Interorgan ammonia trafficking and metabolism. Ammonia is generated in the intestines from nitrogenous compounds from the diet, deamination of glutamine by glutaminase, and metabolism of nitrogenous substances by colonic flora. In normal circumstances, most ammonia is metabolized to urea in the liver. Portal-systemic shunts and liver failure cause a rise in blood ammonia that may affect brain function by inducing several disturbances in astrocytes that may impair mitochondria and the glutamate–glutamine trafficking between neurons and astrocytes. Skeletal muscle is capable to decrease blood ammonia by metabolizing ammonia to glutamine. Kidney has also an important role in determining blood ammonia by excreting urea in the urine and generating ammonia. NH_3, ammonia; Glu, glutamate; Gln, glutamine; GNASE, glutaminase; BBB, blood–brain barrier.

The ability of the muscle to "fix" appreciable amounts of blood-borne ammonia becomes important to regulate arterial ammonia in case of liver failure and highlights the importance of maintaining an adequate muscle mass.

Patients with HE have an increased diffusion of ammonia into the brain in relation to an increase in arterial ammonia (41). In the brain, ammonia is metabolized to glutamine in astrocytes, where ammonia or glutamine exerts their toxic effects. Recent data provide more information on the mechanisms by which ammonia causes neuronal disturbances, but a complete explanation is still lacking (42). Signs of oxidative stress, such as protein tyrosin nitration, have been found in several experimental preparations. In addition to injury to proteins, ammonia induces RNA oxidation, which may have multiple

consequences in neurotransmission and postsynaptic protein synthesis. Such changes may also underlie the pathologically altered oscillatory networks in the brain of HE patients in vivo, as detected by magnetoencephalography (43).

3.2. Inflammation

Patients with acute and acute-on-chronic liver failure develop frequently a marked activation of inflammatory mediators (44). The presence of a systemic inflammatory response syndrome has been linked to the development of HE in fulminant hepatic failure (45) and in cirrhosis (46). The activation of inflammatory mediators, such as cytokines, may modulate the effect of neurotoxins on the brain. The accompanying impairment in renal function can increase circulatory urea levels, with subsequent colonic generation of ammonia via urease-containing bacteria. Peripheral inflammation may signal the brain through the activation of vagal afferents (47). Other mechanisms of transduction of signals into brain are binding of cytokines to receptors in cerebral endothelial cells or direct access of cytokines into brain tissue at sites lacking blood–brain barrier (such as the circumventricular organs) (48). Inflammation may also be directly induced in the brain. Microglial activation and induction of synthesis of proinflammatory cytokines have been shown in experimental models (49). Neuroinflammation has an important role in many neurological diseases. In HE, activation of inflammation in brain tissue may increase blood–brain barrier permeability, result in the generation of intracerebral mediators (such as nitric oxide and prostanoids), and cause astrocytic swelling (13, 50).

3.3. Circulatory Dysfunction

Patients with liver failure, especially those with acute and acute-on-chronic liver failure, exhibit commonly circulatory disturbances characterized by low arterial pressure, low peripheral vascular resistance, and high cardiac index (51). It has been hypothesized that this circulatory dysfunction participates in the pathogenesis of neuronal disturbances (52). There is a close parallelism between renal and cerebral circulation in liver failure. In advanced liver failure, both territories lose the property of vascular autoregulation (53). In patients with cirrhosis and ascites, there is renal and cerebral vasoconstriction, which are probably related to arterial hypotension and to the overactivity of vasoconstrictor systems (54). The clinical experience also links renal failure to HE. In patients with advanced cirrhosis, an increase in serum creatinine and a decrease in serum sodium are the two most important factors involved in the recurrence of HE (55). The experience with patients

with cirrhosis and organic nephropathies suggest that the mechanisms involved in recurrence of HE do not simply correspond to a decrease in the excretion of urea in urine. The increase in creatinine in advanced cirrhosis identifies the presence of circulatory dysfunction (56). It is probably that circulatory dysfunction is a key mechanism in precipitating HE in patients with advanced liver failure, especially among those with acute-on-chronic liver failure.

4. PRINCIPLES OF TREATMENT

HE is a manifestation of severe liver failure; its treatment cannot be separated from the treatment of liver failure, which requires a series of supportive measures, including the general management of a patient with change in mental status. Several measures specifically designed to treat HE appear to be beneficial (57), although many of them have not undergone proper assessment in good clinical trials and have been criticized (58). Treatment of precipitating factors (Table 1) is a mainstay of management, which requires their active search and continuous monitoring.

4.1. Nutritional Measures

Intake of large amounts of proteins should be avoided because they can precipitate HE. However, the classically recommendation of restricting dietary protein intake is no longer valid (59). In patients with cirrhosis, a low-protein diet does not improve the outcome of acute HE (60) and does not reduce its recurrence (61). Protracted nitrogen restriction may be harmful, as witnessed in patients with acute alcoholic hepatitis (62). The current recommendation is to give a diet that contains a normal amount of proteins (0.8–1.2 g/kg/d).

Severe malnutrition, which is common among patients with cirrhosis, is associated with a poor short-term prognosis. A positive nitrogenous balance may improve encephalopathy by promoting hepatic regeneration and increasing the capacity of muscle to detoxify ammonia (39). However, improvement in nutritional status in patients with cirrhosis is difficult. A high-protein intake (>1.2 g/kg/d) may be necessary to maintain nitrogen balance, but can increase blood ammonia and may precipitate HE (63). Modifying the composition of the diet and increasing its calorie/nitrogen ratio may improve tolerance to protein. At isonitrogenous levels, vegetable and dairy products cause less encephalopathy than does meat (64). A high calorie-to-nitrogen ratio, which is characteristic of casein-based and vegetable diets, reduces gluconeogenesis and has anabolic effects on the utilization of dietary

Table 1
Precipitating factors for HE

Precipitating factor	*Possible effects*	*Mechanism of action*	*Associated coprecipitant*
Sepsis	Increase in blood ammonia Enhancement of the effects of putative toxins on the CNS	Protein catabolism Activation of cytokines	Azotemia Arterial hypotension
Gastrointestinal bleeding	Impairment in liver function Increase in blood ammonia	Hepatic hypoperfusion Nitrogen load Disturbances of plasma amino acids	Infection Anemia Arterial hypotension
Hypokalemia	Increase in blood ammonia	Ammonia generation	
Azotemia	Increase in blood ammonia	Ammonia generation	
Dehydration	Increase in blood ammonia	Hepatic hypoperfusion	Hypokalemia Azotemia
Diuretics	Increase in blood ammonia	Hypokalemia Azotemia Dehydration	
Acute hepatitis	Impairment in liver function Enhancement of effects on the CNS	Liver injury Activation of cytokines	
Surgery	Impairment in liver function	Hepatic hypoperfusion	Anesthetics
Constipation	Increase in blood ammonia	Ammonia generation by enteric flora	
Large protein intake	Increase in blood ammonia	Nitrogen load	
Psychoactive drugs	Enhancement of effects on the CNS	Activation of inhibitory neuro-transmission	

CNS, Central nervous system

proteins. The benefits of vegetable-based diets have also been related to the presence of nonabsorbable fiber that is metabolized by colonic bacteria. Branched-chain amino acids show anticatabolic effects in patients with chronic liver diseases, probably due to their ability to serve as an energy substitute for muscle, and because of their actions on muscle protein synthesis and degradation (65).

4.2. Decreasing the Production of Toxins: Prebiotics, Probiotics, and Antibiotics

The observation of a relationship between portosystemic shunting, constipation, and HE leads to the concept that the intestinal flora is an important source of toxins. This was followed by the introduction of intestinal cleansing, prebiotics, probiotics, and antibiotics to treat HE. The goals of these treatments are to increase fecal nitrogen excretion, reduce the generation of ammonia by fecal flora, and decrease the amount of ammonia that reaches portal blood (66). This can be achieved by promoting the growth of saccharolytic flora with little urease activity and reducing the bulk of proteolytic flora. Another possible beneficial effect of modifying the enteric flora is reducing translocation of intestinal bacteria; a decrease in plasma endotoxin has been shown with prebiotics and probiotics (67). Translocation of bacterial products may activate inflammatory mediators (68), worsen hemodynamic parameters, and favor the development of HE.

Lactulose (a nonabsorbable disaccharide) is a prebiotic that was first introduced with the idea of increasing the amount of *Lactobacillus bifidus* in the enteric flora. The mechanism of action is more complex. Administered orally, lactulose and lactitol (a similar nonabsorbable disaccharide) are not broken down by intestinal disaccharidases and reach the cecum, where they are metabolized by enteric bacteria to lactate and acetate (69). These metabolites cause a drop in cecal pH, which is critical for the drugs to be effective, and is associated with catharsis. A similar effect can be obtained by giving different combinations of probiotics that are enriched in lactobacillus or in other "healthy" species (70). The efficacy of prebiotics and probiotics in decreasing blood ammonia and improving minimal HE is similar at short term (71). However, probiotics lack the cathartic effect of nonabsorbable disaccharides and fiber, which make them better tolerable, but could also limit their efficacy. Another alternative that has been proposed is administration of acarbose, a drug that inhibits glucose absorption and modifies the enteric flora. In one study, treatment with acarbose decreased ammonia and improved HE (72).

Several antibiotics that reduce the gram-negative bacilli population have been introduced in the treatment of HE. Neomycin and rifaxim, two antibiotics that are poorly absorbed and decrease blood ammonia, are commonly prescribed as alternatives to nonabsorbable disaccharides (73). In addition to decreasing enteric flora, neomycin causes a reduction in mucosal glutaminase activity and thereby decreases the ability of the mucosa to consume glutamine and produce ammonia (74). There are concerns that long-term therapy with neomycin could result in intestinal malabsorption and renal or auditory toxicity, because it is an aminoglycoside. Rifaximin appears safer for prolonged therapy (75).

Therapies aimed at reducing the production of toxins by the intestinal flora are by far the most commonly used and better studied. Unfortunately, few placebo controlled trials have been conducted. The best results have been observed for treatment of minimal HE (58) and for prevention of recurrence of HE (76). Combination of different therapies may exert some synergism, but the available data are scarce (77). In patients with cirrhosis and an acute episode of HE, the major aims of therapy are controlling the precipitating factor and improving liver function. In this circumstance, many patients receive broad-spectrum antibiotics. The administration of drugs that decrease the intestinal production of toxins may have only a marginal benefit (78).

4.3. New Therapies

The current burden of illness and hospitalization for HE is very high (79), indicating that there is a need for better therapies. New goals of therapy are achieved with drugs that reduce blood ammonia without interfering with enteric flora, new measures for precipitating factors, and liver-support devices.

The generation of ammonia in the small intestine may be reduced by inhibiting glutaminase (80). However, since glutamine is a major energetic substrate of the intestine, this may result in serious adverse effects (81). An alternative mechanism to decrease ammonia is to increase the disposal by stimulating the synthesis of nontoxic nitrogenous compounds. Muscle may become an important organ to enhance ammonia detoxification by conversion to glutamine. L-ornithine–L-aspartate, which has undergone clinical evaluation (82) and is available in several countries, and L-ornithine–L-phenylacetate(83), now under clinical investigation, provide intermediates for glutamine synthesis and decrease plasma ammonia.

Exacerbation of circulatory dysfunction (identified by an increase in creatinine) and hyponatremia are the two most important risk factors for

the development of HE (55). Aquaretic drugs increase plasma ammonia and have shown some promise in improving minimal HE (84). Their putative mechanism of action is through diminishing astrocyte swelling. It is possible that patients with ascites and hyponatremia treated with aquaretics could experience fewer episodes of HE. The administration of albumin alone or combined with vasoconstrictors have shown to be beneficial in preventing circulatory dysfunction in patients with cirrhosis (56) and may secondarily reduce the incidence of HE. In patients with diuretic-induced HE, patients treated with albumin showed a better outcome than those treated with another volume expander (85). The physicochemical characteristics of albumin and the observation of improvement in parameters of oxidative stress suggest that treatment with albumin may decrease the effects of toxins on circulatory, renal, and neurological function (86). Liver support devices, such as the Molecular Adsorbents Recirculating System (MARS), might also play a role. MARS improves the grade of HE (87) independently of changes in ammonia and cytokines, suggesting that other toxins, such as oxygen-based free radicals, might be important.

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5. Patients methods and results

5.4. Supplementary material II

Córdoba J, García-Martínez R, Simón-Talero M

Hyponatremic and hepatic encephalopathies: similarities, differences and coexistence.

Metab Brain Dis • 2010 Mar; 25 (1): 73-80. Epub 2010 Mar 9.

Metab Brain Dis (2010) 25:73–80
DOI 10.1007/s11011-010-9172-3

ORIGINAL PAPER

Hyponatremic and hepatic encephalopathies: similarities, differences and coexistence

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Received: 18 November 2009 / Accepted: 27 November 2009 / Published online: 9 March 2010

Abstract Hyponatremic and hepatic encephalopathy are common causes of metabolic encephalopathy that may coexist in patients with cirrhosis. The clinical picture is common to any metabolic encephalopathy and is characterized by a confusional syndrome that may evolve into coma. Chronic mild or minimal manifestations can be seen in both, but motor symptoms are more common in hepatic encephalopathy. Recent advances show that in addition to clinical manifestations both encephalopathies share some pathogenetic mechanisms. Dysfunction of astrocytes, osmotic changes in the brain and brain edema are present in both situations. Recognition of these abnormalities is important to plan therapy. New drugs that affect brain hydration may be useful for both encephalopathies.

Keywords Metabolic encephalopathy · Hepatic encephalopathy · Liver failure · Cirrhosis complications · Human

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Introduction

Metabolic encephalopathy refers to the impairment in brain's integrated activity in the absence of structural abnormalities (Plum and Posner 1982). The clinical manifestations correspond to a syndrome of global cerebral dysfunction induced by systemic stress, and can vary from mild executive dysfunction or agitated delirium, to deep coma with decerebrate posturing. Normal neuronal activity requires a balanced environment of electrolytes, water, amino acids, excitatory and inhibitory neurotransmitters, and metabolic substrates. Disturbances in this environment cause dysfunction of the ascending reticular activating system and/or its projections to the cerebral cortex, leading to impairment of arousal and/or awareness. The ultimate neurophysiologic mechanisms include interruption of polysynaptic pathways and altered excitatory–inhibitory amino acid balance. Correction of metabolic derangements results in disappearance of clinical manifestations.

Hyponatremic and hepatic encephalopathy are one of the most common causes of metabolic encephalopathy and may coexist in patients with cirrhosis. Several advances in recent years have lead to recognize an important role for the stage of cell hydration in the pathogenesis of both pathologies (Haussinger and Schliess 2008). Changes in astrocyte hydration and compensatory osmotic regulation modify multiple metabolic pathways that may affect neuronal function. The recent development of vaptans, which are antagonists of V2 receptors located in the distal nephron, holds great promise in aiding the treatment of hyponatraemic encephalopathy (Arieff 2001); they may also have beneficial effects in cirrhotic patients with hepatic encephalopathy. This article reviews the similarities (Table 1) and differences (Table 2) in the pathogenesis, clinical manifestations and treatment of hyponatremic and hepatic encephalopathy and discusses the coexistence of hyponatremic and hepatic encephalopathy,

Table 1 Similarities between hyponatremic and hepatic encephalopathy

Microscopy	Astrocyte injury with sparing neurons and olygodendrocytes
Brain edema	Recognizable with neuroimaging
	Compensatory osrmoregulatory changes (decrease in myoinositol)
Biomarkers	Imperfect correlation to plasma sodium or plasma ammonia
Acute manifestations	Confusional syndrome-coma
	Frequently accompanied by nausea
Chronic manifestations	Neuropsychological abnormalities and normal physical exam (minimal-mild encephalopathy)

their relation with brain edema and their importance in the outcome of patients with cirrhosis (Table 3).

Hyponatremic encephalopathy

Hyponatremia is defined as a decrease in the serum sodium concentration to a level below 136 mmol per liter. Hyponatremia can be associated with low, normal, or high tonicity, which refers to the contribution to osmolality of solutes, such as sodium and glucose that cannot move freely across cell membranes. Dilutional hyponatremia, by far the most common form of the disorder, is caused by water retention and is seen in congestive cardiac failure, cirrhosis or nephrotic syndrome. Dilution of body solutes causes hypotonicity, which in turn, leads to transcellular shifts in water and an increase in cell hydration and volume.

Pathogenesis of hyponatremic encephalopathy

The manifestations directly attributable to hyponatremia primarily occur with acute and marked reductions in the plasma sodium concentration (<130 mEq/l) and reflect neurologic dysfunction induced by cerebral edema.

Table 2 Differences between hyponatremic and hepatic encephalopathy

	Hyponatremic encephalopathy	Hepatic encephalopathy
Brain metabolites	Decrease in glutamine	Increase in glutamine
Clinical manifestations	Headache, seizures	Disturbances of motor function (asterixis, parkinsonism...)
Magnetic resonance (diffusion imaging)	Decrease in ADC (intracellular)	Increase in ADC (interstitial)
Sequalae	Myelinolisis after rapid correction	Brain atrophy after recurrent episodes

Table 3 Coexistence of hyponatremic and hepatic encephalopathy

Hyponatremia	Cirrhosis
Prevalence	High among patients with advanced cirrhosis
Prognosis	Associated with decreased survival
Risk factor	Increases the risk of hepatic encephalopathy
	Increases the risk of brain edema post-TIPS
Resistance	Associated to resistance to lactulos in patients with minimal hepatic encephalopathy
Treatment	Correction of hyponatremia with vaptans improves neuropsychological tests

Changes in cell hydration and in the concentration of electrolytes can explain impairment in neuronal function. Synaptic transmission and the generation of the action potential are both dependent on the presence of sodium (Gage and Quastel 1965); amino acid transport systems are almost totally sodium dependent.

The development of cerebral edema is specifically dependent upon the transfer of water from plasma and cerebrospinal fluid into the brain. Microscopically, in experimental models cellular swelling appears to be confined to astrocytes, with sparing of neuronal elements and olygodendrocites (Wasterlain and Torack 1968). The passage of water through the lipid membranes of cells is possible due to the presence of membrane proteins that act as water-pores. One such protein that has been shown to regulate the cellular entry of water in brain is aquaporin-4, which has a strategic localization in astrocytes, particularly in their end-feet and is close apposition to cerebral capillaries. Compared with wild-type mice, knockout mice exhibit considerably less brain edema, morbidity, and mortality after the induction of acute hyponatremia, demonstrating that aquaporin-4 mediates a substantial portion of osmotic water transport into the brain (Manley et al. 2000). The degree of edema may also depend on other factors that regulate the activity of aquaporin-4 (Rama Rao and Norenberg 2007): anchoring proteins, such as α-syntrophin, determine the translocation of aquaporin-4 protein onto the plasma membrane and protein kinase C phosphorylates aquaporin-4 and regulates its activity.

Brain edema also depends on the responses of brain cells to osmotic swelling (McManus et al. 1995). The degree of cerebral edema and therefore the severity of neurologic symptoms are much less with chronic hyponatremia. This protective response, which begins on the first day and is complete within several days, occurs in two major steps (Melton et al. 1987). The initial cerebral edema elevates the interstitial hydraulic pressure, creating a gradient for extracellular fluid movement out of the brain into the cerebrospinal fluid. The brain cells lose solutes, leading to the osmotic movement of water out of the cells and less brain swelling. Most of this volume regulatory response initially consists of

the loss of potassium and sodium salts; this is then followed over the next few days by the loss of organic solutes. Electrolyte movement occurs quickly because it is mediated by the activation of quiescent cation channels in the cell membrane; organic solute loss occurs later because it requires the synthesis of new transporters. The organic solutes (which are called osmolytes) account for approximately one-third of the solute loss in chronic hyponatremia (Lien et al. 1991). Reducing the cell's content of these solutes has the advantage of restoring cell volume without interfering with function.

Clinical manifestations of hyponatremic encephalopathy

The severity of symptoms generally reflects the degree of cerebral overhydration, which depend on the intensity of hyponatremia and the rapidity of the fall in serum sodium. Nausea and malaise are the earliest findings, and may be seen when the plasma sodium concentration falls below 125 to 130 meq/L. This may be followed by headache, lethargy, and obtundation and eventually seizures, coma, and respiratory arrest if the plasma sodium concentration falls below 115 to 120 meq/L. However, there is a significant variation and it has been documented that for almost any given level of plasma sodium there is marked differences in depression of sensorium (Arieff and Guisado 1976).

In experimental animals with acute water intoxication, coma and seizures may be seen when serum sodium is acutely lowered to levels of about 120 mmoles/liter over a period of 2 h (Arieff et al. 1976). When serum sodium is reduced to 122 mEq/liter over 2 to 3 days, however, most animals are asymptomatic. More profound hyponatremia (serum Na=110 mmoles/liter or lower) usually results in varying degrees of lethargy, coma and seizures.

In chronic hyponatremia compensatory mechanisms prevent major cerebral overhydratrion. In this circumstance, symptoms related to hyponatremia can be very subtle and difficult to detect clinically, partly because they can be attributed to those of the associated diseases. In most cases the symptoms are mild and remain unnoticed by patient and physician. However, mild chronic hyponatremia may have important consequences. A study found that contributed to falls, especially in elderly people, probably as a result of attention, posture, and gait impairments (Renneboog et al. 2006). These symptoms may be related to some degree of mild edema, as shown in animals that have been rendered chronically hyponatremic (Arieff et al. 1976).

Treatment of hyponatremic encephalopathy

The main goal of therapy is correction of hyponatremia (Adrogue and Madias 2000). However, the reuptake of brain solutes during correction occurs more slowly than loss of brain solutes during the onset of hyponatremia (McManus et al. 1995). For this reason correction of hyponatremia must be performed slowly. Overly rapid correction may be deleterious, especially in patients with chronic hyponatremia (see below). The adaptation that returns the brain volume toward normal in chronic hyponatremia protects against the development of cerebral edema but also creates a potential problem for therapy. A rapid increase in the plasma sodium concentration can lead to an osmotic demyelination syndrome. These changes can lead to potentially severe neurologic symptoms that are delayed for 2 to 6 days after correction and may be irreversible. An increased susceptibility to osmotic demyelination is observed in cirrhotics. In this setting, myoinositol, the most abundant organic osmolyte, is depleted because of both a decrease in brain glutamine and hyponatremia induced brain cell swelling (Cordoba et al. 1998). The mechanism is not fully understood but depletion in organic osmolytes has been associated with myelinolysis (Lien et al. 1991).

It is currently recommended that the plasma sodium concentration in hyponatremic patients be elevated at a maximum rate of 10 to 12 meq/L during the first day and 18 meq/L over the first 2 days (Sterns et al. 1994). More rapid therapy (1.5 to 2 meq/L per hour) is required for the first few hours in symptomatic patients with acute hyponatremia, but the total rate of correction over the first day should be the same.

Treatment can also be beneficial for patients with chronic mild hyponatremia (120–129) that are apparently asymptomatic, because gradually raising the plasma sodium with tolvaptan has been associated with significant improvement in their health-related quality of life (Schrier et al. 2006).

Hepatic encephalopathy

Hepatic encephalopathy is as a disturbance in central nervous system function due to hepatic insufficiency and/or portal-systemic shunting (Ferenci et al. 2002). Hepatic encephalopathy is a prevalent complication of cirrhosis that is associated with increased mortality and places a high burden of illness and hospitalization (Poordad 2007; Bustamante et al. 1999).

Pathogenesis of hepatic encephalopathy

A large amount of data support a central role for disturbances of neurotransmission and impairment of astrocytes in hepatic encephalopathy. Thus, it has been proposed that injury to astrocytes causes secondarily abnormalities in neurotransmission and results in the

clinical picture of hepatic encephalopathy (Haussinger et al. 2000). Multiple neurotransmitter systems are abnormal (Butterworth 1996). Those that are better characterized include the excitatory glutamate system and the inhibitory GABAergic system, but many others are also affected, such as histamine, serotonine, opioid, etc.

Current hypotheses propose that hepatic encephalopathy is secondary to the exposure of the brain to circulating neurotoxins (Cordoba and Minguez 2008). Among the different toxins that have been proposed ammonia appears to be the most important factor (Butterworth 2003). Ammonia is produced by the gut and an important amount is of bacterial origin. The concentration of ammonia in portal blood is high and undergoes a high degree of extraction in the liver (90%). Concentrations of ammonia are raised in the systemic circulation and in the cerebrospinal fluid of patients with hepatic encephalopathy (Cooper and Plum 1987). Similarly to what is observed in hyponatremia the correlation between plasma ammonia and the degree of hepatic encephalopathy is not tight. This may depend on the rapidity and the severity of the exposure of the brain to ammonia. For instance it is well documented that in patients with fulminant hepatic failure a plasma ammonia above 150–200 mcM is closely related to the development of cerebral herniation (Clemmesen et al. 1999). Other factors, that can be difficult to identify, may act synergistically. Clinical experience shows that delirium secondary to metabolic disturbances is common among hospitalized patients, specially among older and critically ill patients (Inouye 2006), two conditions that are frequent in cirrhotic patients with hepatic encephalopathy.

Recent studies have found oxidative stress in the brain of hepatic encephalopathy (Haussinger and Schliess 2008). Astrocytes exposed to ammonia develop the mitochondrial permeability transition that leads to free radical generation and increased lipid peroxidation. Other factors that can participate in the development of oxidative stress are glutamine, peripheral benzodiazepine receptor, manganese and nitric oxide. The oxidation of lipids, proteins, and nucleic acids is well known to impair cell structure and function. The consequences of oxidative stress in hepatic encephalopathy are unknown, but it is noteworthy that free radicals are known to cause cell swelling in brain slices and cultured astrocytes.

Brain edema in hepatic encephalopathy

Classically, brain edema has been considered a specific feature of fulminant hepatic failure and has been separated from hepatic encephalopathy (Ware et al. 1971). Experimental and clinical data have documented brain edema in models of chronic liver failure (Cordoba et al. 1996) and in patients with cirrhosis (Cordoba et al. 2001; Shah et al. 2008). Astrocyte swelling is considered the underlying constituent of brain edema in these circumstances. In fulminant hepatic failure, astrocyte swelling along with cerebral blood flow disturbances causes the expansion of brain volume within the rigid skull and results in intracranial hypertension. In cirrhosis, astrocyte swelling may induce a series of functional abnormalities that can be responsible for hepatic encephalopathy (Haussinger et al. 2000). Lack of cerebral hyperperfusion and activation of compensatory mechanisms may explain why intracranial hypertension is infrequent in cirrhosis (Donovan et al. 1998).

Brain swelling is present in all the clinical situations that are accompanied by hyperammonemia, such as inborn enzymatic deficits of the urea cycle, Reye's syndrome and hyperammonemia induced by chemotherapy or lung transplantation. The main difference between these situations and liver failure is the concentration of plasma ammonia, usually five times higher than in liver failure. Infusion of ammonia to experimental models reproduces cerebral swelling. Glutamine, the product of ammonia detoxification in astrocytes, has been postulated to be responsible for the development of brain edema through an osmotic mechanism (Brusilow 1986). In support to this hypothesis, inhibition of glutamine synthetase prevents cellular swelling in culture and in animals (Takahashi et al. 1992). In rats after portacaval anastomosis, 6 weeks after exposure to endogenous ammonia and a 2- to 3-fold increase in brain glutamine, the sum of brain organic osmolytes remains constant, principally at the expense of a reduction of *myo*-inositol (Cordoba et al. 1996). Similar principles may be applicable to explain the decrease in brain *myo*-inositol observed in cirrhotic patients (Rovira et al. 2002).

Clinical manifestations of hepatic encephalopathy

The neurologic manifestations are variable and depend on the severity of liver failure and the degree of portosystemic shunting. The most distinctive presentation is an acute episode characterized by the sudden onset of an acute confusional state that can evolve into coma. Disturbances of motor function are common, the most characteristic being the presence of asterixis; pyramidal signs also may be present. Interestingly, a substantial number of patients exhibit initially nausea and malaise, similarly as in acute hyponatremia. The term chronic hepatic encephalopathy is reserved for patients who have frequent episodes of encephalopathy or persistent cognitive (memory loss, confusion, disorientation) or motor disturbances (tremor, apraxia and rarely paraplegia). Minimal hepatic encephalopathy corresponds to those neurologic manifestations not obvious at clinical examination but detected with neuropsychological or neurophysiological tests

(Ortiz et al. 2005). The manifestations of hepatic encephalopathy are similar to those of hyponatremic encephalopathy. Specific signs that can be found are those of liver failure, such as hepatic faetor. Clinical experience suggests that asterixis is much more common in hepatic than in hyponatremic encephalopathy, but a systematic comparison between both has not been performed.

Clinical relevance of brain edema in cirrhosis

Magnetic resonance has confirmed the presence of brain edema in relation to the development of hepatic encephalopathy (Shah et al. 2008). The increase in brain water appears to be mostly located in the interstitial compartment (Kale et al. 2006), which challenges the concept of astrocyte swelling. These techniques are usually not available for clinical use. Conventional techniques indicate indirectly the presence of brain edema. T2-weighted images have shown an increase in the signal along the corticospinal tract (Cordoba et al. 2003) and changes in the volume of leukoaraiosis (Minguez et al. 2007). The better explanation for the increase in the volume of ventricles that has been observed following liver transplantation is the existence of mild chronic brain edema prior to liver transplantation (Garcia-Martinez et al. 2010).

^{1}H-magnetic resonance spectroscopy has identified a pattern of neurospectroscopic abnormalities, characterized by an increase in Glx/Cr and a decrease in Cho/Cr and mIns/Cr, without changes in NAA/Cr. This pattern has only been observed in liver failure (Kreis et al. 1992). A good explanation for the findings of ^{1}H-magnetic resonance spectroscopy is that they reflect an increase in the concentration of glutamine in astrocytes and a regulatory decrease of the concentration of brain organic osmolytes, such as choline-containing compounds and *myo*-inositol.

An unresolved issue is whether low-grade brain edema detected by magnetic resonance has any clinical relevance. Some patients with cirrhosis in deep hepatic coma may develop intracranial hypertension and brain herniation, patients with hyponatremia are at higher risk (Jalan et al. 1997). Studies in patients with overt hepatic encephalopathy are difficult to conduct. The few available have found a larger decrease of *myo*-inositol in overt hepatic encephalopathy (Cordoba et al. 2002), suggesting a more marked activation of regulatory volume decrease to compensate for further astrocytic swelling. Patients with minimal hepatic encephalopathy offer the advantage of studying a situation that is easier to control. The two paradigms that have been studied are the evolution after liver transplantation and the response to an acute ammonia load. Following liver transplantation, brain edema, assessed by MTR, has been shown to normalize in parallel with the correction of neuropsychological disturbances (Cordoba et al. 2001). In a study that administered an oral amino acid challenge resembling gastrointestinal bleeding to cirrhotics without encephalopathy, MTR was found to decrease and neuropsychological tests deteriorated. In accordance with the hypothesis of osmotic induced astrocyte swelling, neuropsychological deterioration was greater in patients with lower *myo*-inositol (Shawcross et al. 2004). These results supports the hypothesis that the neuropsychological effects of induced hyperammonemia is determined by the capacity of the brain to handle ammonia-induced increase in glutamine, which is limited if the osmotic reserve (the astrocytic pool of *myo*-inositol) is low.

One interesting finding on fast-Flair imaging is the presence of T2 hyperintensity along the corticospinal tract in cirrhotics (Cordoba et al. 2003). This image appears to correspond to astrocytic edema along this central motor pathway. Clinical signs of corticospinal impairment (exaggerated deep tendon reflexes, Babinski's sign) are common in overt HE. For this reason, we investigated the relationship between image and function of the corticospinal tract with transcranial magnetic stimulation. We found a delay of neuronal transmission that was corrected after liver transplantation. The degree of impairment and the evolution after liver transplantation correlated with the disappearance of T2 hyperintensity. Thus, signs of astrocytic swelling and neuronal dysfunction could be delimited to a specific neuronal pathway.

Treatment of hepatic encephalopathy

Correction of liver failure and portal-systemic shunting are the ideal strategies to avoid the exposure of the brain to toxins and reverse the neurological disturbance. However, these strategies are difficult to conduct. Treatments addressed to lower blood ammonia are easy to institute and result in clinical benefit (Blei and Cordoba 2001). General measures for patients with delirium are applicable to any form of metabolic encephalopathy (Inouye 2006). Therapy should be focused in providing normal blood flow, temperature, osmolality, and pH for optimal central nervous system function. Once the episode has been resolved preventive measures should be instituted in high-risk patients (Sharma et al. 2009a). In contrast to hyponatremic encephalopathy, rapid correction has not been associated with myelinolysis. However, recurrent episodes may lead to brain atrophy (Amodio et al. 2007).

Coexistence of hyponatremic and hepatic encephalopathy

Various metabolic disorders causing encephalopathy are combined, rather than occur in isolation, especially in critically ill patients. This reflects the interaction among various organ systems, causing multiple metabolic derange-

ments. Any metabolic derangement may act synergistically causing encephalopathy.

Hyponatremia is frequent among cirrhotic patients due to the difficulties in excreting solute-free water, an abnormality that reflects a reduction of effective circulating volume due to arterial splanchnic vasodilation. Sodium may be a good indicator of the intensity of this pathophysiological disturbance. Several studies have shown a good correlation between serum concentration and survival; patients with hyponatremia have a poor survival compared with that of patients without hyponatremia (Llach et al. 1988). Hyponatremia is the expression of dilution of solutes due to excessive water retention and usually is accompanied by ascites. In a study among patients with ascites it was found that approximately half of them had hyponatremia and than those with hyponatremia developed more frequently complications of cirrhosis (Angeli et al. 2006).

The frequency of hepatic encephalopathy is increased among patients with hyponatremia. In the previous study (Angeli et al. 2006), the incidence of hepatic encephalopathy was associated with the levels of sodium in serum (>135 mEq/L:15%, 130–135 mEq/L: 24%, and <130 mEq/L: 38 %). In another study in patients with cirrhosis, serum sodium concentration was an independent predictive factor of electroencephalographic abnormalities (Amodio et al. 2001). Two additional studies have shown that in patients with advanced cirrhosis a low serum sodium concentration at the time of the procedure has been associated with an increased risk of the development of overt hepatic encephalopathy during follow-up (Guevara et al. 2009). Furthermore, the presence of hyponatremia identifies those patients with minimal hepatic encephalopathy that are more resistant to treatment with lactulose (Sharma et al. 2009b).

Astrocyte swelling: the common link between hyponatremic and hepatic encephalopathy

Experimental studies have shown that chronic hyponatremia exacerbates brain edema induced by ammonia (Cordoba et al. 1998). This exacerbation of edema can be explained by the loss of organic osmolytes induced by chronic hyponatremia (Fig. 1). Astrocytes respond to changes in intra or extra cellular osmolality by increasing or decreasing the number of osmotic active solutes inside the cell (McManus et al. 1995). Astrocytes are the cells prominently involved in hyponatremic and hepatic encephalopathy. It is plausible that any change in the state of cell hydration induced by a decrease in extracellular sodium or an increase in intracellular glutamine activates compensatory mechanisms and increase the risk of astrocyte swelling in front of an additional osmotic stress.

The histological picture in adequately fixed preparations of experimental models of hepatic encephalopathy shows astrocyte swelling. Alzheimer type II astrocytosis is found in the brain of patients that die with hepatic encephalopathy. These cells, which exhibit an enlarged and clear nucleus with the chromatin displaced to the side, have been considered the expression of astrocyte swelling in brains fixed under immersion. The degree of Alzheimer type II astrocytosis has been related to the severity of encephalopathy (Norenberg 1996).

Astrocytes play a key role in the maintenance of central nervous system function by virtue of their interactions with other neuronal cells. Astrocytes are involved in the regulation of the extracellular microenvironment and significantly influence neuronal excitability and neurotransmission. A particularly critical function is the uptake of glutamate, which allows terminating the neurotransmitter action of glutamate. An increase in astrocyte hydration and low-grade cerebral edema is sufficient to trigger multiple alterations of astrocyte function. This results in a disturbance of glioneuronal communication and the clinical picture of encephalopathy. Hyponatremia aggravates brain edema, as it exacerbates the differences of osmolality between the intra-astrocytic and the extracellular compartment. The decrease in extracellular osmolality can be compensated by a decrease in the intracellular concentration of brain organic osmolytes, such as glutamine, *myo*-inositol or choline compounds. Cirrhotic patients with

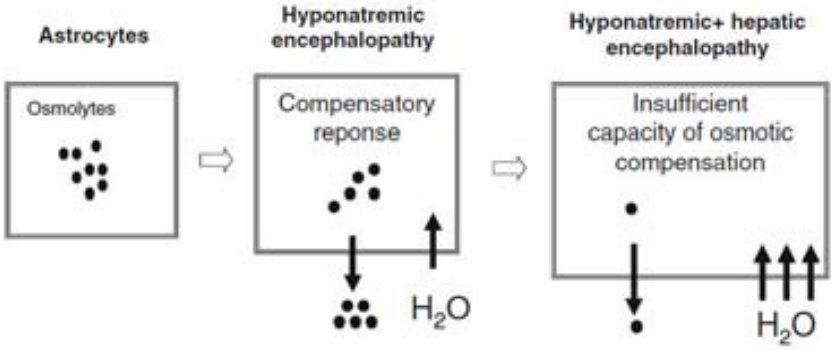

Fig. 1 Schematic representation of changes in astrocyte volume and osmotic response in hyponatremic encephalopathy and in combined hyponatremic and hepatic encephalopathy. The decrease in intracellular osmolytes secondary to hyponatremia diminishes the capacity to respond to an additional osmotic challenge

hyponatremia exhibit larger decreases of *myo*-inositol (Restuccia et al. 2004) and according to the results of amino acid challenge tests may have difficulties handling an acute ammonia load (Shawcross et al. 2004]. Since astrocyte swelling is induced by ammonia, hyponatremia, benzodiazepines or inflammatory cytokines, such a model would explain why rather heterogeneous conditions (e.g., bleeding, electrolyte disturbances, sedatives, infections) can precipitate hepatic encephalopahthy in the cirrhotic patient. Thus, multiple factors would act synergistically on the common pathogenetic mechanism: glial swelling with its functional consequences.

Management of hepatic encephalopathy in the presence of astrocyte swelling

Maintenance of fluid homeostasis is critical to normal brain function. Alterations in the extracellular concentration of electrolytes have direct consequences on neurotransmission. The identification of brain edema in cirrhosis leads to consider measures that reduce it (or at least do not aggravate it) while treating hepatic encephalopathy. Some of the factors that have classically been considered to precipitate hepatic encephalopathy, such as ammonia load, infections or benzodiazepines, may act inducing astrocyte swelling. Since there is no known method to treat astrocyte swelling, therapy is primarily aimed at correcting precipitating factors. A better knowledge of the mechanisms that induce astrocyte swelling may lead to new drugs. Among them, antioxidants are currently under investigation. In the meantime, it should be highlighted that water and electrolyte management should be performed with extreme caution.

In patients with acute liver failure and severe encephalopathy, the increase in serum sodium concentration achieved by the administration of hypertonic sodium chloride was associated with a decrease in the incidence and severity of episodes of intracranial hypertension in comparison with that in a control group of patients receiving standard therapy alone (Murphy et al. 2004).

Rapid shifts in plasma osmolality should be avoided. In patients with hepatic encephalopathy, ascites is better treated with paracentesis than with diuretics. Once hepatic encephalopathy has been corrected, low-dose diuretics may be prescribed, but frequent monitoring is necessary to detect changes in plasma electrolytes and to adjust the dose. In general, cirrhotics exhibit better neurological function when overhydrated than when dehydrated. Patients with encephalopathy appear to be extremely sensitive to changes in effective circulatory volume. Expansion of circulatory volume may explain the beneficial effects observed after infusions of albumin (Jalan and Kapoor 2004). Protracted diarrhea secondary to lactulose or to indiscriminate use of enemas may worsen neurological function through exacerbating astrocytic edema.

Aquaretic drugs, currently under clinical investigation, can correct hyponatremia In a study using satavaptan, the correction of hyponatremia was associated with improvement of neuropsychological tests (Cordoba et al. 2009). Since, the severity of liver failure was not affected by satavaptan, it could be concluded that chronic hyponatremia had a direct effect on cognitive function, which may be improved with the use of aquaretic. It may be worthwhile to investigate in the future whether the administration of vaptans could be an adequate strategy to decrease the risk of developing hepatic encephalopathy.

Acknowledgement CIBERehd is supported by Instituto de Salud Carlos III.

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5. Patients methods and results

5.5. Supplementary material III

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Prognostic Assessment in Patients with Hepatic Encephalopathy

Disease Markers: Diagnostic and prognostic markers in liver cirrhosis

Edited By: Augustin S et al

Prognostic Assessment in Patients with Hepatic Encephalopathy

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Abbreviations

HE	Hepatic Encephalopathy
MELD	Model for End-stage Liver Disease
ALF	Acute Liver Failure
HBV	Hepatitis B Virus
TIPS	Transjugular Intrahepatic Portosystemic Shunts
HCC	Hepatocellular Carcinoma
BCLC	Barcelona Clinic Liver Cancer
AOLF	Acute-on-chronic liver failure
MARS	Molecular Absorbent Recirculating System
EEG	Electroencephalogram
CFF	Critical Flicker Frequency
LT	Liver Transplantation

Financial support: CIBEREHD is supported by Instituto de Salud Carlos III, Madrid, Spain. The data that have generated part of the article have been obtained thanks to the support of FIS 07/0641. Rita García-Martínez has been supported by grant CM07/00109

ABSTRACT

Hepatic encephalopathy (HE) is a common complication of liver failure that is associated with poor prognosis. However, the prognosis is not uniform and depends on the underlying liver disease. Acute liver failure is an uncommon cause of HE that carries bad prognosis but is potentially reversible. There are several prognostic systems that have been specifically developed for selecting patients for liver transplantation. In patients with cirrhosis the prognosis of the episode of HE is usually dictated by the underlying precipitating factor. Acute-on-chronic liver failure is the most severe form of decompensation of cirrhosis, the prognosis depends on the number of associated organ failures. Patients with cirrhosis that have experienced an episode of HE should be considered candidates for liver transplant. The selection depends on the underlying liver function assessed by the Model for End-stage Liver Disease (MELD) index. There is a subgroup that exhibits low MELD and recurrent HE, usually due to the coexistence of large portosystemic shunts. The recurrence of HE is more common in patients that develop progressive deterioration of liver function and hyponatremia. The bouts of HE may cause sequels that have been shown to persist after liver transplant.

Key words Hepatic encephalopathy, prognosis, acute liver failure, acute-on-chronic liver failure, liver transplantation

INTRODUCTION

Hepatic encephalopathy (HE) is usually interpreted as a sign of liver failure and has ominous considerations. However, as for other complications of cirrhosis (e.g. jaundice, hepatocellular carcinoma, hepatorenal syndrome…), the prognosis of patients with HE is not uniform. Establishing the prognosis is difficult and requires a precise assessment of neurological and hepatic function(12). HE is characterized by a myriad of neurological manifestations, diverse underlying liver disorders and a variety of precipitating factors(15); all of them may affect prognosis. The approach to the patient with HE should be performed according to the underlying disorders, as is recognized in the classification (table 1). This article reviews the assessment of prognosis in patients with HE, based on the underlying liver disease and the severity of neurological manifestations. Unfortunately, the majority of studies have not classified the patients according to the type of liver disease and the availability of data in some areas is limited.

Table 1: Classification of Hepatic Encephalopathy *

Liver disease	Types	Subtypes	Features
A-Acute liver failure			Neurological manifestations include typically the presence of intracranial hypertension. The liver was previously healthy, the patient has the possibility to completely regenerate and recover the liver function
B-Portosystemic by-pass			Neurological manifestations secondary to large portosystemic shunts that may be congenital or surgical induced. There is no underlying liver disease.
C-Cirrhosis	Episodic¶	Precipitated	Secondary to gastrointestinal haemorrhage, constipation, excessive protein intake, infection, renal failure, dehydration, electrolyte disturbance.
		Spontaneous	Without recognized precipitating factors. Usually associated with large portosystemic shunts (spontaneous, surgical, TIPS)
	Persistent	Mild	Chronic cognitive or motor manifestations that impact negatively on social and occupational activities but do not cause dependency
		Severe	Chronic manifestations that cause dependency (dementia, paraplegia, parkinsonism…)
	Minimal		Cognitive disturbances detected by neuropsychological or neurophysiological tests that are not evident in the standard neurological examination

* The classification is based on the consensus of Vienna(15).
¶ The term **recurrent HE** refers to patients that have experienced more than 2 episodes of HE within the last six months

ACUTE LIVER FAILURE

The development of HE in a patient that was previously asymptomatic should prompt the diagnosis of acute liver failure (ALF). This is a rare condition in

which rapid deterioration of liver function results in altered mentation and coagulopathy(37). The presence of HE is a requirement for the diagnosis; in other words, ALF cannot be diagnosed in the absence of HE. The most prominent causes of ALF are drug induced liver injury, viral hepatitis, autoimmune liver disease and shock(34). However, approximately 20% of cases have no discernible cause(24).

ALF often affects young persons and carries a high morbidity and mortality. It is unclear why some patients with the same apparent degree of severity and the same aetiology of liver failure have different outcomes. Indeed after the occurrence of encephalopathy, ALF patients can die without liver transplantation while others will recover either in a few hours or in a few days. Prior to transplantation, most series suggested less than 15% survival; liver transplantation has made it possible to achieve survival greater than 65%(24). The prognosis of ALF depends on many factors. The most important are age, aetiology of ALF and clinical and biological status on admission and at the peak of deterioration.

ALF can occur through distinct pathways according to aetiologies; there is probably more than one mechanism responsible for ALF. High level spontaneous prognosis without liver transplantation (>50%) is seen in patients with paracetamol overdose, acute hepatitis A infection, liver shock, and pregnancy- related ALF. To the contrary, prognosis is poor in patients with ALF due to indeterminate causes, drug-intoxication other than paracetamol, hepatitis B virus (HBV) infection, autoimmune hepatitis, Wilson's disease, and Budd–Chiari syndrome.

There are several prognostic systems that have been specifically developed for selecting ALF patients for liver transplantation(45). Overall, such prognostic scores have proven to have acceptable specificity but have low sensitivity to determine outcome. Since the currently available prognostic scoring systems do not adequately predict outcome and determine candidacy for liver transplant the decision to proceed to liver transplant could not rely only upon them(37). Each centre should establish its own transplant policy according to local epidemiology and experience and use the prognostic criteria as a tool, but not as definitive criteria. A flexible use of them with continuous monitoring seems to be the wiser approach. There is no unanimously accepted prognostic system. The most

widely used are the King's College criteria(33) and the Clichy-Villejuif criteria(6) (table 2). The King's College criteria have shown positive predictive values ranging from just below 70% to nearly 100% and negative predictive values ranging from 25% to 94%(2,47). The Clichy-Villejuif predicted death in acute viral hepatitis cases with a positive predictive value of 82% and a negative predictive value of 98 but subsequent studies have shown these criteria to be less accurate than King's College criteria in predicting outcome(25).

The neurological status influences survival; severe HE (grade 3-4) upon admission and during hospitalization is a significant determinant of poor outcome(24). For this reason, some centres have decided to use the severity of HE as a main determinant to select patients for liver transplant(9). Advanced HE is a marker of the severity of liver function and of the presence of intracranial hypertension, a common complication of ALF that is responsible for an important number of deaths secondary to brain herniation. Recent studies have shown that the concentration of ammonia in plasma can be used to predict the development of intracranial hypertension, which may help to select patients who should undergo intracranial pressure monitoring. The risk of brain herniation is higher for plasma ammonia concentration above 200 microM/L(57).

Table 2: Prognostic criteria for patients with ALF.

Clichy–Villejuif	**Age**	**Criteria**
	Under 30 years:	HE stage 3 or 4 + factor V <20%
	Over 30 years:	HE stage 3 or 4 + factor V <30%
King's College	**Aetiology**	**Criteria**
	Paracetamol	- Lactate >3.5 Mmol/L, - Arterial pH <7.3, or lactate >3 mmol/L after adequate volume resuscitation or - HE stage 3 or 4 + creatinine >300 mcm/L + INR >6.5
	Non-paracetamol	- INR >6.7 or any three of the following: - drug toxicity or indeterminate cause - age <10 or >40 years - jaundice to coma interval >7 days - bilirubin >300 mcm/L - INR >3.5

V

ACUTE HE IN CIRRHOSIS

The importance of HE in determining the prognosis of patients with cirrhosis has been acknowledged in the most widely used system that assesses the severity of liver failure: the Child-Pugh scoring system. However, the experience with patients that have undergone portosystemic shunts or Transjugular Intrahepatic Portosystemic Shunts (TIPS) showed that the development of HE in these patients is independent of survival. Clinical experience also indicates that there is a subgroup of patients with cirrhosis that in spite of developing multiple episodes of HE have a long-life. In accordance to these experiences, the MELD score, which has replaced the Child-Pugh system in the selection of patients for liver transplant, does not include HE.

Patients with cirrhosis presenting with acute HE should undergo a diagnostic process that assesses the presence of precipitating factors and the severity of liver function (figure 1).

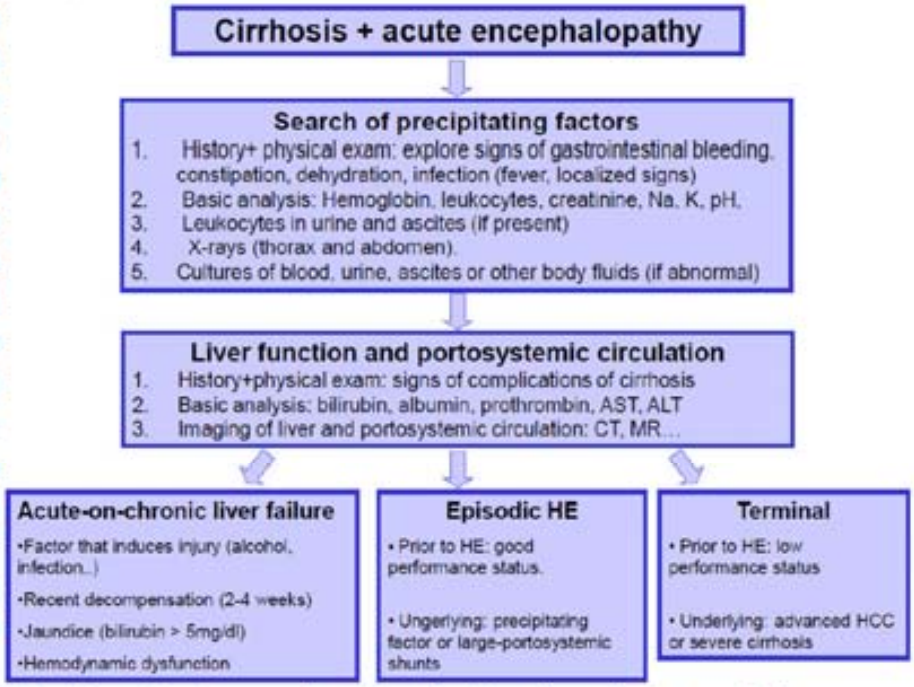


Figure 1: The assessment of a patient with cirrhosis and a acute change in mental state should be initiated by investigating the existence of precipitanting factors. The assessment should be completed with blood tests and imaging studies that evaluate liver function and portosystemic circulation. According to the results the patients are classified as: a) episodic HE b) acute-on-chronic liver failure or c) terminal liver disease, and managed accordingly.

It is possible to classify patients according to underlying liver disease and the previous performance status. For those with hepatocellular carcinoma (HCC), the Barcelona Clinic Liver Cancer (BCLC) system is the most appropriate(30). In patients with cirrhosis that have not developed HCC a classification in stages has been proposed according to the presence of ascites and varices(14). These

stages have been established by combining data from 2 large natural history studies, recently refined by the results of an analysis of the prognostic significance of infection in cirrhosis (figure 2)(3).

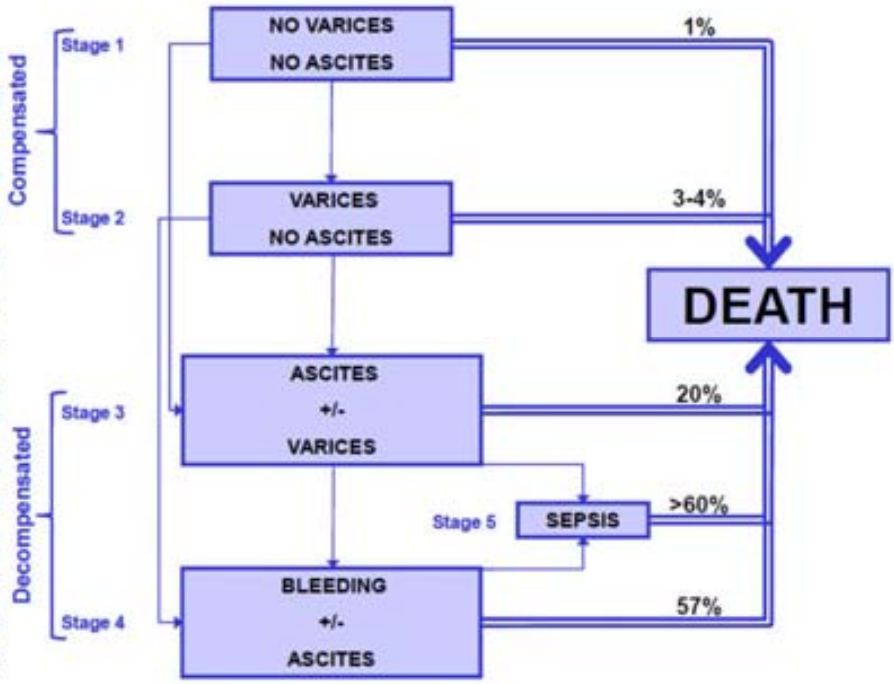


Figure 2: Clinical outcome in cirrhosis and classification in five stages according to the presence of cirrhosis, varices and sepsis: stage 1 without varices or ascites, stage 2 with varices and without ascites, stage 3 with ascites ± varices and stage 4 with bleeding ± ascites. Mortality at 1-year increases in each stage. The development of sepsis is more likely in stages 3 and 4 and the risk of dying is higher. This situation is purposed as stage 5 *(modified from Arvaniti V et al (3)).*

In this classification stage 1 is defined by the absence of ascites and oesophageal varices (mortality at 1 year: 1%), stage 2 by the presence of oesophageal varices without bleeding (increase in mortality: 3%), stage 3 by the presence of ascites with or without oesophageal varices (increase in mortality: 20%) and stage 4 by the occurrence of variceal bleeding with or without ascites (increase in mortality to 57%). Newly proposed stages 5 (development of infections) and 6 (hepatorenal syndrome) define stages of more advanced severity. Interestingly, HE is not present in any of these stages, indicating that while HE is frequent at the time of death, is not a reliable indicator of prognosis in cirrhosis. This may be explained by the role of portosystemic shunting in the pathogenesis of HE and by the presence of precipitating factors that may directly affect prognosis.

ACUTE-ON-CHRONIC LIVER FAILURE

A major pathogenic element in the development of HE is the presence of portosystemic shunting, which may be intrahepatic or extrahepatic(12). Severe

liver diseases cause intrahepatic shunts due to endothelization of sinusoids and insufficient liver mass. They are recognized by the development of coagulopathy (longer prothrombin time) and jaundice (high bilirubin). The most characteristic extreme example of this situation is acute-on-chronic liver failure (AOLF) and terminal irreversible cirrhosis secondary to advanced HCC. Patients that develop HE as the final event of liver failure are usually identified in this terminal situation before the occurrence of HE; they develop a progressive decline in their quality of life and show a poor performance status for weeks before the development of HE

AOLF has been coined to refer to a situation that is poorly defined: severe liver failure that develops in a relatively short period of time (typically less than 4 weeks), secondarily to a precipitating event (e.g. acute alcoholic hepatitis), in a patient with previously compensated cirrhosis(26). The severity of liver failure is recognized by high bilirubin, prolonged prothrombin time and the development of failure of other organs (kidney failure, hypotension, respiratory failure…).

Patients with AOLF are critically ill and are usually managed in a critical care environment. Due to the lack of diagnostic criteria, the prognosis of AOLF has not been specifically studied. In a population that probably corresponds to AOLF (cirrhotic patients with MELD >18 and signs of systemic inflammatory response) the in-hospital mortality is around 50%(10,53).

The most important factor that determines prognosis in AOLF is the development of multiorgan failure. Scoring systems that have been developed for critically ill patients (SOFA, APACHE II and III) have shown a better reliability than the Child-Pugh or the MELD to identify patients with bad prognosis. These systems provide operational criteria to define extra-hepatic organ failure. It has been shown that patients with 2 organ failures or undergoing the artificial support of 2 organic systems have a high mortality (approximately 75%). This mortality approaches 100% for 3 or more failing organs(55).

One important determinant of prognosis for patients with AOLF and severe HE (grade 3-4) is the lack of improvement of HE during the first week of treatment. This was clearly shown in a clinical trial that investigated the effects of MARS (Molecular Absorbent Recirculating System) therapy for severe HE(22). The study included 70 patients (MELD 30 ± 10) that were randomized to receive MARS (up to 5 days) or standard medical therapy alone. Treatment

with MARS resulted in a more rapid improvement of HE and for those with MELD>30 in a better 2-week survival. The authors found that in this population, which had a predicted mortality above 75%, the two factors that were predictors of a 4-week survival were performance of liver transplantation and improvement of HE by 2 grades in the 5 days study period.

In conclusion, patients with HE and AOLF have a bad prognosis and if possible liver transplant should be a priority. For those awaiting transplant, MARS could become part of a bridging strategy. Patients with AOLF that develop more than two organ failure or exhibit lack of improvement of severe HE after 5 days of treatment have a very poor prognosis. In this situation, supportive therapies may be considered futile and the therapeutic efforts could be limited.

EPISODIC HE

Episodic HE in cirrhotic patients is associated with short life expectancy. One study that investigated survival after the first episode of HE found a cumulative survival a one year of 42% and at 3 years of 23%(8). These results were obtained in the 1990s in a liver unit with a large experience in management of cirrhosis. Since the data were obtained in a referral centre with a transplant program, they may be biased towards more severe patients. However, the data are in agreement with the results previously reported by other authors(11,46). The authors proposed that the prognosis in cirrhotic patients developing HE has not substantially changed during the last decades and that all patients that have developed HE should be considered liver transplant candidates.

The outcome of HE in patients with cirrhosis that do not fulfil the criteria for AOLF is usually determined by the precipitating factor. This concept proceeds from the experience in clinical trials(7,51). The precipitating factor is a clinical event that does not cause a direct injury to the liver or to the portal-systemic circulation but is responsible for the acute change in the mental state. Precipitating factors appear to act by increasing the generation of putative toxins or enhancing the effects of the toxins on the central nervous system. They are temporally related to the development of HE and their correction to the re-establishment of consciousness. Several factors are traditionally considered under this category (gastrointestinal bleeding, constipation, excessive protein

intake, dehydration, electrolyte disturbances, renal failure and infection), and are thought to explain the majority of episodes of HE. However, a significant number of episodes are not related to a precipitating factor(5).

Patients that have survived to an episode of HE should be evaluated for liver transplant. The system to decide if a patient is an appropriate candidate is not different from the currently used in most centres. The prognosis after HE is clearly related to the severity of liver failure. In the study by Bustamante(8) the authors related survival to bilirubin, albumin, prothrombin time, urea and potassium. This supports the use of MELD in deciding which patients that have developed HE should undergone liver transplant. Nevertheless, one retrospective study shows that HE provides additional prognostic information than the one given by MELD(50). Future studies should confirm this finding, before modifying the current transplant policy.

LARGE PORTOSYSTEMIC SHUNTS

The development of HE may be determined by large extrahepatic portosystemic shunts. The experience with surgical porto-caval anastomosis and with TIPS indicates that these are high-risk patients for HE. According to studies in patients with TIPS, the prognosis in this situation, as in patients without TIPS, is determined by parameters of liver function that are included in the MELD(31). The development of severe HE (grade 3-4) is associated with a higher degree of mortality, probably because identifies hospitalized patients with a severe decompensation(50). Interestingly, the prognosis is better in patients with TIPS than in those without TIPS, supporting the notion than in the presence of large portosystemic shunts the severity of HE is less important than in the presence of other precipitating factors.

There is a group of patients with cirrhosis and large spontaneous shunts (non-procedural shunts). The prognosis of these patients appears to be similar to the one of those that have undergone procedural shunts(13). Patients with large spontaneous shunts are usually characterized by good parameters of liver function and do not fulfil the criteria for liver transplant. Large shunts may be suspected by a history of frequents episodes of HE, lack of variceal haemorrhage and relatively preserved liver function (lack of coagulopathy and jaundice)(40). These patients should undergo imaging of the portosystemic

circulation. The survival of patients with large spontaneous portosystemic shunts has not been specifically investigated. The experience in patients with surgical shunts suggests that this condition has a much better outcome than the development of HE in the absence of large shunts.

RECURRENCE OF HE

HE is characterized by its elevated tendency to relapse; it has been estimated that approximately half of the patients that survive to an episode of HE will recur during the following year(48). One of the factors that have been proposed to identify the subjects that will suffer a new episode of HE is the presence of minimal HE. However, part of the prognostic significance can be attributed to the presence of more severe liver failure among patients with minimal HE(21). The combination Child-Pugh B/C and minimal HE, detected by electroencephalogram (EEG)(1) or by critical flicker frequency (CFF)(42) identifies those patients at a higher risk of recurrence.

In patients with minimal HE the presence of a high ammonia value after an oral glutamine challenge is associated with the development of HE(43). This finding was explained by a higher activity of intestinal glutaminase, an enzyme that is present in the intestinal mucosa and deaminates glutamine. The activity of glutaminase may determine the hyperammonemia that follows the digestion of proteins. One interesting observation has been the identification of specific polymorphisms in the glutaminase gen that may increase the activity of intestinal glutaminase and through this mechanism predisposes to the occurrence of episodic HE in affected individuals(44). If confirmed, genetic polymorphism should be included in the group of predisposing conditions. Apart from identifying high-risk patients, the assessment of the polymorphism may initiate the era of personalized medicine in the field of HE.

Several studies in cohorts of patients with advanced HE have demonstrated that those variables that are more closely related to the occurrence of HE are an increase in serum creatinine and a decrease in plasma sodium(19,28,39). The most plausible explanation is a decrease in the capacity of the kidney to remove ammonia and an increase in the susceptibility for the developing brain

edema, a key component of HE. This finding has lead to propose therapies for circulatory dysfunction to prevent the recurrence of HE.

Patients with procedural portosystemic shunts typically stop suffering gastrointestinal bleeding, but develop HE. Approximately, one third of patients submitted to a TIPS will experience HE(38). Non-selective portal-systemic shunts (porto-caval, mesocaval) produce more encephalopathy than do selective shunts (distal splenorenal). However, selectivity of splenorenal shunts is lost in the long-term. Elderly patients and those that have poor liver function are at higher risk for post-shunt encephalopathy. There is no hepatic functional test that identifies with confidence those individuals that will develop HE. Reduction of the diameter of TIPS is associated with improvement of HE. Interestingly, patients with loss of portal perfusion before TIPS are protected against post-TIPS HE(23).

SEQUELS OF HE

The metabolic nature of HE described in the 1950s(36) together with the observation of recovery between episodes led to the traditionally belief that HE is fully reversible. Recent studies, most of them performed in transplanted patients but also in cohorts of cirrhotic subjects, showed increasing evidence that challenges this classical view.

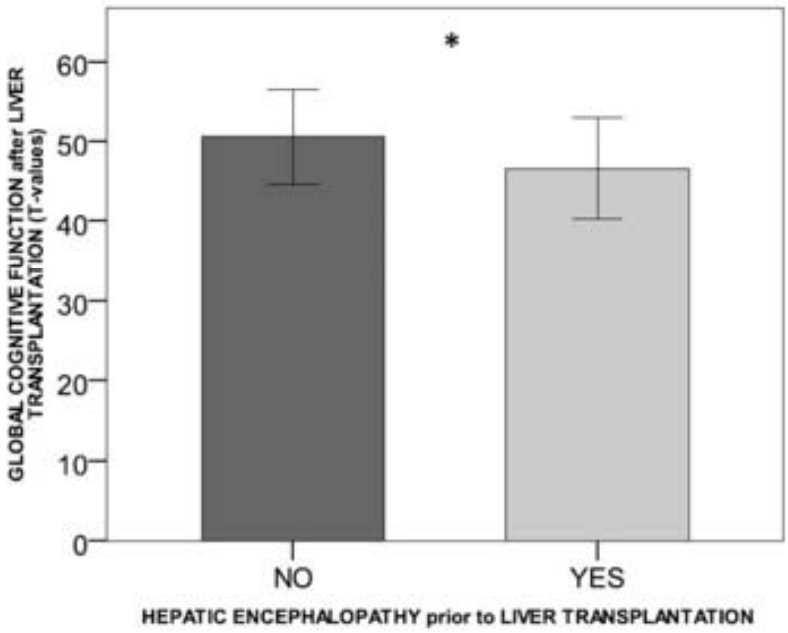
In a recent study that included a large cohort of cirrhotic patients(4), the authors found that those subjects that developed episodes of HE did not improve psychometric parameters with repeated testing (lack of "learning effect"). The same feature was observed with different psychometric tests in different populations (28,41) confirming persistence of deficits after HE.

Since the implementation of liver transplantation (LT) it has been demonstrated its ability to improves HE, even in patients with severe manifestations(27,56). However, studies that have assessed neuropsychological function following LT found a heterogeneous outcome with persistent cognitive deficits (18,32,49,52). Many other factors can impact in the postransplant cognitive function such as pretransplant (alcohol aetiology, prior cerebrovascular disease), peritransplant (ischemia) and postransplant events (immunosuppression, infections, stroke…). One study that performed a

prospective assessment up to nine years after LT observed that cognitive function at long-term was associated with vascular risk factors and signs of small-vessel cerebral disease in MR images(17). However, these recent studies support the notion that HE is associated with permanent sequels(18).

The origin and nature of these persistence deficits are not well known. Different neuroimaging techniques have shown some degree of brain atrophy in patients with chronic HE(29,58) as well as neuropathological studies(20). The prevalence and the degree of atrophy were higher among alcoholic patients. This feature could be explained by the fact that alcohol cause a dose-related brain shrink which is partially reversible with abstinence(35). A recent prospective study performed in a group of patients before and after LT showed an association between prior HE with posttransplant cognitive deficits (figure 3) and smaller brain volume. In addition, the smaller brain volume after LT correlated with lower levels of N-acetyl-aspartate/cr considered a neuronal marker (18). A plausible explanation for these finding is that the chronic exposition to toxins involved in the pathogenesis of HE could cause loss of brain tissue. This hypothesis is supported by the neuropathological demonstration of neuronal loss in the most severe cases of HE(16,54).

Figure 3: The development of hepatic encephalopathy in patients before liver transplantation is associated with worse cognitive function after liver transplant (*p<0.05). (Modified from Garcia-Martinez et al (18))



The concept that the episodes of HE may lead to irreversible decline in cognitive function has important consequences. It has been recently shown that

lactulose and rifaximin decrease the risk of the recurrence of HE (5,48). Thus, secondary prophylaxis with these drugs is recommended to decrease the number of further episodes of HE. Additional benefits may include preventing neuropsychological decline and may extent to the post-liver transplant period. In addition to this, is important to prevent "premature aging" of the brain by identifying and treating vascular risk factors, such as diabetes mellitus and arterial hypertension, and prescribing the minimal possible dose of immunosupressors.

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5. Patients methods and results

5.6. Supplementary material IV

Rita García Martínez, Juan Córdoba

Acute-on-Chronic liver failure: the brain.

Current Opinion In critical Care 2011 (in press)

Acute-on-Chronic Liver Failure: The Brain

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Financial disclosure:

CIBEREHD is supported by *Instituto de Salud Carlos III*.

Rita Garcia-Martinez is the recipient of grant CM07/00109 (Instituto de Salud Carlos III) and a grant from the Spanish Association for the Study of the Liver (AEEH).

Abbreviations:

ACLF	Acute-on-chronic Liver Failure
HE	Hepatic Encephalopathy
GABA	γ-Aminobutyric Acid
ICU	Intensive Treatment Unit
TIPS	Transjugular Intrahepatic Portosystemic shunt
SOFA	Sequential Organ Failure Assessment
ePTFE	Expanded Polytetrafluoroethylene Stent-graft
SIRS	Systemic Inflammatory Response Syndrome
APACHE	Acute Physiology and Chronic Health Enquiry
MELD	Model for End-Stage Liver Disease
HESA	Hepatic Encephalopathy Scoring Algorithm
LOLA	L-ornithine L-aspartate
OP	Ornithine-phenylacetate

Abstract:

Purpose of review: Brain disturbances, which are considered a form of hepatic encephalopathy are common in acute-on-chronic liver failure.

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Recent findings: Patients with hepatic encephalopathy exhibit sings of energy impairment that may participate in the development of disturbances in neurotransmission. Ammonia participates in the genesis of brain edema and in the development of oxidative stress injury to astrocytes. Neuroinflammation is a new element that has been described in experimental models. These mechanisms are involved in the genesis of cognitive sequels that may persist after liver transplantation. Clinical trials have demonstrated the value of drugs that decrease the production of ammonia in the intestines to prevent encephalopathy. In addition, improvement of circulatory dysfunction with the use of albumin and vasoconstrictors may prevent hepatic encephalopathy in acute-on-chronic liver failure. New drugs that act by enhancing ammonia disposal through the synthesis of nitrogenous metabolites have shown promising results.

Summary: A better knowledge of the pathogenesis of brain disturbances in acute-on-chronic liver failure provides the rationale for using ammonia focused therapy in the prevention and treatment of encephalopathy. New therapies addressed to correct brain edema, circulatory dysfunction and inflammation may also be useful for encephalopathy and may improve the neurological outcome.

Keywords: *hepatic encephalopathy, acute-on-chronic liver failure, brain edema, ammonia, neuroinflammation.*

INTRODUCTION

Disturbances of brain function are common in patients with acute-on-chronic liver failure (ACLF). The most frequent manifestation is an acute confusional syndrome that can evolve to coma. This neurological syndrome is usually considered a form of hepatic encephalopathy (HE), due to its clinical resemblance to the neurological manifestations present in patients with cirrhosis. In cirrhosis, the development of HE is related to precipitating factors that increase the exposure of the brain to toxins. In patients with ACLF, additional aspects of major pathophysiological importance are: systemic inflammatory response, circulatory dysfunction and failure of other organs that can cause directly disturbances to brain function. The current article reviews recent findings in the pathogenesis of neurological disturbances, recent data on the outcome of HE and better knowledge on the effects of different therapies.

DISTURBANCES IN THE BRAIN

Several alterations have been described in brain in the setting of HE.

Disturbances in neurotransmission

HE is considered to result from abnormalities of neurotransmission. This hypothesis is supported by potential reversibility of HE and the lack of neuronal damage in histological preparations. Multiples disturbances in neurotransmission have been described, including the excitatory glutamatergic and inhibitory GABAergic systems. One study showed, for the first time, that the increase in the GABAergic tone induced by hyperammonemia relates to a specific disturbance in behavior(1). The administration of a GABA blocker directly to the cerebellum restored the learning impairment present in rats with chronic hyperammonemia. However, the relevance of neurotransmitter abnormalities was challenged by data from autopsied material. Densities and affinities of multiple receptors were not homogeneous among different patients that died with HE(2). There were regional abnormalities compared to controls, but it was not possible to identify a specific pattern.

Energy impairment

The brain is the tissue with the highest energy requirements of the body and depends entirely on the process of glycolysis and respiration within its own cells to synthesize its energy demands. Patients with HE show a decrease in consumption of oxygen and glucose that is accompanied by a parallel decrease in cerebral blood flow(3). In patients with HE, the oxygen delivery is approximately twice the oxygen consumption, indicating that oxygen delivery is not a limiting factor for oxygen consumption. Consequently, cerebral blood flow seems to be reduced as a result of diminished cerebral oxygen requirement during HE, and not vice versa. The primary event in the pathogenesis of HE could be inhibition of cerebral energy metabolism by

ammonia, but it is not possible to establish whether the decrease in oxygen consumption is the cause or the consequence of encephalopathy. As in other metabolic encephalopathies, a decrease in neuronal function will decrease energy requirements. Alternatively, ammonia may inhibit oxidative metabolism in mitochondria and cause secondarily neuronal dysfunction(4).

Disturbances in energy metabolism may have an important role in the development of brain edema in ACLF. Brain microdialysis has been applied to investigate energy metabolites in patients with acute liver failure that had an intracranial catheter for monitoring intracranial pressure(5). A high lactate/pyruvate ratio, an indicator of impaired brain energy metabolism, has been demonstrated in the absence of hypoxia and has been correlated to ATP degradation products. Possible explanations for these findings include inhibition of rate limiting steps in the Krebs' cycle and compromised oxidative phosphorylation in the mitochondrial inner membrane. Alternatively, newly synthesized lactate may be induced by activated glycolisis generated by excessive glutamatergic activation (Figure 1). Irrespective of the mechanism being involved, lactate can decrease brain pH and cause injury to the astrocyte, leading to the accumulation of water in the intracellular compartment(6).

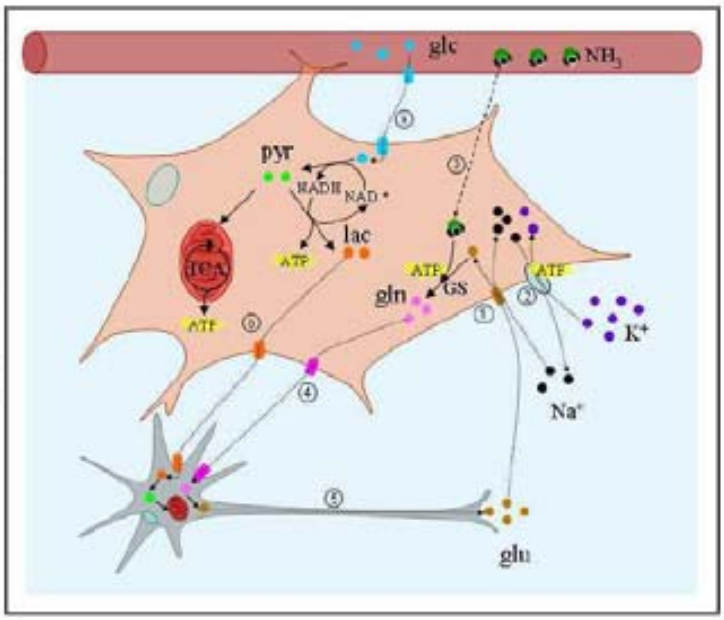

Figure 1: Interaction between ammonia, the glutamate-glutamine cycle and energy metabolism in astrocytes and neurons. Glutamate (Glu) is reuptaken by from the synaptic cleft by the astrocyte (1) in cotransport with Na^+(2). The excess Na^+ is interchanged with K^+ through a Na/K pump (3). Ammonium (NH_3) enters the astrocyte from blood through passive diffusion and combines to Glu to synthesize glutamine (gln), a reaction (4) that is catalyzed by glutamine synthetase (GS). Gln is transported to the neuron where is transformed again to Glu, a major neurotransmitter, and closes the glutamine-glutamate cycle. Glucose (glc) enters the astrocyte from blood (a) and is transformed into pyruvate (pyr) to produce ATP in mitochondria. A significant amount of pyr is transformed to lactate (lac) that is shuttled to the neuron (b). Lactate reaches the neuron where is transformed into pyruvate to provide ATP through the tricarboxylic acid cycle (TCA).

Brain edema

Cerebral herniation secondary to brain swelling is a typical feature of acute liver failure. Intracranial hypertension can be demonstrated with pressure-catheters in patients with advanced encephalopathy before the development of specific signs (pupillary abnormalities, decerebrated postures, bradykardia…). Sophisticated

magnetic resonance techniques have demonstrated that cerebral edema develops in ACLF, but usually to a lesser extent than in acute liver failure. The accumulation of water is located in the astrocytes and is related to the effects of ammonia that is metabolized into glutamine. It has been proposed that the transport of glutamine into the mitochondria yields to high levels of ammonia inside the mitochondria and induces oxidative stress. The administration of L-histidine inhibits competitively the transport of glutamine and prevents brain edema in experimental models(7).

The development of brain edema relates to the intensity and duration of the rise in plasma ammonia. However, other factors, such as multiple organ failure and hyponatremia may enhance the risk of cerebral herniation. According to a study in 87 patients with acute liver failure, clinically relevant brain edema is seldom seen with ammonia plasma concentration below 150 micromol/l, except for patients with high SOFA scores(8). Hyponatremia, a common finding in ACLF, may exacerbate astrocyte swelling due to differences in osmolality between the intracellular and the extracellular compartment(9). The enhancement of brain edema may be the explanation why hyponatremia is the most important risk factor for the development of HE among patients with advanced cirrhosis(10●). In cirrhosis, hyponatremia is associated with poor short-term prognosis. One study that included 126 consecutive cirrhotic patients admitted to the intensive care unit (ICU) found a higher frequency of complications and lower in-hospital survival among those with sodium below 135 mEq/l(11). The treatment of hyponatremia with vaptans(12), which are a new family of drugs that block the receptor of antidiuretic hormone may improve brain function. An additional factor that may have a role in the development of brain edema in ACLF is an increase in blood-brain-barrier permeability(13). A possible explanation is disruption of tight-junction proteins in brain endothelial cells caused by the effect of inflammatory mediators activated in ACLF(14).

SYSTEMIC MECHANISMS THAT AFFECT BRAIN FUNCTION

Liver failure causes increase plasma levels of several toxins (efficiently metabolized by liver under normal conditions) that can reach the central nervous system where cause brain disturbances. In addition, other factors commonly presents in liver failure may deteriorate neurological function.

Ammonia

Ammonia has been historically considered the most important factor in the genesis of hepatic encephalopathy. In normal conditions, ammonia is produced by the gut and an important amount is of bacterial origin (Figure 2). A large fraction of ammonia produced in the intestine is produced from the deamination of glutamine by glutaminase. This enzyme has become the focus of major interest, because the concentration of ammonia in peripheral blood in patients with cirrhosis relates to the

activity of intestinal glutaminase. In addition, Romero-Gomez et al. found that specific polymorphisms in the glutaminase gene identify patients at a higher risk for the development of HE(15••). They performed mutation scanning of the glutaminase gene in a group of 109 patients with cirrhosis and identified a section in the promoter region where base pairs were repeated (a microsatellite). The association between 2 long alleles of the microsatellite and HE was confirmed in a validation cohort of 177 patients (adjusted hazard ratio: 3.1). Functional studies showed higher luciferase activity in cells transfected with the long form of the microsatellite, supporting the concept that the long microsatellite enhances glutaminase transcriptional activity. These results may be useful for designing biomarkers to identify patients at higher risk for HE and suggest that the administration of inhibitors of intestinal glutaminase may become a new therapy for HE.

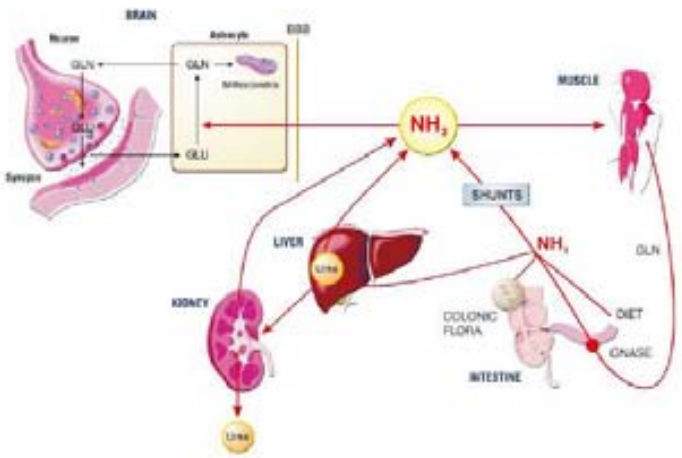

Figure 2: Inter-organ ammonia trafficking and metabolism. Ammonia is generated in gut from nitrogenous compounds from the diet, deamination of glutamine by glutaminase and metabolism of nitrogenous substances by colonic flora. Normally, ammonia is metabolized in the liver. Portal-systemic shunts and liver failure cause a rise in blood ammonia that may affect brain function by inducing several disturbances in astrocytes that may impair mitochondria and the glutamate-glutamine trafficking between neurons and astrocytes. Muscle is capable to decrease blood ammonia by metabolizing ammonia to glutamine. Kidney has also an important role by excreting urea in the urine and generating ammonia.
NH_3: ammonia, Glu: glutamate, Gln: glutamine, GNASE: glutaminase, BBB: blood-brain-barrier.

Patients with HE have an increased diffusion of ammonia into the brain. It has been hypothesized that there may be a specific increase in the permeability of the blood-brain-barrier. One study examined cerebral perfusion and ammonia metabolism with positron emission tomography in patients with different grades of liver fibrosis and did not find changes in the permeability to ammonia(16). Thus, the higher diffusivity is simply explained by a higher concentration of arterial ammonia. Ammonia levels are high in patients with HE, specially among those with large portosystemic shunts. The identification of these shunts may be specially relevant among some patients, as shown by the demonstration of episodic HE after liver transplantation, in spite of

normal liver function, in patients with persistent portosystemic shunts(17). Occlusion of the shunts may be a valid approach to treat resistant HE. In patients with transjugular intrahepatic portosystemic shunt (TIPS) the diameter may be reduced with hourglass-shaped expanded polytetrafluoroethylene (ePTFE) stent-graft(18).

Inflammation

Patients with ACLF develop frequently a marked activation of inflammatory mediators. The presence of a systemic inflammatory response syndrome (SIRS) has been linked to the development of HE. In a study that included 100 patients admitted to the ICU, of whom half of them developed progressive multiorgan failure and died, the SIRS score and Sequential Organ Failure Assessment (SOFA) score were significantly higher in those with grade 4 HE, who were also less likely to survive(19). The activation of inflammatory mediators, such as cytokines, may modulate the effect of neurotoxins on the brain or signal the brain through the activation of vagal afferents.

A completely new concept is the induction of inflammation in the brain. Activation of microglia and the synthesis of proinflammatory cytokines in the brain has been demonstrated in experimental models of HE(20). Activation of inflammation may increase blood-brain barrier permeability, result in the generation of intracerebral mediators, such as nitric oxide and prostanoids and cause astrocytic swelling. These observations may have therapeutic implications. Hypothermia, which is a therapy that is used in resistant intracranial hypertension, may act by decreasing neuroinflammation(21•). There are a series of anti-inflammatory drugs that may be effective for decreasing neuroinflammation and may improve the outcome of HE in ACLF(20;22) .

OUTCOME

HE is usually interpreted as a sign of liver failure with ominous consequences. However, as other liver failure complications, the outcome is not uniform and is intimately related to the underlying liver disease.

Prognosis

The most important factor that determines prognosis in patients with ACLF and HE is the development of multiorgan failure. Scoring systems that have been developed for critically ill patients (Acute Physiology and Chronic Health Enquiry –APACHE- II and III, SOFA) have shown a better reliability than the Child-Pugh or the Model for End-Stage Liver Disease (MELD) to identify patients with bad prognosis. As shown in a retrospective analysis of seventy-one patients with HE admitted to the ICU, the presence of arterial hypotension, use of vasopressors and acute renal failure identified those patients that exhibit higher mortality. Patients with isolated HE (without other signs of organ failure), even requiring intubation, have lower mortality rates(23). Lack

of improvement of HE during the first week of treatment is usually an ominous sign. For this reason, it is important to perform a continuous monitoring of the mental status, which can be done with the West-Haven criteria or the Glasgow Coma Scale. The Hepatic Encephalopathy Scoring Algorithm (HESA) is a system that provides an objective assessment of the cognitive parameters present in the West-Haven criteria(24). The HESA allows a more reliable assessment of the severity of HE, which is especially useful for multicenter clinical trials.

Sequels

The exposure to neurotoxins involved in the pathogenesis of HE may cause loss of brain tissue, which may explain the persistence of neuropsychological deficits after the episodes of HE(25•;26). Neurocognitive abnormalities can extent to the post-liver transplant period, as was documented in a group of liver transplant recipients(27••). Those that had suffered from overt HE prior to liver transplant exhibited lower cognitive performance. This observation was corroborated in one study that investigated prospectively cognitive function and brain size after liver transplant. In addition to worst cognitive function those that developed episodes of HE before liver transplant showed smaller brain volume(28).

TREATMENT

Treatment of HE requires a supportive therapy which is common to those patients with altered cognition level, decrease in plasma levels of toxins and treatment of precipitanting factors and other common elements in liver cirrhosis which can impair brain function.

Decreasing the production of gut toxins

The goal of the treatment of HE is to increase fecal nitrogen excretion, reduce the generation of ammonia by fecal flora and decrease the amount of ammonia that reaches portal blood(29). Another possible beneficial effect is reducing translocation of intestinal bacteria that activate inflammatory mediators, worsen hemodynamic parameters and favor the development of HE(30). Two large clinical trials in patients with stable cirrhosis(31;32••) have shown the value of modifying the gut flora in the prevention of HE (table 1).

In spite of the absence of similar clinical trials, the same principles appear to be valid for ACLF. It seems recommendable to prescribe preventive therapy for HE in patients admitted for ACLF. Non-absorbable disaccharides (lactulose and lactitol) are prebiotics that increase the amount of microorganisms thought to be healthy in the enteric flora, such as lactobacillus bifidus. Probiotics, achieve the same effect by directly administering a mixture of live microorganisms. Both have shown similar efficacy in decreasing blood ammonia and improving minimal HE(33). Rifaximin is a

minimally absorbed oral antimicrobial agent that reduces ammonia-producing enteric bacteria and appears to be safe for prolonged therapy. Combination of lactulose and rifaximin may exert some synergism. However, it is not known, if rifaximin is necessary if the patient receives broad-spectrum antibiotics, as is commonly prescribed in patients with ACLF due to concomitant infections.

Table 1: Clinical trials for the prevention of HE in cirrhosis

Study	Patients	Treatment	Outcome
Bass NM et al.(32••)	Cirrhosis + 2 episodes of HE in the previous 6 months (n=299)	a) Rifaximin 550 mg bid b) Placebo bid 90% patients were treated with lactulose that was allowed in both arms	Rifaximin reduced the risk of an episode of HE over a 6-month period (from 46% to 22%, HR: 0.42)
Sharma BC et al.(31)	Cirrhosis who recovered from an episode of HE (n=140)	a) Lactulose b) Placebo	Lactulose reduced the recurrence of HE over a median follow up of 14 months (from 47% to 20%)

Circulatory dysfunction

Patients with ACLF exhibit circulatory dysfunction characterized by low arterial pressure, low peripheral vascular resistance and high cardiac index(34). Exacerbation of circulatory dysfunction is an important precipitating factor of HE(10). A low concentration of plasma albumin has been associated with the development of circulatory dysfunction. The benefits of the administration of albumin in the prevention of circulatory dysfunction after large volume paracentesis or spontaneous bacterial peritonitis have been attributed to the oncotic effects of albumin. However, albumin has important anti-oxidant effects that may relevant in ACLF. One study that included 34 patients with ACLF measured the functional capacity of albumin using electron paramagnetic resonance spectroscopy(35•). The study found marked impairment of the albumin function, suggesting changes in the structure of the molecule. These abnormalities correlated with disease severity and prognosis. These findings provide the basis for investigating the use of albumin, alone or combined with vasoconstrictors, in the treatment and prevention of HE in patients with ACLF.

New therapies

The ability of the muscle to "fix" appreciable amounts of blood-borne ammonia becomes important to regulate arterial ammonia in case of liver failure and highlights the importance of maintaining an adequate muscle mass. L-ornithine-L-aspartate (LOLA) and ornithine-phenylacetate (OP) provide substrates for glutamine synthesis and appear to stimulate the synthesis of glutamine in muscle. LOLA, which is available in several countries, has been investigated in patients with acute liver failure in a

placebo-controlled trial that included two hundred one patients(36). The administration of LOLA did not improve survival and had no effects on plasma ammonia and HE. One explanation for the negative results could be the severity of liver failure, which may limit the efficacy for therapies based on decreasing plasma ammonia through the synthesis of glutamine. An alternative explanation, is that the newly synthesized glutamine is recycled in the intestine, where is transformed into glutamate and releases ammonia. OP provides phenylacetate (part of the OP molecule) which reacts with glutamine to synthesize phenyl-acetyl-glutamine that is eliminated in the urine and avoids the recycling of glutamine. OP, which is at initial phases of clinical development, has shown encouraging results in experimental models(37••).

CONCLUSION

ACLF causes an increase in the exposure of the brain to several substances that under normal circumstances are efficiently metabolized by the liver; those that have a high "first-pass" metabolism are the most important, as shown by the important role of portal-systemic shunting in the development of HE. Brain disturbances in ACLF include abnormalities in the neurotransmission, energy impairment, brain edema and neuroinflammation. HE is a manifestation of severe liver failure; its treatment cannot be separated from the treatment of liver failure, which requires a series of supportive measures, including the general management of a patient with change in mental status. Measures specifically designed to treat HE appear to be beneficial, although many of them have not undergo proper assessment in clinical trials. Current therapy is focused on decreasing blood ammonia. Correction of circulatory dysfunction with albumin and vasoconstrictors and improvement of hyponatremia with vaptans may improve HE and may become standard therapies in the future.

KEY POINTS

- Disturbances of brain function are very common in ACLF.
- Impairment of brain energy and development of brain edema appear to be central in the pathogenesis of encephalopathy.
- Recent data suggest that neuroinflammation may have a significant role in brain disturbance
- Ammonia-focused therapy constitutes the basis of current therapy, supported by recent large clinical trials.
- Emerging therapies include therapy for circulatory dysfunction, correction of hyponatremia with vaptans and new drugs for hyperammonemia.

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6. Discussion

Hepatic encephalopathy is a common complication of liver cirrhosis and its pathogenesis is incompletely clarified. Although many advances in recent years have led to a better understanding of the disease, an in-depth knowledge of its cause is still awaited perhaps partly due to lack of accurate methodology. The brain is not readily available for pathological studies and the reproduction of the entire syndrome in animal models is still unsatisfactory[28]. Multiple obstacles hinder further investigations in defining HE as a fully reversible disease.

The study of possible sequels of hepatic encephalopathy and its structural basis were addressed in the present research using the available non invasive technology. Essentially, this research confirmed the improvement of cognitive and neuroradiological abnormalities related to liver failure after LT which has been previously assessed.

Importantly, we observed a heterogeneous outcome in the cognitive function and confirmed the association of HE with a worse neurological function. In addition, we found a relationship between HE and smaller brain volume which could represent a structural basis for this permanent damage. Furthermore, an indirect measure of presence of neurons such as N-Acetyl-Aspartate/creatine suggests neuronal cell loss.

It is worth noting that although these sequels are mild and in average, the cognitive function in LT recipients is good, these defects could have important implications as the subjects are also at risk of other neurological insults.

I. Cognitive function after LT

Previous studies performed in patients showed an improvement in the neurological[9,29,95,102,107,115,121,125] and radiological abnormalities[39,139,142] during the first year after LT. The same findings were reproduced in our studies. We identified an improvement in all the explored cognitive indexes during the first year post-LT in both of our studies. However, we identified 2 patients in the first study and 7 in the second one who did not achieve a normal cognitive function. The second study found that prior to transplant medical conditions such as alcohol abuse, diabetes mellitus and prior HE were linked a worse posttransplant neurological status. The assessment performed at long-term (nine years after LT) indicate that after 1 year, the neurological function remains stable unless posttransplant *de novo* comorbidities impair the cognition. In fact, the prevalence of vascular risk factors in this population is high and the patients are at risk of cardiovascular events[53,83,106,150]. So, the patients with the most prominent expression of small vessel cerebrovascular disease (largest increase in WMLs) exhibited a neurological deterioration in the long-term follow-up period with memory decline. An accumulation of cardiovascular risk factors was significantly higher among these subjects. In summary, prettransplant conditions and post-LT events influence the cognitive outcome (figure 9).

There are important limitations in these studies. As the neurological function after LT is affected by several factors, it is difficult to assess each condition separately. As a result, estimation of the burden

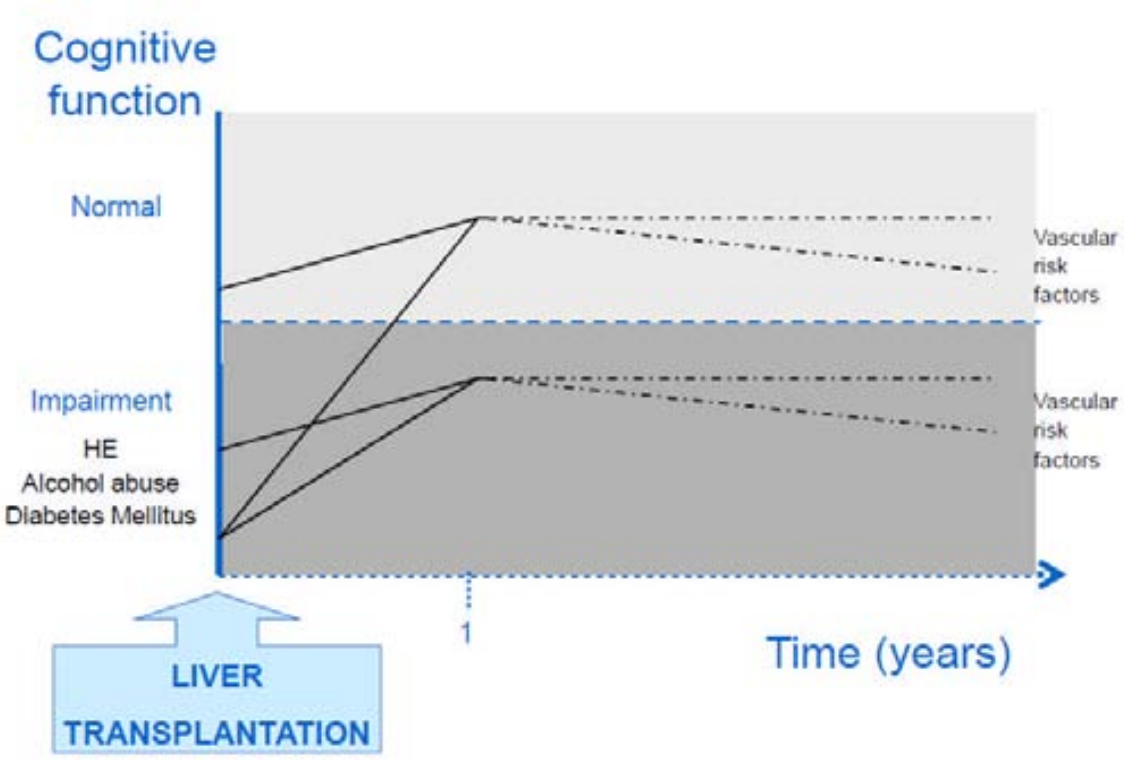

Figure 9.
Evolution of cognitive function after LT. An improvement associated to the correction of liver failure during the first year is expected. Accumulation of pretransplant conditions increases the risk of permanent sequels. After 1 year, the cognition is stable unless de novo postransplant comorbities impair the neurologic state.

of HE on the posttransplant cognition is difficult to ascertain. From our results we can conclude that presence of HE is linked to a permanent impairment of mainly motor function.

On the other hand, other factors that could affect cognition may not have been properly investigated in our studies, in part due to methodological difficulties. Perioperative ischemia and changes in brain fluxes could also harm brain structure and function. Posttransplant neurological events (seizures, central nervous system infections, other metabolic encephalopathies…) may also be responsible of neurological outcome. Immunosuppressive drugs may directly cause cognitive impairment. All the participants in our studies consumed immunosuppressive drugs. However, different regimens or plasma levels were not associated with the brain function. Although we cannot exclude their participation, our results suggest that HE increase the susceptibility to persistent deficits.

II. Brain volume (BV) after LT

Neuroimaging techniques used in human studies with diseases characterized by cognitive decline revealed different abnormalities providing the structural basis for the neurological dysfunction.

Two-dimensional measurement of ventricular size is an indirect measure of brain volume in the absence of hydrocephalus[163] . In the longitudinal assessment performed in the group of 22 patients (study I) we observed an enlargement of lateral ventricles at short and long-term after LT. In parallel we observed an improvement in the neuropsychological function at short-term and a stable function at long-term with deterioration in the subgroup with higher cardiovascular risk suggesting different pathogenic mechanisms. There is a growing body of evidence that imply the presence of low grade brain edema in cirrhosis which improves with liver transplant [39,68,70,84,112,126,139,146]. Our results are in concordance with these findings. The resolution of low grade brain edema is the most plausible explanation for the decrease in the brain volume with improvement of neurological function. After one year the decrease in the BV was related to the age of the patients and the cerebrovascular disease indicating that an appropriate treatment of the cardiovascular risk factors could be useful in preventing the cognitive decline.

Interestingly, we observed a smaller BV in the patients compared with healthy controls matched by age. However, we did not find any association with prettransplant conditions in this relatively small cohort. In the second study, an accurate MR volumetric method[151,152]

linked the prior HE to the posttransplant normalized BV. So, patients with prior HE exhibited a smaller posttransplant normalized brain volume compared with those without prior HE and with same age. The lack of longitudinal assessment together with other factors such as ischemia and immunosuppression that may have not been adequately investigated in this study makes us to interpret this finding with caution. Nevertheless, prior neuroradiological[18,101,173] and neuropathological[65,91] studies described the presence of brain atrophy in cirrhotic patients even in those with no history of alcohol abuse. Our results are in agreement with the previous reports and support the notion that HE causes loss of brain parenchyma.

III. Role of HE in the cognitive function and brain volume after LT

The findings of this research indicate that HE is associated with a permanent detrimental effect on the cognitive function. In the same group of patients, HE is associated with a smaller normalized brain volume. To the best of our knowledge this is the first report that defines a link between HE and permanent functional impairment at the same time with a smaller BV. In spite of the limitations of this research that requires further investigations, this study support the hypothesis of a permanent damage associated with HE due to loss of brain tissue. The relationship between the smaller brain volume and the lower concentration of N-Acetyl-Aspartate/creatine (NAA/Cr) indicate that neurons are involved in the decrease of brain volume.

NAA is synthesized from *aspartate* and *acetyl-coenzyme A* in neurons and is involved in synthesis of myelin by oligodendrocytes and bioenergetic metabolism of neuron in mitochondria[113] (Figure 10).

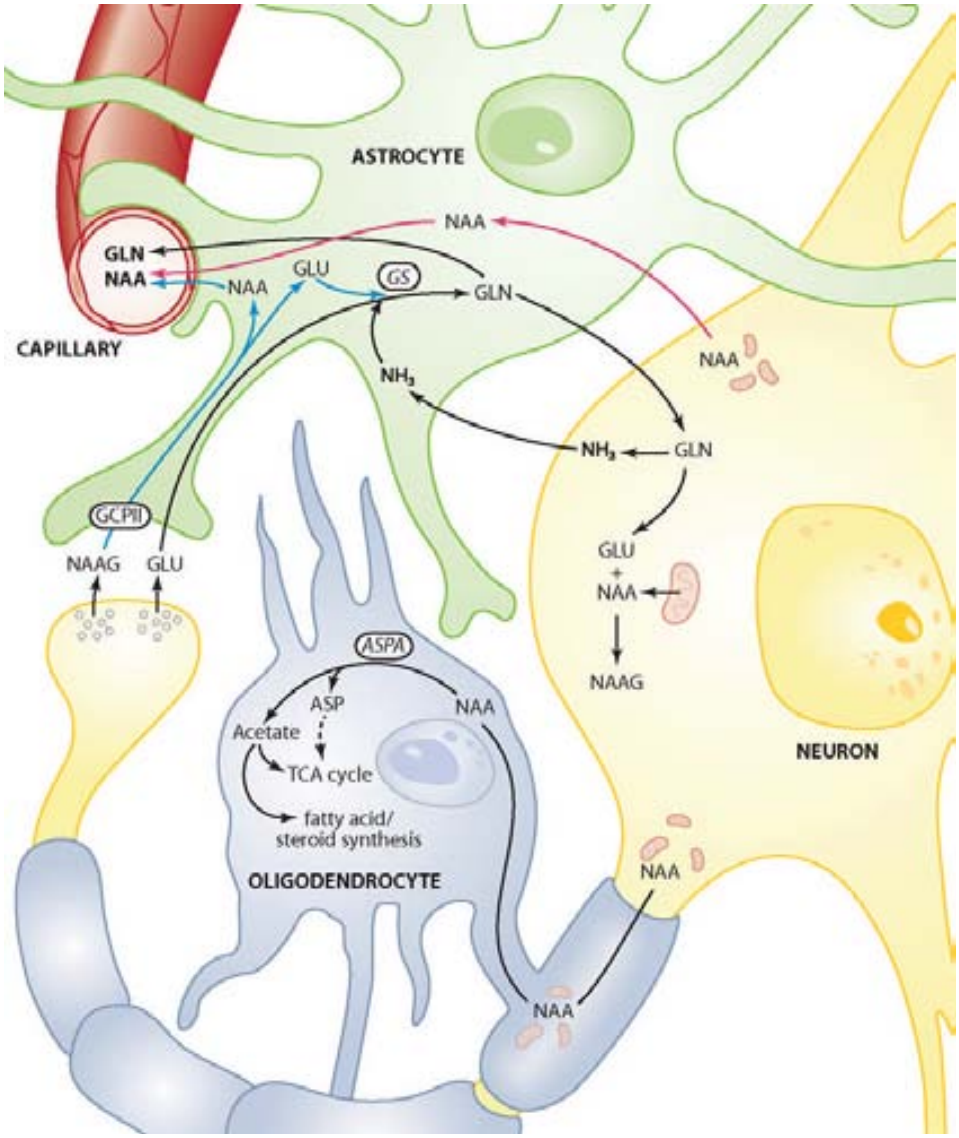

Figure 10.
Synthesis and metabolism of NAA in brain. NAA is synthesized in neuronal mitochondria and can either be transported to oligodendrocytes for fatty acid synthesis or energy production or can be used for synthesis of N-acetylaspartylglutamate (NAAG) in neurons which is release with other transmitters. Then can be hydrolyzed by astrocytes into NAA and glutamate[113].

NAA is considered a neuronal marker that indicates presence and/or function of neurons. It has been demonstrated that there is a decrease of NAA/cr in several neurodegenerative diseases as Alzheimer disease[86,168], multiple sclerosis[31,98], epilepsy[21,166] or stroke[54,55].

Loss of neurons in HE is the most plausible explanation for the results of this research and this is supported by the following evidence:

a. *Mechanisms of neuronal cell death are activated in HE.*

- Oxidative/nitrosative stress and mitochondrial dysfunction may lead to cell death as was shown in multiple neurodegenerative process[79]. Evidence for their activation in liver failure[76] and their participation in HE[17,120] derive from human observations[59], animal studies[94] and cell culture investigations[60].
- Energy impairment and increased of brain lactate[34,160] demonstrated in brain during liver insufficiency could be implicated in neuronal cell death[80].
- Inflammation have been involved in the progression of neurological[164] and neurodegenerative diseases[46] by an exacerbation of local process that may lead to cell death. In liver failure, systemic inflammation exacerbates neurotoxicity induced by ammonia[32,148]. On the other hand, neuroinflammation and microglial activation participate in the pathogenesis of neurodegenerative diseases[79] and have been recently reported in animal models of HE[135]. Treatment with anti-inflammatory drugs suggests a protective action[82].

These mechanisms of cell death existing in HE may act synergistically[27] as have been reported in cultured rat astrocytes[58].

b. *Demonstration of neuronal cell death in other neurological manifestations of chronic liver disease*[26]. Loss of neurons in chronic liver failure has been reported in acquired hepatocerebral

degeneration and cerebellar degeneration in absence of alcohol abuse. Almost all the reported cases to date of AHCD had suffered repeat episodes or a prolonged single episode of coma prior the pathological study. If not all, many of the pathogenic mechanisms involved in these entities are present in HE.

Fortunately, the neurological irreversible damage associated with HE is small and the sequels of mild intensity. However, this concept can have important implications:

- The classical consideration of HE as a pure gliopathy should be revised.
- In patients in the waiting list for LT (in risk of other posttransplant neurological insults), the reduction of neurological damage in the prettransplant stage could be beneficial. Those patients with low MELD score and comorbidities could be specifically helped. The actions include re-evaluation of LT priority in cases of same expected survival and promote preventive therapies.
- In patients who are not transplant candidates, implementation of preventive therapies such as nonabsorbable disaccharides[147] or rifaximin[15] may prevent the cognitive decline.

Development of new drugs addressed to treat or prevent development of HE would be useful in the prevention of cognitive decline and improve the quality of life in cirrhotic patients.

7. Conclusions

I. Cognitive function after LT is heterogeneous and associated to pre and posttransplant conditions. Hepatic encephalopathy is associated with cognitive sequels predominantly in the motor function.

II. A smaller brain volume after LT is associated with previous HE suggesting that HE can lead to loss of brain tissue.

III. The loss of brain volume may be secondary to neuronal damage, as supported by the association between the area peak of N-Acetyl-Aspartate (a noninvasive neuronal marker) in magnetic resonance spectroscopy and the normalized brain volume. This concept is supported by the existence of activated mechanisms of neuronal death in experimental HE and the demonstration of neuronal degeneration in neuropathological preparations of material from autopsies.

IV. Other prettransplant comorbidities such as alcohol abuse and diabetes can impair brain function together with some posttransplant conditions as cerebrovascular disease and may act synergistically with HE in causing impaired cognitive function.

V. Prevention of HE may abrogate the cognitive decline and improve quality of life in cirrhotic patients.

8. Future investigations

The present research links HE with neuropsychological sequels and smaller brain volume which strongly suggests loss of brain tissue during its occurrence, particularly neuronal loss. However, establishing this causality requires further investigations.

The next step will be to demonstrate loss of brain tissue after HE in longitudinal studies. To date, the most accurate neuroimaging techniques are sensitive to detect low grade brain edema and changes in MR signals related to the deposit of paramagnetic substances. Validation and interpretation of these results may be difficult. Alternatively, invasive studies in animal models with the reproduction of the syndrome with wide range of severity and duration and investigating neurons and together with markers of neuronal cell death can provide us with very useful information.

On the other hand, improving clinical management with preventative therapies and new drugs that can minimize brain damage may have beneficial neurological effects. Albumin is the main scavenger in the plasma with important antioxidant and anti-inflammatory properties[131]. Albumin function in cirrhosis is impaired[78]. Understanding the basis of this injury is currently under investigation. The benefits of albumin infusion are being assessed in a clinical trial (http://clinicaltrials.gov/ct2/show/NCT00886925?term=albumin+AND+hepatic+encephalopathy&rank=1). In parallel, analysis of albumin structure and function are been carried out in collaboration with Professor Jalan at Royal Free and University College London (London).

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