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# Hepatic Encephalopathy

## Clinical Challenges and Opportunities

Edited by
Lorenzo Ridola and Oliviero Riggio

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# Hepatic Encephalopathy: Clinical Challenges and Opportunities

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Editors

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*Editorial*

# Hepatic Encephalopathy in the 21st Century: Still an Emerging Topic

**Lorenzo Ridola * and Oliviero Riggio**

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Why write about hepatic encephalopathy (HE) in the twenty-first century? It is in front of this question that we found ourselves, once invited to serve as guest editors for the Special Issue of the Journal of Clinical Medicine entitled "Hepatic Encephalopathy: Clinical Challenges and Opportunities". Scientific research has made important advances, the pathogenesis of HE is more deeply known, and the role of toxins such as ammonia (still relevant!), as well as those of systemic inflammation, are being discussed, even in a very heated way. Overt HE has been deeply defined, while the Minimal Hepatic Encephalopathy (MHE) (and covert) are becoming increasingly important, due to the high impact on the daily life both of the patient and caregiver. Progress is also remarkable regarding the pharmacological approach. In fact, secondary prophylaxis appears to be consolidated and effective, while for episodic HE, the importance of identifying and treating precipitating factors, as an "ideal first cause" of HE, is now affirmed and widely accepted [1–3]. Nonetheless, there are still many gray areas and issues that need further study, especially regarding the role of treatment in patients with minimal HE [4–6]. In fact, in this specific setting, guidelines suggest treating patients on a case by case basis. More generally, the ideal design of therapeutic studies also remains debated. In fact, the existing literature on HE medical management still suffers from a lack of standardization, and this heterogeneity makes the pooling of data difficult or meaningless. There is still an unmet need for "robust" controlled clinical trials on treatment effects on HE, because decisive clinical studies are few, although the number of patients and their resource utilization remain high [6].

In this Special Issue, it was therefore decided to give space to contributions addressing the most innovative topics in HE. The pathogenesis of HE is, indeed, not completely clear. A series of observations, based on clinical and therapeutic features, suggest that for any substance to be involved in the pathogenesis of HE, it should originate in the gut, possibly by the action of bacteria, be found in the portal circulation, be increased in peripheral blood as a consequence of failing liver or portal systemic shunting, and finally should exert its effect on the brain. Unfortunately, any attempt to clarify the nature of the substance(s) involved has, until now, not been completely satisfactory. Ammonia meets all the above mentioned criteria, but its correlation with HE is not always found. Because of these considerations, the role of other potential toxins, such as tryptophan derivatives, has been investigated over the years [7] and the effects of systemic inflammation and its mediators have been better studied. However, even today, ammonia plays a predominant role in the pathogenesis of HE and this is also confirmed by the effectiveness of treatments aimed at ensuring the metabolism or the prompt elimination of nitrogen derivatives. There is growing interest in the modulation of the gut–liver–brain axis in the therapy of complications of liver cirrhosis, including HE. Fecal transplantation in this setting of patients has also recently been proposed with encouraging results [8,9]. This certainly represents a new frontier in the management of advanced liver diseases. Concerning HE pathophysiology, in this issue, the relationship between hepatic dysfunction and the progression of brain energy crisis in hepatic encephalopathy has also been addressed.

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Another unsolved problem, which still heavily complicates the management of cirrhotic patients with portal hypertension, is the occurrence of HE after transjugular intrahepatic portosystemic shunt (TIPS) placement. A TIPS is widely adopted to treat complications of portal hypertension such as recurrent variceal bleeding or refractory ascites, by shunting blood flow, bypassing the liver and, consequently, reducing portal pressure, with the aim to reduce mortality and bridge patients to liver transplant. TIPSs represent often a life-saving procedure, but are characterized, due to the blood diversion directly into systemic circulation, by the development of HE, particularly in the first period immediately after the procedure. To date, the role of drug therapy in prophylaxis of HE after TIPS is not yet clear and supported by strong scientific evidence [1,2,10]. More recently, approaches characterized by modulation of the stent caliber or choosing reduced caliber shunts have also been proposed [11–14]. However, even this evidence does not yet allow us to formulate sufficiently "robust" recommendations and the choice to close TIPSs for refractory HE is very hard and needs to be counterbalanced with the competing risk of newly developing those complications of portal hypertension that were the indication to TIPS placement. Recently, the role of spontaneous portosystemic shunts (SPSSs) also appears to be of growing interest, both for the causative role in determining HE, and due to the possibility to treat shunt-induced HE by closing or reducing the caliber of those collaterals [15–18]. Indeed, the search for SPSSs has entered the process of classification of patients with a history of HE.

A frequent complication of cirrhosis is malnutrition, which is associated with the progression of liver failure, and with a higher rate of complications, including infections, hepatic encephalopathy, and ascites. It is well known that sarcopenia, a condition in which muscle mass and function are reduced due to the patient's poor nutrition and reduced physical activity, also has a prognostic impact on cirrhosis and its complications [19]. A historical dietary approach of encephalopathic patients was characterized by a close protein restriction. Nevertheless, recently published European Association for the Study of the Liver guidelines on nutritional management of cirrhotic patients [20] state that nutritional status and the presence of sarcopenia should be evaluated in patients with HE and to avoid protein restriction in patients with HE. To date, the relationship between sarcopenia and HE looks better defined, as well as the importance of an adequate nutritional intake, but the role of an intervention deserves more convincing results. For example, studies aimed to assess the role of physical exercise in improving sarcopenia and consequently HE should, in our opinion, be strongly encouraged.

In summary, HE is a condition characterized by a heavy burden both on the patient and caregiver, and on health systems. Therefore, considerable interest is currently focused on the use of administrative data. This information is available in databases of health care systems, and the retrieval and analysis of these data allow us not only to provide an immediate picture of the dimension of HE's burden, but also to identify prognostic factors associated with the development of hepatic encephalopathy. This knowledge, in the near future, may allow for better stratification of patients at risk and starting early therapeutic interventions.

It is therefore time to consider HE under a new perspective, in which some different new factors should be considered to have a determinant/causative and prognostic role. Indeed, patients at risk for HE should be considered, not only those with severe liver disease, or a previous history or with minimal/covert HE, or TIPSs, but also those with sarcopenia, nutritional deficit, or bearing SPSSs. It is important to consider cirrhotic patients under this whole panorama in order to identify very high-risk patients, in which other factors with a different and non-"classical" management should be respectively searched and adopted.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Patients with Minimal Hepatic Encephalopathy Show Altered Thermal Sensitivity and Autonomic Function

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**Abstract:** Cirrhotic patients may experience alterations in the peripheral nervous system and in somatosensory perception. Impairment of the somatosensory system could contribute to cognitive and motor alterations characteristic of minimal hepatic encephalopathy (MHE), which affects up to 40% of cirrhotic patients. We assessed the relationship between MHE and alterations in thermal, vibration, and/or heat pain sensitivity in 58 cirrhotic patients (38 without and 20 with MHE according to Psychometric Hepatic Encephalopathy Score) and 39 controls. All participants underwent attention and coordination tests, a nerve conduction study, autonomic function testing, and evaluation of sensory thresholds (vibration, cooling, and heat pain detection) by electromyography and quantitative sensory testing. The detection thresholds for cold and heat pain on the foot were higher in patients with, than those without MHE. This hyposensitivity was correlated with attention deficits. Reaction times in the foot were longer in patients with, than without MHE. Patients with normal sural nerve amplitude showed altered thermal sensitivity and autonomic function, with stronger alterations in patients with, than in those without MHE. MHE patients show a general decrease in cognitive and sensory abilities. Small fibers of the autonomic nervous system and thermal sensitivity are altered early on in MHE, before large sensory fibers. Quantitative sensory testing could be used as a marker of MHE.

**Keywords:** minimal hepatic encephalopathy; nerve conduction; thermal sensitivity; autonomic testing

## 1. Introduction

Hepatic encephalopathy (HE) is defined as a 'brain dysfunction caused by liver insufficiency and/or portosystemic shunting' [1]. HE may be classified as covert or overt. The terms minimal or covert hepatic encephalopathy are used when cognitive and motor alterations induced by liver cirrhosis are not evident but may be unveiled using psychometric or neurophysiological tests. Overt hepatic encephalopathy is applied when neurological alterations are more evident. The scale most often used for grading the extent of HE is the West Haven criteria, which distinguishes between four grades of clinically overt HE. Currently, some experts suggest differentiating between covert HE (MHE plus grade I HE according to West Haven criteria) and overt HE (grades II–IV) [1].

Around 30–50% of cirrhotic patients present minimal hepatic encephalopathy (MHE), characterized by attention deficits, psychomotor slowing, and mild cognitive impairment, which impair quality of life and reduce life span [1–6]. MHE patients have difficulty performing everyday tasks such as driving [7,8]. They also have higher risk of falls, accidents, and hospitalization, creating a greater economic burden on the health and social care systems [9–11]. MHE is undetectable by traditional clinical methods and is currently diagnosed with a battery of psychometric tests called the psychometric hepatic encephalopathy score (PHES) [12,13]. Although these psychometric tests are a first-line tool for diagnosing MHE, several reports indicate a subset of cirrhotic patients who show mild cognitive and motor deficits not detected by the PHES [14–18].

Cirrhotic patients may show alterations in the peripheral nervous system, for example in somatosensory perception [19]. Somatosensory perception is composed of different modalities, which can be grouped in three subsystems. The first collects information from cutaneous mechanoreceptors such as fine touch, pressure, and vibration. The second subsystem receives proprioceptive information from specialized receptors associated with the muscles, tendons, and articulations, called proprioceptors. The third system derives from receptors that inform of pain, temperature changes, and gross touch. All these data are conducted through afferent fibers of pseudounipolar neurons of the spinal ganglion, reaching the posterior spinal medulla, where crossing may take place directly (protopathic sensibility) or at the brain stem (epicritic sensibility). Finally, the afferents end in a contralateral manner to the location of the received stimulus, in the corresponding somatotopic region of the cortex.

The autonomic nervous system controls the functioning of the different body systems within the organism. This system, through its three efferent components—sympathetic, parasympathetic, and enteric—innervates the heart muscle, the smooth muscle of all organs, and the exocrine and endocrine glands. The most frequent clinical manifestations of autonomic dysfunctions are cardiovascular, digestive, sudomotor, ocular, genitourinary . . . , and there are several functional tests to evaluate autonomic function, such as the assessment of the cardiovascular system (RR interval, valsalva maneuver, . . . ), and the sudomotor system, among others [20].

Clinically, polyneuropathy is defined by a series of motor, sensory, and autonomic symptoms. To diagnose peripheral polyneuropathy and classify the type of fiber and modality affected (small or large fibers, sensory, motor, or both) sensory and motor nerve conduction studies are performed, which can often identify the primary pathological process.

Several sensory nerve conduction studies have shown that up to 70% of cirrhotic patients could be affected by sensorimotor peripheral neuropathy [19,21]. Polyneuropathy associated with liver cirrhosis is included within the chronic axonal polyneuropathies of toxic-metabolic origin, characterized by length-dependent or dying-back axon degeneration, initially affecting the sensory fibers and with a pattern of distal predominance [22]. Some chronic axonal polyneuropathies with predominantly sensory or autonomic alterations may affect only a small caliber axonal fibers, potentially undetectable in nerve conduction studies. These small caliber fibers include sympathetic and parasympathetic unmyelinated fibers that are involved in conducting pain and temperature sensation [20,23].

Alteration of these fibers can be evaluated by autonomic electrophysiologic studies such as electrodermal activity related to sweating (sympathetic skin response), heart rate variability (RR interval measurement) and thermotesting.

Previous studies using neurophysiological methods have found impairments in both central and peripheral parts of the somatosensory system in patients with liver cirrhosis and overt HE [24].

Analyzing somatosensory system function is of interest in MHE patients because this system is involved in awareness, attention, and motor response [25], areas which are altered in these patients. Impaired function of the somatosensory system could contribute to the development of mild cognitive and motor deficiencies in MHE. This theory is supported by sensory deficits manifested in patients with cognitive impairment-associated diseases such as Alzheimer's and Parkinson's [26,27].

Mild cognitive impairment was linked to early changes in the primary somatosensory cortex, which has been proposed as a sensitive marker for cognitive decline [26].

A better characterization of sensory perception, nerve conduction, and autonomic function could enable detection of MHE at earlier stages and with greater sensitivity. The aim of this study was to evaluate and characterize thermal, vibration and heat pain sensitivity in cirrhotic patients and healthy controls, and to assess any differences between patients with and without MHE. We evaluated different neurological functions such as attention, concentration, mental processing speed, working memory, and bimanual and visuomotor coordination, using specific psychometric tests. These functions are altered at early stages of MHE [14]. We assessed the correlations of performance in these functions with alterations in sensory perception and autonomic function. We also performed sensory and motor nerve conduction studies and autonomic function testing to classify the type of fiber affected (small or large fibers, sensory, motor, or both), and pinpoint the primary pathological process.

## 2. Experimental Section

### *2.1. Patients and Controls*

Fifty-eight patients with liver cirrhosis were consecutively recruited from outpatient clinics of Clinico and Arnau de Vilanova hospitals in Valencia, Spain. Cirrhosis diagnosis was based on clinical, biochemical, and ultrasonographic data. The exclusion criteria were overt hepatic encephalopathy, recent alcohol intake (<6 months), infection, recent antibiotic use or gastrointestinal bleeding (<6 weeks), recent use of drugs affecting cognitive function (<6 weeks), presence of hepatocellular carcinoma, and neurological or psychiatric disorders. Patients with insulin-dependent diabetes were also excluded, as they presented more severe polyneuropathies than diabetic patients taking oral antidiabetic drugs. Thirty-nine healthy volunteers without liver disease were also included. Exclusion criteria for all groups were acute or chronic pain and any signs of superficial inflammation or injury in the left foot or hand, to avoid interference with the sensitivity results. All participants were included after signing a written informed consent. Study protocols were in accordance with the ethical guidelines of the Declaration of Helsinki and were approved by the Research Ethics Committees of both hospitals (2018/210).

After performing psychometric tests, patients were classified as with or without MHE (see below) and were referred to the neurophysiology unit to undergo electrophysiological and quantitative sensory testing. These tests were performed within the following week after the PHES was performed, in order to minimize possible cognitive fluctuations. The composition and characteristics of the groups are given in Table 1.

**Table 1.** Composition of the different groups and etiology of liver disease.

| | Control | NMHE Patients | MHE Patients |
|---|---|---|---|
| Number of subjects | 39 | 38 | 20 |
| Sex (male/female) | 13/24 | 36/2 | 18/2 |
| Age † | 64 ± 2 | 60 ± 1 | 64 ± 1 |
| Etiology of cirrhosis | | | |
| Alcohol | | 18 | 10 |
| HCV/HBV/HCV + alcohol | | 13/0/1 | 4/1/0 |
| NASH/NASH + alcohol | | 1/3 | 4 |
| other | | 2 | 1 |
| Diabetes (without/DM NID) | | 31/7 | 12/8 |
| Child Pugh score (A/B/C) | | 29/9/0 | 14/6/0 |
| MELD score † | | 9.4 ± 2.7 | 9.1 ± 2.3 |
| Lactulose | | 3 (8%) | 4 (20%) |
| Beta-blockers | 2 (5%) | 15 (39%) | 5 (25%) |
| Polyneuropathy (no/yes) (%) | 37/2 (95/5) | 24/14 (63/37) | 12/8 (60/40) |

† Values are expressed as mean ± SD. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively; HBV: hepatitis B virus; HCV: hepatitis C virus; NASH: non-alcoholic steatohepatitis; DM NID: diabetes mellitus without insulin dependence; MELD: model end-stage liver disease.

### 2.2. Neuropsychological Assessment

MHE was diagnosed by the Psychometric Hepatic Encephalopathy Score (PHES) [13]. The global PHES scores were calculated with Spanish normality tables (http://www.redeh.org/TEST_phes.htm), adjusting for age and education level. Patients were defined as having MHE with a score $\leq -4$ points.

To evaluate other cognitive abilities, further psychometric and motor tests were performed: the Stroop test (congruent, neutral, and incongruent tasks) for selective attention and cognitive flexibility; the d2 test, which evaluates selective/sustained attention and mental concentration; bimanual and visual-motor coordination tests; the Symbol Digit Modalities Test (Oral SDMT), for mental processing speed; Digit Span, evaluating immediate and working memory; and Letter-Number Sequencing, which evaluates working memory in greater depth than Digit Span. These tests were performed as in Giménez-Garzó et al. [14] on the same day as the PHES.

### 2.3. Neurophysiological Studies of Large Caliber Fibers: Nerve Conduction Study

The electrophysiological study of sensory and motor nerve conduction was undertaken with Synergy, version 22.0.0.144 and components: UltraPro S100 version 1 and UltraProS100 DSP version 591. Nerve conduction studies allowed us to determine the conduction of motor and sensitive fibers, including large and myelinated fibers, which have the greatest conduction velocity. This study allowed the detection and classification of the type of progressive polyneuropathy and to further identify the origin of sensory deficits.

Each laboratory should elaborate their own independent protocol of neurophysiological evaluation due to the differences that exist between testing equipment, techniques, and individual characteristics of the study population. The conduction study protocol used for the diagnosis of polyneuropathy was based on those described by Falck and Stålberg [28] and Preston and Shapiro [29].

The parameters measured were amplitude, latency, and conduction velocity. The latency measures nerve conduction time, in milliseconds (ms), from which the stimuli begins, to the initial moment of the evoked response; amplitude is the median value, in millivolts (mV), of the negative peak and positive peak of the evoked response, it assessed the number of stimulated axons; and conduction velocity, expressed in m/s, was calculated by measuring two stimulated points of the same nerve and dividing it by the difference between proximal latency and distal latency.

The sensory nerves explored were the unilateral ulnar, unilateral superficial radial, bilateral sural, and bilateral superficial peroneal nerves. The motor nerves examined were the unilateral ulnar, bilateral peroneal nerves, and bilateral posterior tibial nerves. Data obtained from nerve conduction measures were used to detect large nerve fiber damage, i.e., polyneuropathy. The diagnosis of polyneuropathy was based on the alteration of 3 or more nerves in 2 different extremities. See Supplementary Information for a more detailed explanation about the method used for nerve conduction studies.

### *2.4. Neurophysiological Studies of Small Caliber Fibers: Autonomous Nervous System and Quantitative Sensory Testing (QST)*

To evaluate whether small caliber fibers are affected in MHE, we performed neurophysiological tests including study of the autonomous nervous system (sympathetic skin response, RR interval) and Quantitative Sensory Testing.

#### 2.4.1. Autonomous Nervous System

Sympathetic skin response (SSR) can be used to explore sudomotor function, which is part of the thermoregulation system [30]. SSR was analyzed using the Synergy UltraPro S100. Registering electrodes were placed on the right palm with a reference electrode on the back of the hand. The electrodes used were common to the EMG technique, with a low frequency filter of 0.5 Hz. The stimulus used was small electric stimulation, and the amplitude (in mV) and latency (in ms) of response were registered. The amplitude of SSR was considered abnormal when it was lower than 1.1 mV. This threshold was calculated from the mean of amplitude of sympathetic skin response of controls minus 2 standard deviations.

We also studied heart rate variability as a measure of the cardiovascular autonomic nervous system. We determined resting heart rate via the vagal tone, and heart rate variability, which consisted of variation of the RR interval of the electrocardiogram, by evaluating fluctuations in heart rate. Duration of RR intervals recorded over time reflects the influence of both sympathetic and parasympathetic nervous systems in heart rate modulation. Among the different activation maneuvers, we performed the Valsalva maneuver, hyperventilation, and orthostatic tests (from lying to sitting position) [31]. Registering electrodes were placed on both wrists above the radial artery.

#### 2.4.2. Quantitative Sensory Testing (QST)

QST was performed using CASE IV System WR Testworks (Supplementary Figure S1). Case IV was an automated diagnostic device, which detects and characterizes disease-altered sensory thresholds of sensory receptors, nerve fibers, central nervous system tracts, and/or cerebral association areas [32–35]. In this study, we assessed two somatosensory pathways: the leminiscal pathway, tested by way of vibration, and the ventrolateral pathway, evaluated through heat-pain and cooling perception. These thermal and nociceptive sensations were transmitted through small sensory nerve fibers [36], which can be classified by diameter and myelination. Vibration travels through large diameter sensory myelinated fibers (A alpha), while cooling perception mainly is relayed by small diameter myelinated fibers (A delta), and heat-pain is transmitted by unmyelinated (C fibers) [23]. The parameters registered were: vibration detection threshold (VDT), mediated by large diameter sensory myelinated fibers, A alpha; cooling detection threshold (CDT) measuring mainly small diameter myelinated fibers, A delta; and heat pain detection threshold (HPDT), mediated by small diameter unmyelinated C fibers. Supplementary Figure S2 shows the algorithm used, which determines how the stimuli are presented. The calculation of sensory thresholds is detailed in Supplementary Materials.

A total of six tests were performed: three modalities (VDT, CDT, and HPDT) on two test sites, hand and foot, in that order. Results were represented by the sensory thresholds of each individual test in just-noticeable difference (JND), corresponding to the mean stimuli level just detectable by the subject. A normal range was calculated from data

collected from the control group, and tests outside the normal range were summed up for each individual patient. The total time to complete each test was measured in seconds.

### 2.5. Statistical Analysis

Values are given as mean ± standard error of mean (SEM), unless otherwise specified. D'Agostino and Pearson omnibus normality test was used to test variable normality. Between-group differences were analyzed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test. For non-parametric variables the Kruskal-Wallis test was performed, followed by Dunn's multiple comparisons test. Results were analyzed by GraphPad PRISM Version 7. The probability level accepted for significance was $p < 0.05$. Bimanual and visual-motor coordination tests were analyzed using univariate analysis of covariance (ANCOVA) with age included as covariate, followed by post-hoc Bonferroni. Analyses of contingency tables were performed by Fisher's exact test. We evaluated the predictive capacity of QST parameters for MHE using ROC (receiver operating characteristic) curves. Pearson correlation analysis and ROC analyses were performed using the SPSS software, Version 20 (SPSS Inc, Chicago, IL, USA) and two-sided $p$-values < 0.05 were considered significant.

## 3. Results

### 3.1. Neuropsychological Assessment

After PHES, cirrhotic patients were stratified into 38 patients without MHE (NMHE) and 20 with MHE (Table 1).

Selective and sustained attention (measured by Stroop and d2 tests) and mental processing speed (by oral SDMT) were altered in both groups of patients compared to controls, but patients with MHE performed worse in these tasks than NMHE patients (Table 2).

**Table 2.** Performance in neuropsychological tests.

| | Controls | NMHE Patients $p$ vs. Control | MHE Patients $p$ vs. Control | MHE Patients $p$ vs. NMHE | Global ANOVA $p$ Values |
|---|---|---|---|---|---|
| PHES Global score | 0.0 ± 0.2 | −0.8 ± 0.2 | −7.5 ± 0.7 *** | <0.001 | <0.001 |
| Bimanual coordination (min) | 2.2± 0.1 | 2.3 ± 0.1 | 3.4 ± 0.3 *** | <0.001 | <0.001 |
| Visual-motor coordination (min) | 2.7 ± 0.1 | 2.9 ± 0.1 | 3.7 ± 0.2 *** | <0.001 | <0.001 |
| d2 Test | | | | | |
| TR Values | 378 ± 19 | 322 ± 11 * | 271 ± 14 *** | 0.03 | <0.001 |
| TOT Values | 366 ± 16 | 292 ± 12 ** | 245 ± 15 *** | 0.05 | <0.001 |
| CON Values | 146 ± 7 | 114 ± 6 * | 89 ± 10 *** | 0.04 | <0.001 |
| TA Values | 142 ± 7 | 117 ± 6 * | 98 ± 7 ** | ns | 0.001 |
| Stroop test | | | | | |
| Congruent Task † | 103 ± 4 | 94 ± 3 | 81 ± 4 ** | 0.04 | 0.003 |
| Neutral Task † | 78 ± 3 | 69 ± 2 * | 57 ± 2 *** | 0.001 | <0.001 |
| Incongruent Task † | 47 ± 2 | 37 ± 2 ** | 30 ± 2 *** | 0.02 | <0.001 |
| Oral SDMT (correct pairings) | 44 ± 2 | 38 ± 1 * | 26 ± 2 *** | <0.001 | <0.001 |
| Digits Span-Total score | 14 ± 0.7 | 12 ± 0.6 * | 11 ± 0.7 ** | ns | 0.007 |
| Letter-Number Sequencing test (right answers) | 9 ± 0.6 | 7 ± 0.6 | 5 ± 0.7 ** | 0.02 | 0.001 |

Values are expressed as mean ± SEM. Differences between groups were analyzed using one-way ANOVA followed by post-hoc Tukey. For bimanual and visual-motor coordination tests, univariate analysis of covariance (ANCOVA) was performed, with age included as a covariate, followed by post-hoc Bonferroni. Significant differences compared to controls are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. MHE and NMHE: patients with and without Minimal Hepatic Encephalopathy: respectively; PHES: Psychometric Hepatic Encephalopathy Score; d2 test: TR: Total number of characters processed; TOT: Total correctly processed; CON: Concentration performance; TA: Total right answers. † Stroop test: Congruent task: number of words read in 45 s; Neutral task: number of colors read in 45 s; Incongruent task: number of items completed in 45 s. Oral SDMT: Symbol digit modalities test (oral version).

Patients with MHE also showed impairment in working memory tasks such as Digit Span and Letter-Number Sequencing tests, performing worse than NMHE patients in the

latter test (Table 2). Finally, bimanual, and visual-motor coordination were poorer in MHE patients than in NMHE patients ($p < 0.001$).

### 3.2. Nerve Conduction Study

Based on nerve conduction studies, polyneuropathy was detected in 22 cirrhotic patients (38%): 14 without MHE (37% of NMHE patients) and eight with MHE (40% of MHE patients) (Table 1). Proportions of polyneuropathy between groups were not statistically different (Fisher exact test $p > 0.99$). Ten NMHE patients and four patients with MHE had mild to moderate sensory axonal polyneuropathy. Four patients from each group showed sensory-motor axonal neuropathy. There were two controls who presented mild large fiber neuropathy. In order to avoid possible bias in the results when comparing to controls, we included these controls in the analysis in Figure 1, as well in Table 3.

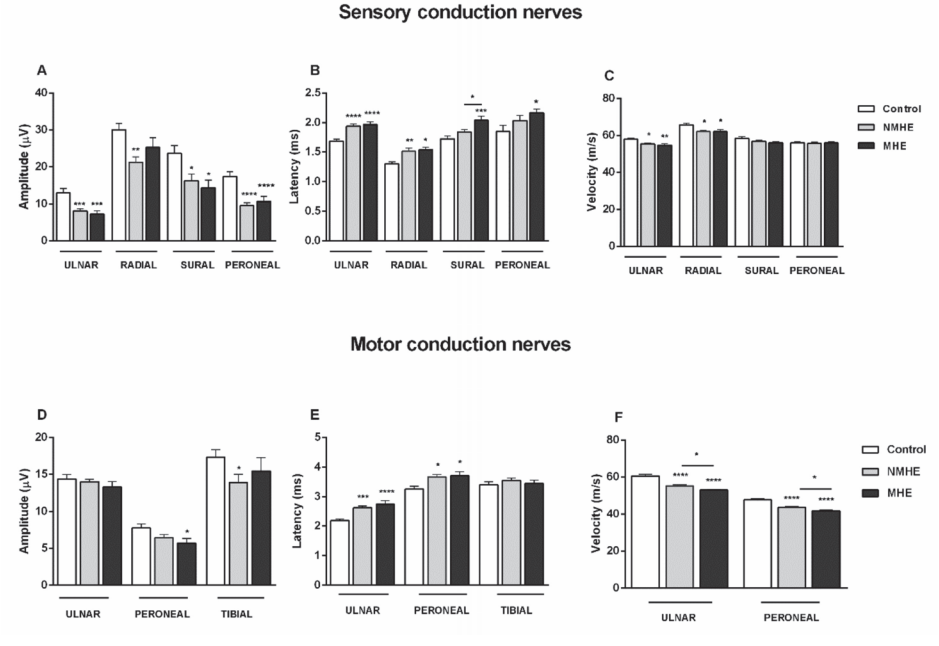


**Figure 1.** Cirrhotic patients with and without MHE show alterations in sensory and motor nerve conduction. (**A**–**C**): Sensory-nerve conduction. (**A**) Amplitude; (**B**) Latency; (**C**) Nerve conduction velocity. Data are for these sensory nerves: ulnar, radial superficial, sural, and superficial peroneal nerves. (**D**–**F**): Motor-nerve conduction. (**D**) Amplitude; (**E**) Latency; (**F**) Nerve conduction velocity. Data for ulnar, peroneal, and tibial motor nerves are shown. MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

**Table 3.** Comparison of QST parameters and autonomic testing in controls and cirrhotic patients.

| QST Parameters | | Controls | NMHE Patients $p$ vs Control | MHE Patients $p$ vs Control | MHE Patients $p$ vs. NMHE | ANOVA Global $p$ Values |
|---|---|---|---|---|---|---|
| Vibration detection (JND) | hand | 7 ± 0.5 | 9 ± 0.5 ** | 10 ± 0.5 ** | ns | <0.0001 |
| | foot | 13 ± 0.6 | 17 ± 0.4 **** | 17 ± 0.6 **** | ns | <0.0001 |
| Cooling detection (JND) | hand | 7 ± 0.3 | 10 ± 0.5 **** | 11 ± 0.8 **** | ns | <0.0001 |
| | foot | 8 ± 0.4 | 13 ± 0.7 **** | 16 ± 1 **** | 0.04 | <0.0001 |
| Heat pain 0.5 (JND) | hand | 16 ± 0.5 | 18 ± 0.5 ** | 19 ± 0.7 ** | ns | 0.0006 |
| | foot | 18 ± 0.4 | 19 ± 0.3 ** | 21 ± 0.5 **** | 0.01 | <0.0001 |
| Heat pain 5.0 (JND) | hand | 20 ± 0.5 | 22 ± 0.4 ** | 22 ± 0.7 * | ns | 0.004 |
| | foot | 21 ± 0.3 | 22 ± 0.2 | 23 ± 0.6 ** | 0.04 | 0.005 |
| Vibration detection time (s) | hand | 127 ± 1 | 132 ± 2 * | 138 ± 3 ** | ns | 0.004 |
| | foot † | 127 ± 2 | 132 ± 2 | 141 ± 4 *** | 0.04 | 0.0007 |
| Cooling detection time (s) | hand † | 141 ± 1 | 154 ± 5 | 155 ± 3 ** | ns | 0.004 |
| | foot † | 144 ± 2 | 176 ± 8 ** | 213 ± 15 **** | 0.01 | <0.0001 |
| Heat pain time (s) | hand | 115 ± 8 | 182 ± 11 *** | 189 ± 18 *** | ns | 0.0001 |
| | foot | 125 ± 8 | 149 ± 9 | 187 ± 14 *** | 0.01 | 0.0009 |
| **Autonomic testing** | | | | | | |
| R-R Interval variation (%) | Basal † | 3.2 ± 0.7 | 3.1 ± 0.5 | 2.0 ± 0.2 | ns | 0.264 |
| | Hyperventilation | 11.4 ± 2.0 | 5.9 ± 0.8 * | 7.1 ± 1.8 | ns | 0.021 |
| | Valsalva | 15.6 ± 2.1 | 9.3 ± 1.3 | 9.9 ± 2.0 | ns | 0.389 |
| | Orthostatic test | 8.2 ± 2.5 | 5.5 ± 1.0 | 3.5 ± 0.7 | ns | 0.130 |
| Sympathetic skin response | Amplitude | 4.1 ± 0.6 | 2.6 ± 0.3 | 1.5 ± 0.3 ** | 0.047 | <0.001 |
| | Latency | 1.40 ± 0.04 | 1.57 ± 0.04 ** | 1.59 ± 0.06 * | ns | 0.015 |

Values are expressed as mean ± SEM. Between-group differences were analyzed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test, with the exception of non-parametric variables, (†) in which Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. Differences compared to control group are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. QST: quantitative sensory test; MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively; s: seconds, JND: just noticeable differences.

Figure 1 shows the results of the nerve conduction study. Nerve sensory amplitudes were reduced in cirrhotic patients in all nerves studied, and latencies in ulnar and radial nerves were increased compared to controls (Figure 1A,B). Sural nerve latency was increased in MHE compared to NMHE patients. Nerve conduction velocity was reduced in ulnar and radial sensory nerves in both patient groups (Figure 1C). Nerve motor amplitude was reduced in the peroneal nerve in MHE patients (Figure 1D), and latencies were increased in ulnar and peroneal nerves in cirrhotic patients (Figure 1E). Conduction velocity in these motor nerves was reduced in both patient groups compared to controls, being significantly lower in MHE than NMHE patients (Figure 1F).

### *3.3. Quantitative Sensory Testing (QST)*

QST total results for each group are displayed in Table 3. We observed higher thresholds and longer detection times in NMHE patients compared to controls in all modalities, except for heat pain 5.0 thresholds in the foot, and detection times for vibration and cooling in the hand, and heat pain in the foot (Table 3). Patients with MHE showed higher thresholds than NMHE patients in cooling detection ($p = 0.04$), heat pain 0.5 ($p = 0.01$) and heat pain 5.0 ($p = 0.04$) when the foot was the test site (Table 3). The proportion of patients in which over half the tests were outside the normal range was higher ($p = 0.007$) in MHE (67%) than in NMHE patients (26%).

Control subjects finished each test in around 120 s, the NMHE group taking slightly longer (125–180 s) and the MHE group longer still (130–220 s). Significant differences

between patients were found in vibration detection time ($p = 0.04$), cooling detection time ($p = 0.01$) and heat pain time ($p = 0.01$) when the foot was the test site (Table 3).

Regarding autonomic function, there was a general decrease in all parameters of heart rate variability (R-R interval), being significantly reduced in NMHE patients compared to controls in the hyperventilation test ($p < 0.05$). The amplitude of SSR was significantly reduced in patients with MHE compared to controls ($p < 0.01$) and NMHE patients ($p < 0.05$) (Table 3).

To test the influence of sex on QST parameters, we performed an analysis of QST parameters comparing results from by sex in the control group. Although there was a higher proportion of women in the control group than in the patient groups (Table 1), we did not find any significant differences between males and females in the variables analyzed (Supplementary Table S1).

### *3.4. Comparisons between Patients with and without MHE with Normal Sural Nerve Amplitude*

We selected sural nerve amplitude as a representative value of the nerve conduction study, as it is the most distal nerve studied in lower limbs, and one of the first nerves to be affected in large fiber polyneuropathy. Sural nerve amplitude values were considered normal or pathologic according to reference values from our laboratory (cut-off: 15 μV). Sural nerve amplitude was affected in 42% of NMHE patients and 40% of patients with MHE, indicating an impairment of the large sensory fiber. Supplementary Table S2 shows the results of nerve conduction studies in controls and patients with normal sural nerve. Patients with MHE presented longer latencies than controls in ulnar and radial sensory nerves. Ulnar motor nerve latency was increased in both NMHE and MHE patients compared to controls, whereas the conduction velocities of ulnar and peroneal motor nerves were decreased in both groups of patients compared to controls, being significantly lower in MHE than in NMHE patients in ulnar motor nerve.

Looking for early markers of MHE based on neurophysiological parameters, we checked for signs of small caliber fiber alterations in patients without affected sural nerve amplitude, by assessing sympathetic skin response, RR interval variation, and QST (Table 4, Figure 2).

A greater affectation was found in patients with MHE compared to NMHE in the modality of conduction heat pain detection in the foot, with a trend towards significance ($p = 0.06$) in the sensitive sensory threshold value (0.5 JND) and a significant increase in the maximum value reached during the heat pain test (5.0 JND) ($p = 0.04$). Patients with MHE had higher thresholds in thermal detection in cooling when the foot was the test site ($p = 0.007$), and also took a longer time to detect temperature changes-foot cooling detection time-($p = 0.007$) compared with NMHE patients (Table 4; Figure 2). Moreover, 54% of MHE patients had abnormal scores in more than half of the QST tests performed, while for NMHE patients, the percentage was 28%.

There were no statistically significant differences between NMHE and MHE patients in vibration detection, but both cirrhotic patient groups had higher thresholds than controls in the two test sites (foot and hand). The same result was found for cooling detection when the hand was the test site (Table 4; Figure 2).

When analyzing autonomous functions among patients with normal sural nerve amplitude (without large fiber impairment), we found greater alterations in MHE than in NMHE patients, reflected in a decrease in heart rate variability (RR interval) (small fiber involvement) both in the baseline study at rest ($p = 0.007$) and in the orthostatic test or passive tilt test ($p = 0.04$) (Table 4).

Sympathetic skin response amplitude was abnormal in 36% of MHE patients and 11% of NMHE patients. Patients with MHE showed lower response potential in amplitude than NMHE patients ($p = 0.03$) (Table 4, Figure 2). Both NMHE and MHE patients showed lower amplitudes ($p < 0.05$, and $p < 0.0001$, respectively) and longer latencies ($p < 0.05$) than controls (Table 4; Figure 2).

We also tested the possible contribution of alcoholic etiology to the effects observed. We grouped the patients with alcoholic etiology together with those with mixed etiologies such as NASH + alcohol and virus + alcohol, to determine if there were differences between past alcohol abuse and other etiologies. There were no differences between patients with alcoholic-induced cirrhosis and those of other etiologies in the parameters analyzed in quantitative sensory system and autonomic studies (Supplementary Table S3).

Regarding the relationship between neuropathy and liver disease severity, no patients in this study were in Child Pugh C grade; indeed, 74% were in Child-Pugh A grade and 26% in B. We found no significant differences in QST parameters and autonomic testing when grouping according to liver disease severity (Supplementary Table S4).

Given that diabetes was another possible confounding factor, we grouped patients with normal sural nerve amplitude by presence or absence of this condition. Analysis of QST and autonomic variables showed no significant differences between patients with and without diabetes (Supplementary Table S5).

**Table 4.** Comparison of QST parameters and autonomic testing in patients with normal sural nerve amplitude.

| *QST* Parameters | | Controls | NMHE Patients *p* vs. Control (*n* = 18) | MHE Patients *p* vs. Control (*n* = 11) | MHE Patients *p* vs. NMHE | ANOVA Global *p* Values |
|---|---|---|---|---|---|---|
| Vibration detection (JND) | hand | 7 ± 0.5 | 9.5 ± 0.7 ** | 9.3 ± 0.7 * | ns | 0.0008 |
| | foot | 13 ± 0.6 | 16 ± 0.5 * | 17 ± 0.8 ** | ns | 0.002 |
| Cooling detection (JND) | hand | 7 ± 0.3 | 9.4 ± 0.6 *** | 11 ± 1 **** | ns | <0.001 |
| | foot | 8 ± 0.4 | 11 ± 1 *** | 15 ± 1.4 **** | 0.004 | <0.001 |
| Heat pain 0.5 (JND) | hand | 16 ± 0.5 | 17.5 ± 0.8 | 19 ± 1 * | ns | 0.04 |
| | foot | 18 ± 0.4 | 19 ± 0.5 | 20 ± 0.5 ** | 0.06 | 0.01 |
| Heat pain 5.0 (JND) | hand | 20 ± 0.5 | 22 ± 0.6 * | 22 ± 0.4 | ns | 0.013 |
| | foot | 21 ± 0.3 | 21 ± 0.2 | 22 ± 0.3 | 0.04 | 0.04 |
| Vibration detection time (s) | hand | 127 ± 1 | 132 ± 3 | 135 ± 3 * | ns | 0.04 |
| | foot † | 127 ± 2 | 134 ± 3 * | 137 ± 2 ** | ns | 0.001 |
| Cooling detection time (s) | hand † | 141 ± 1 | 149 ± 7 | 156 ± 5 * | ns | 0.014 |
| | foot † | 144 ± 2 | 156 ± 6 | 205 ± 22 **** | 0.007 | <0.001 |
| Heat pain time (s) | hand | 115 ± 8 | 166 ± 16 ** | 167 ± 20 * | ns | 0.004 |
| | foot | 125 ± 8 | 153 ± 13 | 160 ± 16 | ns | 0.06 |
| **Autonomic testing** | | | | | | |
| RR Interval variation (%) | Basal † | 3.7 ± 0.7 | 4.3 ± 0.9 | 1.8 ± 0.1 * | 0.007 | 0.008 |
| | Hyperventilation | 9.5 ± 1.4 | 6.7 ± 1.3 | 6.8 ± 2 | ns | 0.15 |
| | Valsalva | 12 ± 2 | 11 ± 2 | 10 ± 3 | ns | 0.95 |
| | Orthostatic test | 5 ± 1 | 6.9 ± 1.4 | 2.6 ± 0.4 | 0.04 | 0.05 |
| Sympathetic skin response | Amplitude | 4.6 ± 0.5 | 3.1 ± 0.4 * | 1.4 ± 0.2 **** | 0.03 | 0.0001 |
| | Latency | 1.4 ± 0.02 | 1.5 ± 0.03 * | 1.5 ± 0.06 * | ns | 0.04 |

Values are expressed as mean ± SEM. Between-group differences were analyzed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test, with the exception of non-parametric variables, (†) in which Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. Differences compared to control group are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. QST: quantitative sensory test; MHE, NMHE: patients without and with minimal hepatic encephalopathy, respectively; s: seconds, JND: just noticeable differences.

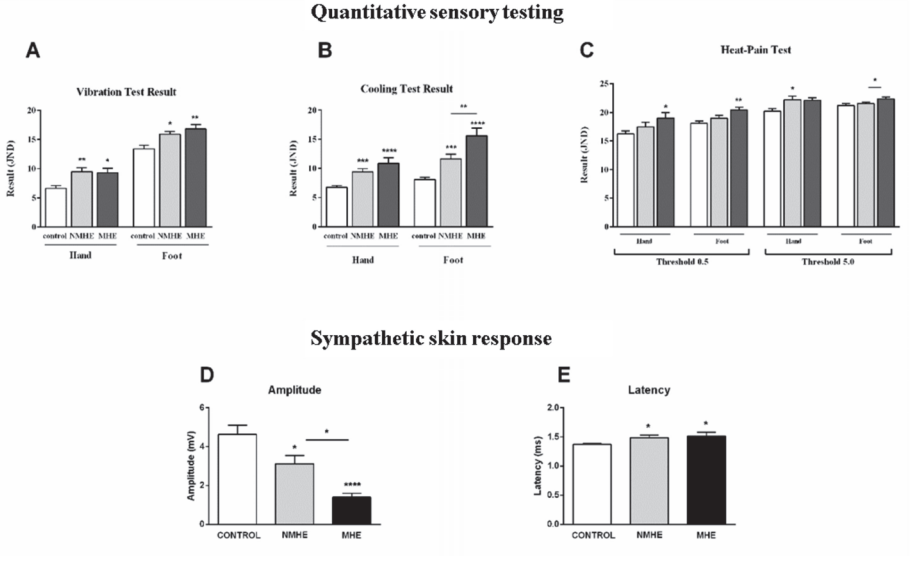


**Figure 2.** MHE patients with normal sural nerve amplitude show alterations in thermal sensitivity and sympathetic skin response compared to NMHE patients and controls. (**A**–**C**) Quantitative sensory test (QST) parameters: (**A**) Vibration detection threshold, (**B**) Cooling detection threshold, and (**C**) Heat Pain detection threshold, at 0.5 and 5.0 thresholds. (**D**–**E**) Sympathetic skin response: (**D**) Amplitude, (**E**) Latency. JND: just noticeable differences; MHE, NMHE: patients with and without minimal hepatic encephalopathy, respectively. Results are the mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

### *3.5. Correlations between QST Parameters, Autonomic System, and Psychometric Tests*

We analyzed whether changes in QST parameters correlated with performance in psychometric tests by assessing different cognitive abilities (Table 5), finding vibration detection time in foot and hand, cold detection time in hand, and heat pain detection 0.5 in foot to be correlated with the PHES score.

Neutral and incongruent tasks from the Stroop test assessing cognitive flexibility and selective attention showed good correlations with QST parameters, especially with cooling detection threshold (hand: $r = -0.473$, $p < 0.001$; foot: $r = -0.370$, $p = 0.002$, vs. incongruent task).

Performance in d2 test, assessing selective and sustained attention and concentration was associated with time duration in vibration (hand), cooling (foot), and heat pain (foot) tests. Significant correlations were also found between performance in d2 test and thresholds in vibration (foot) and cooling in both hand and foot (Table 5).

Mental processing speed, measured by Oral SDMT, correlated with time duration in all QST tests, except for vibration detection time in hand and heat pain detection time in foot. This test also correlated with cooling detection threshold, in both hand and foot (Table 5).

Working memory, measured by Digit Span and Letter-Number Sequencing tests, was associated with cold detection time in foot and heat pain detection time in hand. Vibration and cooling thresholds in both hand and foot, and heat pain detection 5.0 in hand also correlated with working memory tests.

Motor coordination tests showed weak correlations with QST parameters.

There were significant correlations between sympathetic skin response amplitude and PHES score ($r = 0.43$; $p = 0.02$), and Stroop congruent and neutral tasks ($r = 0.441$; $p = 0.027$, and $r = 0.51$; $p = 0.01$, respectively).

Sympathetic skin response amplitude correlated with vibration threshold in foot ($r = -0.361$; $p = 0.018$) and cooling threshold in hand ($r = -0.380$; $p = 0.013$), and also with heat pain detection 0.5 in foot ($r = -0.317$; $p = 0.049$).

**Table 5.** Correlations of QST parameters with psychometric tests.

| *QST* Parameters | | PHES Score | Stroop Test | d2 Test | | Oral SDMT | Digit Span | Letter-Number Sequencing | Coordination Tests |
|---|---|---|---|---|---|---|---|---|---|
| | | | | d2-TOT | d2-CON | | | | |
| Vibration detection (JND) | hand | | | | | | −0.23 (0.06) | −0.40 (0.002) | |
| | foot | | −0.31 (0.01) | −0.27 (0.03) | −0.26 (0.04) | −0.23 (0.06) | −0.39 (0.001) | −0.29 (0.02) | 0.23 (0.07) § |
| Cooling detection (JND) | hand | −0.33 (0.004) | −0.47 (<0.001) | | −0.31 (0.01) | −0.36 (0.002) | −0.25 (0.04) | −0.38 (0.002) | 0.23 (0.05) |
| | foot | | −0.37 (0.002) | −0.25 (0.04) | −0.28 (0.02) | −0.36 (0.002) | −0.29 (0.02) | −0.30 (0.02) | |
| Heat pain 0.5 (JND) | hand | | −0.21 (0.08) | −0.25 (0.04) ‡ | | −0.27 (0.03) | | | |
| | foot | −0.34 (0.005) | −0.22 (0.07) | | | | | | 0.29 (0.02) § |
| Heat pain 5.0 (JND) | hand | | | | | −0.24 (0.06) | −0.32 (0.01) | −0.28 (0.03) | |
| | foot | | | | | | | | |
| Vibration detection time (s) cmidrule2-10 | hand | −0.32 (0.008) | −0.30 (0.02) | −0.34 (0.006) | −0.34 (0.009) | | | −0.25 (0.06) | 0.24 (0.06) § |
| | foot | −0.35 (0.003) | −0.24 (0.05) | | | −0.25 (0.06) | | | 0.24 (0.06) § |
| Cooling detection time (s) | hand | −0.22 (0.06) | −0.26 (0.03) | −0.26 (0.04) ‡ | | −0.38 (0.001) | | | 0.25 (0.04) |
| | foot | −0.24 (0.04) | −0.29 (0.01) | −0.31 (0.01) | −0.32 (0.009) | −0.34 (0.004) | −0.41 (0.001) | −0.40 (0.001) | |
| Heat pain time (s) | hand | | −0.25 (0.04) † | | −0.24 (0.05) | −0.33 (0.006) | −0.34 (0.006) | −0.30 (0.02) | |
| | foot | | | −0.29 (0.02) | −0.29 (0.02) | | | | |

The $r$ values ($p$-values) of the Pearson correlation analysis are shown. Significant and near-significant correlations are shown. Values for Stroop test are for Incongruent task, except for (†), which are for Neutral task. ‡ Values for d2-TR, Total number of characters processed; § values for visual-motor coordination test; values for bimanual coordination test. QST: quantitative sensory test; JND: just noticeable differences; s: seconds; PHES: Psychometric Hepatic Encephalopathy Score; d2 test: TOT: Total correctly processed; CON: Concentration performance; Oral SDMT: Symbol digit modalities test (oral version).

### *3.6. Predictive Capacity of QST Parameters for MHE*

We performed a ROC analysis, and found that cooling detection in the foot had a significant predictive capacity for detecting MHE, with the following area under the ROC curve (AUC): Cooling detection in foot (JND): AUC: 0.739; $p = 0.017$; 95% CI (0.543–0.936) and for Cooling detection time in foot (s): AUC: 0.820; $p < 0.001$; 95% CI (0.543–0.936).

## 4. Discussion

The main findings of this study are: (a) Hyposensitivity is present in cirrhotic patients but is higher in patients with MHE; (b) MHE patients have impaired thermal sensitivity

in the foot, both in cooling and heat pain detection; (c) This impairment correlates with worse performance in attention, mental processing speed, and working memory tests; (d) Thermal sensitivity and autonomic function, involving small caliber nerve fibers, are early alterations associated with MHE, which appear before sural nerve amplitude (large nerve fiber) is altered.

To our knowledge, the present results provide first time evidence of impaired detection of cold and temperature changes in MHE patients compared to those without MHE. Although lower sensitivity to vibration and thermal stimuli was observed in the two patient groups compared to the control group in both hand and in foot, MHE patients had greater hyposensitivity when the foot was the test site.

The different results found in lower limbs and upper limbs is explained because toxic-metabolic polyneuropathies such as those derived from liver diseases, are characterized by length-dependent involvement, therefore the lower distal limbs are the first to be affected. Alterations reported in hepatic encephalopathy are included within the chronic axonal polyneuropathies of toxic-metabolic origin, characterized by length-dependent dying-back degeneration of axons, initially affecting the sensory fibers and with a pattern of distal predominance. In nerve conduction studies, the main finding is a decrease of the SNAP (sensory nerve action potential) and CMAP (compound muscle motor action potential) amplitudes, affecting the lower extremities more, with normal conduction velocity and distal latency, although conduction velocity can be reduced to 70–80% of the reference values if there is a decrease in CMAP amplitude greater than 50%, associated to a selective loss of fast-conducting fibers [37].

Our results are in contrast with a previous study from Brenner et al. [24] where the hand was used as the test site, the result of which found impaired thermal perception in grade 2 HE patients, but no differences in MHE patients. They concluded that these alterations emerge mainly in advanced stages of the disease. Like Brenner et al., we also found no differences between the two patient groups when the test was performed on the hands. However, by testing the foot we found greater hyposensitivity in MHE patients, suggesting that in these patients the distal sensory fibers become impaired earlier than the proximal ones found in the hands, which don't deteriorate until more advanced grades of HE [24].

The differences found between hand and foot test sites indicate that the foot is more sensitive in distinguishing between cirrhotic patients with or without MHE, while the hand seems to have more sensitivity to detect cirrhotic patients who have not yet developed MHE.

Peripheral neuropathy is a frequent complication of liver cirrhosis [19,21,22], potentially affecting more than 70% of cirrhotic patients; in our study, however, only 38% of cirrhotic patients presented peripheral neuropathy. This lower incidence could be due to differing cirrhotic patient profiles, given that previous studies found this high incidence in patients with end-stage liver disease awaiting liver transplantation [19], whereas patients in the present study all had compensated cirrhosis. Moreover, neuropathy grade has been linked to liver disease severity, with a higher neuropathy score in Child-Pugh class C patients than those in A and B [19]; in this study, nonetheless, no patients were in Child Pugh C grade, in fact most were in Child-Pugh A (74%), so we can reasonably rule out liver disease severity as underpinning the effects observed in this study.

Patients with liver disease are at high risk of developing other metabolic syndromes such as diabetes, in which small fiber neuropathy is often concomitant. In this study we show that most alterations observed in small fibers were not influenced by diabetes.

The studies of the autonomic nervous system (RR interval, sympathetic skin response) and of thermal sensitivity are neurophysiological studies useful for evaluating function of small fibers.

The term small fiber neuropathy refers to a group of neuropathies characterized by a selective or predominant disorder of the poorly myelinated peripheral A delta afferent fibers and the unmyelinated C fibers [38]. In the somatosensory nervous system, these fibers

transmit information on temperature, pain, and itching, and in the neurovegetative nervous system they are involved in sudomotor, thermoregulatory, cardiovascular, gastrointestinal, urogenital, and other functions [39].

In this study we show that MHE is more associated with alterations in small fiber function (cooling and heat pain thresholds) rather than large fiber function (vibration threshold).

Patients with MHE with normal sural conduction do not present alterations in large nerve fibers; however, in our study, we found that they present alterations in thermal sensitivity and autonomic function, which imply alterations in small nerve fibers, which are involved in these functions. This would indicate that in MHE patients, alterations in thermal perception and autonomic nervous system precede alterations in large fibers, given the impairment in QST parameters and RR interval variation found in MHE patients compared to NMHE patients.

The autonomic or vegetative nervous system is part of the central and peripheral nervous system involved in regulating involuntary functions of the organism, maintaining internal homeostasis and adapting responses to variations in the external and internal environment. The sudomotor function is part of the thermoregulation system, a complex homeostatic system integrated in the hypothalamus. By studying this system we can assess possible abnormalities in autonomic nervous system function [30]. Within the patient group with normal sural nerve amplitude (no distal large fiber impairment) sympathetic skin response amplitude was altered in 36% of MHE patients compared to 11% in NMHE patients. MHE patients also presented more changes in heart rate variability (RR interval) (small fiber involvement) in both the baseline study at rest, and the orthostatic test or passive tilt test. These data indicate involvement of both the sympathetic and parasympathetic nervous systems, although with greater influence on the latter, in MHE.

Taken together, these results suggest that the small fibers of the autonomic nervous system (sympathetic skin response, RR interval, and thermal sensitivity) are altered in early stages of MHE while changes to the large sensory fibers (sural nerve) take place later on.

The correlations found indicate that deficits in attention, mental processing speed, and working memory are associated with an impaired QST response. Although the PHES was defined as the gold standard to detect MHE [12,13], it was later shown that it fails to detect certain mild cognitive alterations and classifies patients with these alterations as NMHE [14–17]. The fact that NMHE patients also presented significant impairments in QST parameters compared to controls could be associated with presence of early attention impairment undetected by PHES [14,15].

It is difficult to ascertain whether impairment is central or peripheral: both components are likely to be altered. The correlation with the Stroop test suggests that the attention needed to synchronize activity between high- and low-order areas of the parietal cortex to enhance relevant sensory signals may be deficient. This points to central impairment, in agreement with previous studies in which patients with overt HE showed alterations in thermal sensitivity strongly correlated with central impairment [25]. The significant correlations found between time detection in all QST modalities and attention tests performed could also be pointing not to a deficit in sensing (peripheral impairment), but to a deficit in reporting due to attention dysfunction (central impairment). Therefore, these results would indicate that the delayed response in thermal and vibration detection could be due to alterations in mental processing speed and attention.

The correlation of SSR amplitude with cooling and thermal thresholds might well reflect a problem with awareness level regulation due to presence of MHE.

MHE patients experience psychomotor slowing [17] and longer reaction times, which could contribute to the longer times needed by MHE than NMHE patients to perform the QST tests, mainly when the foot was the test site.

Cognitive impairment and autonomic dysfunction may share a common underlying pathologic mechanism. Impaired autonomic function is present in patients with mild cognitive impairment or several different types of dementia: Alzheimer's disease, frontotemporal

dementia, dementia with Lewy bodies, and Parkinson's disease with dementia [40]. Mild cognitive impairment has been associated with orthostatic blood pressure dysregulation. Nicolini et al., [41] suggested that the underlying physiopathological mechanism could be the disruption of central autonomic control due to damage of the right insula or locus coeruleus damage. The insular cortex is involved in thermosensory function and pain [42,43]. In a previous study by magnetic resonance we found that MHE patients showed gray matter reduction in the right insula, which correlated with PHES score, attention tests, and inflammation [44]. This alteration could contribute to the thermosensory and autonomic impairments associated with MHE.

Quantitative sensory testing of cooling detection in the foot (both JND and detection time) has a significant predictive capacity for detecting MHE, as indicated by the obtained ROC curves. Although we cannot discern whether the results of cooling detection time were due to a peripheral deficit in "sensing" or to a central deficit in "reporting" due to the attention dysfunction, we consider this parameter to be of value in clinical practice and a useful element to consider in the diagnosis of MHE, because although it does not pin point the origin of the pathophysiology, it does seem to predict the presence of MHE. QST testing also gives us a more comprehensive way of evaluating MHE, allowing an easy, low cost, and non-invasive way to check on a patient's evolution and incrementing opportunities to alter prognosis early on.

A limitation of this study was that there are no 'pure' cirrhotic patients, because they usually present other comorbidities, such as diabetes mellitus, arterial hypertension, kidney complications . . . MHE patients have central alterations, peripheral neuropathy (a frequent complication of liver cirrhosis) and a high probability of coinciding with other metabolic syndromes such as diabetes, which in turn, frequently present small fiber neuropathy. Consequently, it is difficult to differentiate whether the polyneuropathy present in cirrhosis is secondary to diabetes mellitus or cirrhosis. In this study, there were no significant differences between those with or without diabetes in early alterations of thermal sensitivity found in MHE patients, which could indicate a main role of MHE in these alterations.

Some medications, such as lactulose, could change the presence of MHE. As shown in Table 1, 3 NMHE patients (8% of total NMHE) and 4 MHE patients (20% of total MHE) were taking lactulose. Although it was reported in the literature that lactulose may improve MHE [45,46], this does not seem to be the case in our study, as four patients taking lactulose presented MHE.

Regarding the alterations in the autonomic function, it was also difficult to differentiate the contribution of liver cirrhosis from the use of medication for arterial hypertension, like beta-blockers. However, the differences in autonomic function observed between NMHE and MHE patients would not be due to beta-blockers, given that the proportion of MHE patients having this medication were lower (5/20; 25%) than NMHE patients (15/38; 39%)

## 5. Conclusions

In summary, patients with MHE experience a general decrease in cognitive and sensory abilities. The small fibers of the autonomic nervous system and cooling sensitivity are affected at early stages of MHE, predominantly distal and in the lower limbs, before the large sensory fibers become altered. We found early alteration in the sympathetic skin response in MHE patients, with lower prevalence in patients without MHE. This could be considered an early marker of pathophysiology, and could be useful for early detection of patients susceptible to developing MHE. Cirrhotic patients are much more likely to present a variety of comorbid syndromes such as diabetes and polyneuropathy, which worsen further prognosis. QST testing would help to detect these issues early on, as well as the presence of MHE, a point at which prognosis can still be altered. This early detection would allow patients to receive treatment and to improve their quality of life. Quantitative sensory tests could be a practical and functional clinical tool to use as a complementary test to detect MHE.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2077-0383/10/2/239/s1, Supplementary methods: Quantitative Sensory Testing (QST); Neurophysiological studies of large fibers: nerve conduction study. Supplementary References. Figure S1. Quantitative Sensory Testing components; Figure S2. One—Time—Period 4, 2, 1 Stepping Algorithm; Table S1. QST and autonomic testing parameters comparing males and females in the control group. Table S2. Parameters of sensory and motor nerve conduction in controls and patients with normal sural nerve Table S3. Comparison of QST parameters and autonomic testing between patients with alcoholic etiology and with other etiologies, in the group of patients with normal sural nerve; Table S4. Contribution of liver disease severity to results observed in patients with normal sural nerve; Table S5. Comparison of QST parameters and autonomic testing in patients with normal sural nerve amplitude with and without diabetes.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

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## Abbreviations

| | |
|---|---|
| HE | hepatic encephalopathy |
| MHE | minimal hepatic encephalopathy |
| PHES | psychometric hepatic encephalopathy score |
| SDMT | symbol digit modalities test |
| EMG | electromyography |
| QST | Quantitative Sensory Testing |
| SSR | sympathetic skin response |
| VDT | vibration detection test |
| CDT | cold detection test |
| HPDT | heat pain detection test |
| JND | just noticeable differences |
| NMHE | patients without MHE |

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# A Look into Liver Mitochondrial Dysfunction as a Hallmark in Progression of Brain Energy Crisis and Development of Neurologic Symptoms in Hepatic Encephalopathy

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**Abstract:** Background: The relationship between liver disease and neuropathology in hepatic encephalopathy is well known, but the genesis of encephalopathy in liver failure is yet to be elucidated. Conceptually, the main cause of hepatic encephalopathy is the accumulation of brain ammonia due to impaired liver detoxification function or occurrence of portosystemic shunt. Yet, as well as taking up toxic ammonia, the liver also produces vital metabolites that ensure normal cerebral function. Given this, for insight into how perturbations in the metabolic capacity of the liver may be related to brain pathology, it is crucial to understand the extent of ammonia-related changes in the hepatic metabolism that provides respiratory fuel for the brain, a deficiency of which can give rise to encephalopathy. Methods: Hepatic encephalopathy was induced in starved rats by injection of ammonium acetate. Ammonia-induced toxicity was evaluated by plasma and freeze-clamped liver and brain energy metabolites, and mitochondrial, cytoplasmic, and microsomal gluconeogenic enzymes, including mitochondrial ketogenic enzymes. Parameters of oxidative phosphorylation were recorded polarographically with a Clark-type electrode, while other measures were determined with standard fluorometric enzymatic methods. Results: Progressive impairment of liver mitochondrial respiration in the initial stage of ammonia-induced hepatotoxicity and the subsequent energy crisis due to decreased ATP synthesis lead to cessation of gluconeogenesis and ketogenesis. Reduction in glucose and ketone body supply to the brain is a terminal event in liver toxicity, preceding the development of coma. Conclusions: Our study provides a framework to further explore the relationship between hepatic dysfunction and progression of brain energy crisis in hepatic encephalopathy.

**Keywords:** hyperammonemia; liver; mitochondria; ketogenesis; gluconeogenesis; brain energy crisis

## 1. Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric disorder developing in patients with severe liver disease. Although the relationship between liver damage and neuropathology has been known for over a century [1,2], the genesis of encephalopathy as a result of impaired liver function is not yet completely clear.

Conceptually, the main cause of HE is the accumulation of ammonia in the blood (hyperammonemia) due to impaired detoxification function of the liver or by portosystemic shunt, allowing gut-derived neurotoxins that bypass the liver to reach the systemic circulation, thereby gaining access to the brain [3].

Other factors such as bacteremia, fungal infection [4], alkalosis [5], products of abnormal amino acid metabolism [6], and various toxic substances [7] may participate in disease development, but elevated blood ammonia entering the brain unhindered is believed to be the main toxic causative agent responsible for altered cerebral functions and clinical manifestations of HE [3,6].

The molecular mechanisms of encephalopathy in the setting of hyperammonemia have not yet been fully elucidated and there is no specific medication for treating this abnormality, despite considerable knowledge of the syndrome.

It has been established that ammonia-related neurotoxicity is characterized by brain energy failure [8], disruption of mitochondrial oxidative phosphorylation [9] and calcium homeostasis [10], an imbalance among multiple neurotransmitter systems [11,12], disturbances of antioxidant defense systems [13,14], systemic neuroinflammation [15], and abnormalities in the pattern of cerebral blood flow [16]. Therefore, HE pathogenesis is viewed as a complex and multifactorial network of interdependent organ systems.

Thus, the impact on functional brain activity as a result of liver dysfunction may be wider than predicted based on increased blood ammonia levels due to the inability of the liver to perform its detoxification function. This hypothesis prompted us to investigate an alternative explanation for the effect of liver malfunction on brain pathology.

It is known that the liver not only takes up toxic ammonia, but also plays a unique role in blood glucose homeostasis [17]. Given that glucose is the main brain energy metabolite, despite the recognized importance of some non-glucose substrates [18], the liver is essential in homeostatic control of blood glucose levels. This process maintains adequate amounts of glucose for the brain and reflects its obligate dependence on the liver, indicating that under physiological conditions, the liver elaborates vital metabolites that assure normal cerebral function [19]. As a result, unsteady blood glucose and oxygen delivery to the brain for even a short period causes brain damage, while chronic deficiency in these substrates leads to irreversible brain injury, thereby provoking coma development and death [20].

Owing to the large stores of glycogen and glycogenolysis and gluconeogenic pathways, the liver produces free glucose from glycogen and gluconeogenic substrates, which is released into the circulation to serve as a fuel source for the organs and particularly, the brain [19]. If the blood contains insufficient amounts of glucose, only the liver [21] is able to produce ketone bodies, which are utilized as an additional energy source in the brain.

Nevertheless, both liver ketogenesis and gluconeogenesis are understudied areas (with the exception of a few studies) in terms of their impact on the adequate supply of the brain with energy substrates and clinical manifestations of HE [20]. Moreover, based on scattered and conflicting data from patients with fulminant liver failure or chronic liver diseases [22–24], it is impossible to assess how the reduction in ketone body and glucose output in the liver may be related to brain pathology. It should be noted that HE studies on animal models also show contradictory results. After portacaval shunt in rats, plasma levels of acetoacetate and β-hydroxybutyrate were reduced [25], or not significantly different from those in the sham-operated group [26]. In the animal model of hyperammonemia initiated by a long term diet containing ammonia, the level of ketone bodies in the blood rose significantly, hence, synthesis and export of ketone bodies by the liver in the hyperammonemic rats were supposed to increase [27].

In our studies performed in rat models of "pure" hyperammonemia produced by infusion of ammonium salts, we observed a significant decrease in the levels of both acetoacetate and β-hydroxybutyrate in the liver and blood of feeding animals, which suggested that hepatic ketogenesis can be inhibited immediately after increasing ammonia levels in the liver [28].

Given the importance of hepatic metabolic activity for providing fuel to the brain and an effective understanding of how perturbations in the metabolic capacity of liver cells may be related to brain pathology in HE, we therefore focused on hepatic gluconeogenesis and ketogenesis in animal models, investigating acute ammonia intoxication in which the concentration of ammonia in the blood quickly

increases to the level found in idiopathic hyperammonemia following lung transplantation [29] and many other human pathologies [30].

Since these metabolic processes are energy-dependent and closely related to mitochondrial function and fatty acid oxidation [31], we sought to study the effect of acute hyperammonemia on the hepatic mitochondrial function, including oxidative phosphorylation, fatty acid oxidative capacity, and how fluctuation of glucose and ketone body levels in the blood can affect brain bioenergetics and coma development. This research will advance understanding of the role of liver mitochondria in brain energy metabolism.

## 2. Experimental Section

### 2.1. Experimental Design

This study was conducted in accordance with the ethical principles formulated in the Helsinki Declaration for care and use of laboratory animals and the Regulations of the European Science Association (revised in the instruction 86/609/EC and formulated in the Order of the Ministry of Health of the Russian Federation of 19.06.2003 № 267 "Regulations in laboratory practices").

### 2.2. Animals

Groups of eight male Wistar rats weighing 200–220 g were used. The animals were housed in a vivarium, four per split-level cage (40 × 30 × 20 cm) at room temperature under a natural light regime. Well-fed animals were fed a standard diet. For hepatic gluconeogenic and ketogenic process activation during fasting, some animals were starved twenty-four hours before the beginning of the experiment.

The ammonia group was injected intraperitoneally with a lethal dose of ammonia acetate of 12 mmol/kg. As observed previously [32], the animals exhibited hyperventilation, clonic convulsions, and coma soon after ammonia injection and died in 15 ± 2 min. In this study, the rats were decapitated immediately after the first seizure, which usually occurred 10 min after ammonia injection. In some experiments, the decapitation procedure was carried out at 5 or 15 min after ammonia loading. To obtain the values for the zero time point, rats were injected with ammonium acetate and decapitated immediately.

Control group animals were decapitated at different time points after injection of saline or sodium acetate.

### 2.3. Preparative and Analytical Methods

Blood was drawn from the retro-orbital plexus into citrate-treated tubes. Plasma was deproteinized with 6% $HClO_4$ and 40% ethanol and neutralized with KOH to pH 6.

Ammonia was estimated by a microfluorimetric method as described by Kosenko et al., 2008 [33].

Glucose, ketone bodies, oxaloacetate, and adenine nucleotides were determined by fluorometric enzymatic methods, as described earlier [28].

BioVision fluorometric assay kits (Catalog # K646-100), based on glucoamylase that hydrolyzes glycogen to glucose, were used to determine glycogen levels in freeze-clamped liver and brain samples.

Glycerol concentration was measured by an assay kit (Sigma, CN FG0100) according to the manufacturer's instructions, using the procedure with a coupled enzyme assay.

Free fatty acids (FFA) were determined by the Free Fatty Acid Quantitation Kit (Sigma, CN MAK044) using a coupled enzyme assay according to the manufacturer's instructions.

### 2.4. Freeze-Clamped Liver and Brain Neocortex

After decapitation, tissue specimens from liver and cortex tissues were removed and freeze-clamped as soon as possible in liquid nitrogen, and then, ground in a mortar into a fine powder. Three milliliters of 6% cold (−20 °C) perchloric acid/40% ethanol mixture were added to 0.3 g

of tissue powder and were extracted for 5 min at −5 °C. Analytical techniques for preparation of liver and brain tissue extracts were similar to those used for blood plasma extraction.

### 2.5. Isolation of Liver Mitochondria Using a Self-Generated Percoll Gradient

Mitochondria were isolated from the remainder of the liver (after freeze clamping) by a combination of differential and self-generated Percoll gradient centrifugation, according to a protocol developed by Graham [34].

Mitochondrial protein was determined by the Lowry method (Lowry et al. 1951) with bovine serum albumin as standard [35].

### 2.6. Mitochondrial Purity Assessment

A quantitative estimate of mitochondrial fraction purity was made using marker enzymes: lactate dehydrogenase (contamination of cytosol), glucose-6-phosphatase (microsomal marker), urate oxidase (peroxisome marker), and 5′-nucleotidase (plasma membrane marker). The enzyme activities were determined by previously described methods [36].

β-Galactosidase, used for detection of lysosome admixture, was determined according to the method described by Graham (1993) [37].

Contamination of the mitochondrial samples with peroxisomes, microsomes, cytoplasm, plasma membranes, and lysosomes was 0.29 ± 0.075%, 0.43 ± 0.07%, 0.15 ± 0.04%, 0.25 ± 0.09%, and 0.34 ± 0.01%, respectively.

The yield of succinate dehydrogenase was more than 90% of total activity (390 ± 12.3 and 19.1 ± 2.1 nmol/min x mg protein in mitochondria and homogenate, respectively) consistent with Graham's findings [38].

Together with a relatively low contamination of the mitochondria with other cellular organelles, the results indicate a high-purity mitochondrial fraction.

### 2.7. Study of Mitochondrial Respiration

Parameters of oxidative phosphorylation were recorded polarographically with a Clark-type electrode using the analytical techniques described earlier [36].

### 2.8. Determination of Pyruvate Carboxylase Activity (EC 6.4.1.1)

Liver mitochondria were disrupted by osmotic shock in 2 volumes of 5 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EGTA-$K^+$ and 0.1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.15 μM aprotinin, 1.5 μM, pepstatin, 10 μM leupeptin and by three subsequent cycles of freezing–thawing. After the final thawing step, the sample was centrifuged at 20,000g for 10 min at +4 °C, and the resulting supernatant was used to determine enzyme activity by oxidation of NADH at 340 nm using malate dehydrogenase (MDH) as a coupling enzyme [39].

### 2.9. Determination of Phosphoenolpyruvate Carboxykinase Activity (EC 4.1.1.32)

Liver phosphoenolpyruvate carboxykinase (PEPCK) activity was determined in a cytosolic fraction obtained from the postmitochondrial supernatant after centrifugation at 100,000× g for 30 min at +4 °C. An assay was performed by coupling the PEPCK with MDH and by NADH oxidation at 340 nm [40].

### 2.10. Microsome Isolation and Glucose-6-Phosphatase Activity (EC 3.1.3.9)

The microsomal fraction was obtained with a standard procedure of differential centrifugation without further purification. To remove the lysosome, the postmitochondrial supernatant was centrifuged at 20,000× g for 20 min at 4 °C and the resulting supernatant was centrifuged at 100,000× g for 30 min at 4 °C. The microsomal pellet was washed once with 20 mL of incubation medium. The final pellet was resuspended in the medium, containing 0.225 M sucrose, 10 mM HEPES, pH 7.4

(SH solution), 0.1 mM DTT, 0.1 mM PMSF, 0.15 μM aprotinin, 1.5 μM, pepstatin, and 10 μM leupeptin at a concentration of 10 mg/mL, and was used immediately to determine microsomal intactness by estimating the latency of "low Km" mannose-6-phosphatase activities in untreated microsomes [41]. For the glucose-6-phosphatase (G-6-Pase) assay, microsomes (50 μg of protein) were disrupted in SH solution containing 0.1% Triton X-100 at 0° for 20 min under constant gentle stirring. Enzyme activity was measured by following inorganic phosphate liberated at 700 nm using the method described by Baginski, 1974 [42].

### 2.11. Activity of Enzymes of Ketone Body Synthesis in the Liver

For the enzymes of the 3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) pathway, the liver mitochondria were disrupted, and enzymes were solubilized using a technique similar to those used for pyruvate carboxylase determination.

### 2.12. Acetoacetyl-CoA Thiolase (EC 2.3.1.9)

The enzyme was determined using the method described by Williamson, 1968 [43] with minor modifications. The standard reaction mixture contained 50 mM Tris-HCl (pH 8.1), 5 mM $MgCl_2$, 60 μM CoA, 10 μM acetoacetyl-CoA, 0.5 mM DTT, and 40 mM KCl. After pre-equilibration at 30 °C, the reaction was initiated by adding 20–40 μg of the sample in a total volume of 0.5 mL and the change in absorption at 313 nm was monitored for 10 min.

### 2.13. HMG-CoA Synthase (E.C.4. l.3.5)

Enzyme activity was estimated spectrophotometrically by measuring the initial rate of acetyl-CoA-dependent disappearance of acetoacetyl-CoA at 300 nm according to Quant et al., 1989 [44], with minor modifications. The assay system contained 50 mM Tris/HCl, pH 8.2, 0.1 mM DTT, 5 mM acetyl phosphate, 10 μM acetoacetyl-CoA, 100 μM acetyl-CoA, 20–50 μg of sample protein and 10 units of phosphate acetyltransferase (EC 2.3.1.8). HMG-CoA synthase activity was measured as the difference in the rate before and after acetyl-CoA addition. As mitochondrial HMG-CoA synthase is inhibited by $Mg^{2+}$ [45], the assay was performed in the absence of $MgCl_2$, where acetoacetyl-CoA has an $\varepsilon 300 = 3.6 \times 10^{3}$ $M^{-1}cm^{-1}$ [46].

### 2.14. HMG-CoA Lyase (E.C.4.1.3.4)

HMG-CoA lyase activity was measured by a slightly modified Wanders procedure [47]. In short, 50–100 μg of the sample were incubated at 30 °C in 1 mL of medium containing 50 mM glycylglycine (pH 8.2), 5 mM $MgCl_2$, 0.1 mM DTT, and 2 mM D,L-3-hydroxy-3-methylglutaryl-CoA. At the time point of 30 min, the reaction was stopped by perchloric acid precipitation (0.3 mL, 13%/40% ethanol) and the extracts were prepared using a technique similar to those used for the liver tissues, then, immediately used for fluorometric determination of acetoacetate [48].

### 2.15. 3-Hydroxybutyrate Dehydrogenase (E.C.1.1.1.30)

3-hydroxybutyrate dehydrogenase (HBDH) activity was measured in submitochondrial particles [49] by the method described earlier [50].

### 2.16. Analysis of Ketogenesis in Mitochondria

The rate of liver ketogenesis was measured in the isolated liver mitochondria using the technique of Takeyama et al., 1990 [51], and the end products of ketogenesis acetoacetate and β-hydroxybutyrate were determined by fluorometric enzymatic methods as described earlier [28].

### 2.17. Statistical Analysis

The results were expressed as mean ± SEM (standard error of the mean). Statistical processing of the results was performed using the program Prizm 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The normality of the distribution of variables was confirmed by the Kolmogorov–Smirnov test. Pairwise comparisons were carried out using the Student's *t*-test, and multiple comparisons were performed using the ANOVA and Bonferroni corrections.

## 3. Results

### 3.1. Metabolic Changes in the Liver and Blood during Starvation

To establish how the presence of ammonia modifies the homeostatic function of the liver associated with maintaining glucose and ketone bodies levels in the blood under fasting conditions, we need to study the metabolic characteristics of starved animals without ammonia. We first examined concentrations of key metabolites in the blood and liver of control non-starved and starved animals (Figure 1).

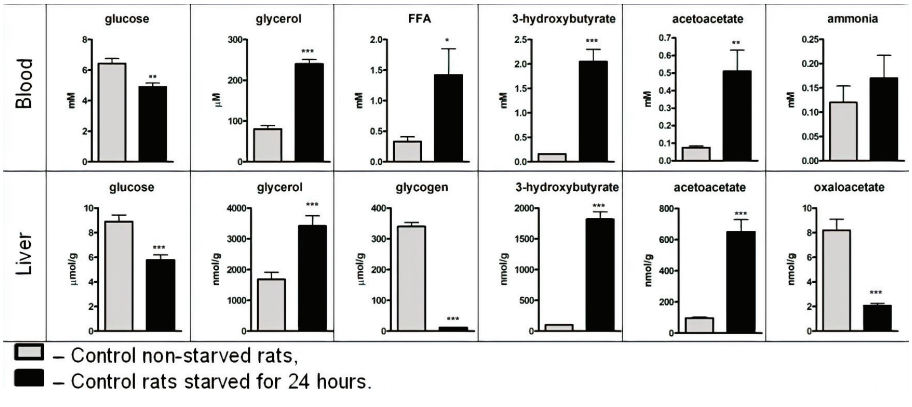


**Figure 1.** Metabolites in the blood and liver of non-starved rats and rats starved for 24 h. Values are the means ± SEM ($n$ = 8). Significant differences in the values in "Control" are estimated by the Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

As shown, 24 h after starvation, glycogen in the liver was significantly depleted (Figure 1). Simultaneously, there was a multifold increase in the concentration of glycerol, acetoacetate, and 3-hydroxybutyrate in the liver and blood; ammonia levels remained constant; FFA in the blood increased; glucose levels became lower, but still remained within the reference values. These results are consistent with the well-documented increase in hepatic gluconeogenesis, ureagenesis, and ketogenesis in starved rats.

### 3.2. Effect of Acute Ammonia Intoxication on Levels of Liver and Blood Glucose, Ketone Bodies, FFA, and Ammonia

We next examined whether ammonia could affect glucose and ketone body levels during fasting-induced hepatic gluconeogenesis and ketogenesis.

As shown in Figure 1, the concentration of ammonia in the blood of control starved rats was 0.170 ± 0.047 mM and increased to 2.86 ± 0.4 mM (Figure 2) 10 min after ammonia injection, which was close to the level observed in non-starved rats with hyperammonemia [52] and in individuals with different pathologies [29,30].

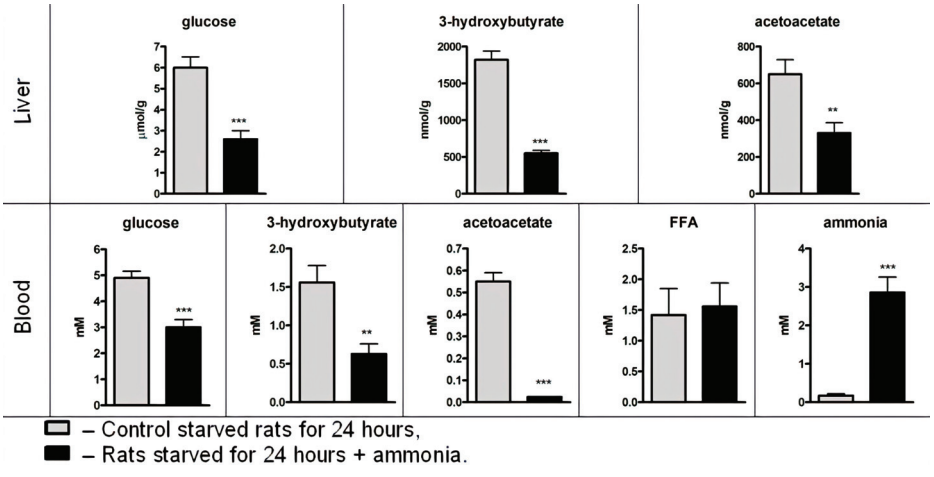


**Figure 2.** Metabolites in the blood and liver of starved rats 10 min after injection of ammonium acetate. Values are the means ± SEM (n = 8). Significant differences in the values in the group "24 h starvation" are estimated by the Student's *t*-test: ** $p < 0.01$, *** $p < 0.001$.

Hepatic glucose, 3-hydroxybutyrate, and acetoacetate levels rapidly decreased, and 10 min after, the ammonia injection showed a 57%, 70%, and 49% decline, respectively, compared to control (Figure 2), suggesting that production of these metabolites in the liver was inhibited and a decrease in their blood levels was expected. Indeed, although the concentration of FFA in the plasma of rats with hyperammonemia was accessible to the hepatic mitochondrial β-oxidation and to the maintenance of ketogenesis and gluconeogenesis, 3-hydroxybutyrate concentration decreased by 60%, acetoacetate virtually disappeared from the blood and the blood glucose level decreased to 61% of control (Figure 2). These results imply that ammonia could disturb either the oxidative function of mitochondria or the metabolic pathway of HMG-CoA.

In order to identify the possible role of liver mitochondria in disrupting the ketogenesis and gluconeogenesis processes, we next studied the effect of acute hyperammonemia on the hepatic mitochondrial function, including oxidative phosphorylation and fatty acid oxidative capacity, first evaluating the intactness and purity of isolated mitochondria.

### *3.3. Intactness and Coupling Efficiency of Isolated Mitochondria*

The intactness of isolated mitochondria was controlled by measuring the parameters of oxidative phosphorylation. The $V_3$, $V_4$, and $V_{unc}$ rates during oxidation of glutamate + malate as respiratory substrates were 72.74 ± 1.91, 10.44 ± 0.84, and 97.2 ± 3.83 ng atoms O/min per 1 mg of mitochondrial protein, respectively. The respiratory control index (RCI) and ADP/O ratio were 7.2 ± 0.64 and 2.87 ± 0.21, respectively (see Table 1). The RCI values upon oxidation of other substrates—pyruvate + malate, succinate, oleate + carnitine, palmitoylcarnitine, and octanoate—were close to the theoretical values (Table 1) and indicated intactness and coupling of mitochondria at all three points of oxidative phosphorylation.

### *3.4. Oxidative Phosphorylation in Hyperammonemia*

The ammonia concentration in liver mitochondria as calculated per intramitochondrial water [53] was 15 ± 4.23 mM in control rats and 95.7 ± 7.5 mM in animals with hyperammonemia.

Upon oxidation of pyruvate + malate in liver mitochondria of hyperammonemic rats, $V_3$, RCI and ADP/O ratio were significantly lower (41%, 36%, and 16%, respectively) than the corresponding values for control samples (Table 1). The $V_4$ and $V_{unc}$ rates did not differ statistically across the two

groups. With glutamate + malate or succinate in the presence of rotenone, the $V_3$ rate also significantly decreased in hyperammonemia by 36% and 38%, respectively, but with only glutamate + malate, RCI fell slightly (22%), while the ADP/O ratio was essentially unchanged in the presence of both substrates in ammonia-treated rats. With the substrates of lipid nature palmitoylcarnitine, octanoate, and oleate + L-carnitine, the respiration rate, $V_3$, in mitochondria from rats with hyperammonemia was markedly and equally inhibited (about 42%) compared to control. Upon oxidation of palmitoylcarnitine, octanoate, and oleate + L-carnitine, RCI in hyperammonemia significantly decreased by 55%, 50%, and 40%, respectively (Table 1).

**Table 1.** Parameters of oxidative phosphorylation in liver mitochondria of control starved animals and animals treated with ammonium acetate.

| Substrate | V3 | V4 | Vu | RCI | ADP/O |
|---|---|---|---|---|---|
| **Control Animals Starved for 24 h** | | | | | |
| Pyruvate + malate | 30.24 ± 1.099 | 10.11 ± 0.11 | 36.2 ± 2.23 | 2.99 ± 0.16 | 3.021 ± 0.13 |
| Glutamate + malate | 88.52 ± 4.37 | 12.24 ± 0.98 | 95.3 ± 4.54 | 7.12 ± 0.54 | 2.87 ± 0.21 |
| Succinate + rotenone | 117 ± 5.45 | 22.8 ± 0.87 | 141 ± 5.31 | 5.13 ± 0.36 | 1.64 ± 0.043 |
| PalmitoyI-L-carnitine | 45.16 ± 1.73 | 10.33 ± 1.81 | 81.04 ± 5.33 | 4.37 ± 0.29 | 1.83 ± 0.44 |
| Octanoate Na | 24.2 ± 0.9 | 4.70 ± 0.11 | 53.7 ± 3.41 | 5.15 ± 0.28 | 2.02 ± 0.06 |
| Oleic acid +L- carnitine | 18.5 ± 0.61 | 6.17 ± 0.23 | 54.4 ± 3.36 | 3.0 ± 0.17 | 2.07 ± 0.19 |
| **Ammonia-Treated Animals Starved for 24 h** | | | | | |
| Pyruvate + malate | 17.84 ± 2.43 *** | 9.32 ± 1.13 | 33.96 ± 2.63 | 1.91 ± 0.21 ** | 2.54 ± 0.09 * |
| Glutamate + malate | 56.98 ± 3.61 *** | 10.32 ± 2.12 | 96.2 ± 3.52 | 5.52 ± 0.27 * | 2.46 ± 0.31 |
| Succinate + rotenone | 72.3 ± 1.33 *** | 19.11 ± 3.33 | 134 ± 6.20 | 3.78 ± 0.53 | 1.77 ± 0.075 |
| PalmitoyI-L-carnitine | 23.9 ± 1.7 *** | 12.1 ± 1 | 102 ± 11 | 1.98 ± 0.12 *** | 2.45 ± 0.15 * |
| Na Octanoate | 14 ± 0.52 *** | 5.5 ± 0.29 * | 49.8 ± 1.5 | 2.55 ± 0.14 *** | 2.7 ± 0.2 ** |
| Oleic acid + L- carnitine | 10.6 ± 0.55 *** | 7.1 ± 1.45 | 54.5 ± 7.5 | 1.8 ± 0.25 ** | 2.7 ± 0.11 ** |

$V_3$, $V_4$ and $V_{unc}$ are the mitochondrial respiration rates in the presence of 200 μM ADP (state 3), in the absence of ADP (state 4), and in the presence of 0.5 μM carbonylcyanide-m-chlorophenylhydrazone (CCCP, state of respiration uncoupling), respectively, and are expressed as ng atom O/min per 1 mg of mitochondrial protein; RCI = $V_3/V_4$ is the respiratory control index. The ADP/O ratio reflects the efficiency of oxidative phosphorylation and is the ratio of the amount of ADP (in nanomoles) phosphorylated with the formation of ATP to the amount of oxygen (in nanogram atoms) utilized in state 3. The substrates were added at final concentrations of: 5 mM sodium pyruvate/2.5 mM potassium malate, 5 mM potassium glutamate/2.5 mM potassium malate, 10 mM potassium succinate/1 μM rotenone, 40 μM palmitoylcarnitine, 5 μM octanoate Na, and 20 μM oleic acid/2 mM carnitine. Values are the means ± SEM (n=8). Significant differences in the values in the group "24 h starvation" are estimated by the Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

These results show that the oxidative metabolism of both lipid and non-lipid substrates is disturbed during a multifold increase in ammonia concentration in the liver mitochondria. From this, we can infer that ammonia has a dramatic impact on mitochondrial oxidative phosphorylation, the main energy supplier in hepatocytes [31].

### *3.5. Adenine Nucleotides and NAD/NADH Ratio in Liver Mitochondria*

To estimate the effect of ammonia on availability of the energy necessary to maintain homeostatic liver function, we measured total mitochondrial adenine nucleotide content, as well as the NAD/NADH ratio, which is known to regulate ketone body and glucose production [54].

The results show that 10 min after injection of ammonia into the starved animals, the ATP content in mitochondria fell by 54% compared to control, while inversely, ADP increased by 38%, and AMP by 49% (Figure 3). The total content of adenine nucleotides (AN) changed insignificantly, while the ATP/ADP ratio dramatically decreased by 71% during this time interval. This indicates that the energy potential of adenine nucleotides in liver mitochondria is decreased by the action of ammonia, but subsequent AN degradation occurs relatively slowly.

At the same time, the redox state of pyridine nucleotides in the mitochondrial matrix changed rapidly; having abruptly dropped upon starvation of control rats from 20 to 7 (data not shown), the NAD/NADH ratio rose immediately up to 13.2 (by 85% higher than the initial value) 10 min after injection of ammonia (Figure 3).

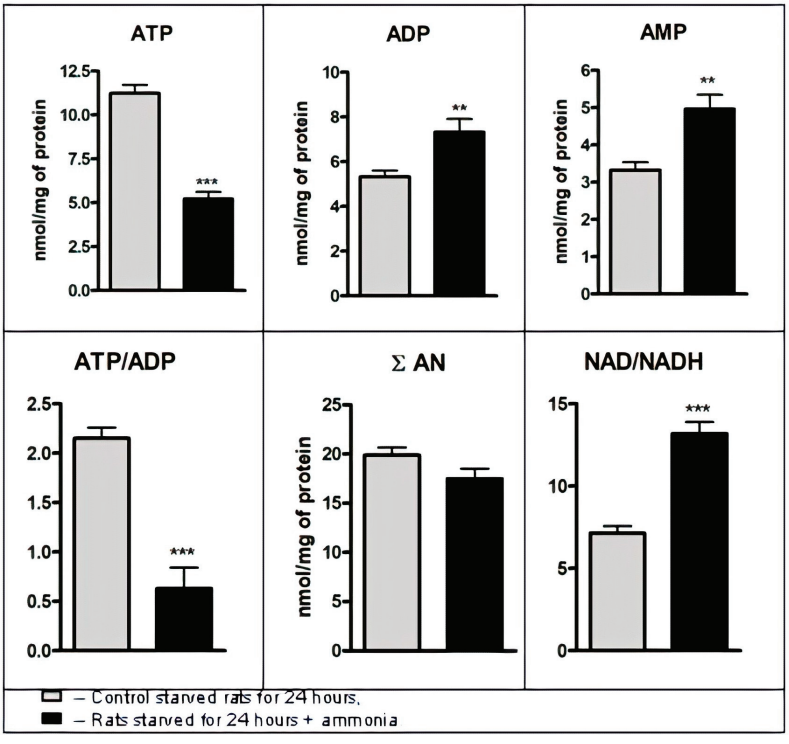


**Figure 3.** Distribution of adenine nucleotides and NAD/NADH ratio in liver mitochondria from control starved rats and starved rats treated with ammonia. Adenine nucleotide content in mitochondria was expressed as nmol/mg of protein. Mitochondrial free [$NAD^+$]/[NADH] ratio was calculated from the components of the glutamic dehydrogenase ([$NAD^+$]/[NADH] = [a-ketoglutarate][$NH_4^+$]/[glutamate], where the equilibrium constant was 3.87 [55]. Values are the means ± SEM ($n = 8$). Significant differences in the values in the group "Control" are estimated by the Student's *t*-test: ** $p < 0.01$, *** $p < 0.001$.

The findings agree with earlier reported results [56] obtained using liver mitochondria from fed rats with hyperammonemia. They suggest that the more oxidized mitochondrial redox state, together with the diminished ATP content and ATP/ADP ratio would result in cessation of energy production followed by a decrease in the activity of pyruvate carboxylase, the first ATP-dependent mitochondrial enzyme that triggers gluconeogenesis [54].

### *3.6. Effect of Ammonia on Pyruvate Carboxylase, Phosphoenolpyruvate Carboxykinase, and Glucose-6-Phosphatase Activity*

We next evaluated whether the ammonia would affect the activity of mitochondrial pyruvate carboxylase (PC) and other key rate-limiting gluconeogenic enzymes located outside the mitochondria. Figure 4 shows that 10 min after injection of ammonia, an abrupt decrease in the activities of PC (67%), PEPCK (87%), and G6Pase (78%) occurred in the liver mitochondria, cytosol, and microsomes, respectively.

These findings show that in starved animals characterized by enhanced hepatic gluconeogenesis, by which normal blood glucose concentration is maintained for at least 24 h upon starvation, the accumulation of ammonia in different liver cell compartments causes a rapid and dramatic decrease in the activities of all key rate-limiting gluconeogenic enzymes; this results in a decline in basal rates of hepatic glucose production and fasting plasma glucose concentrations (Figure 2).

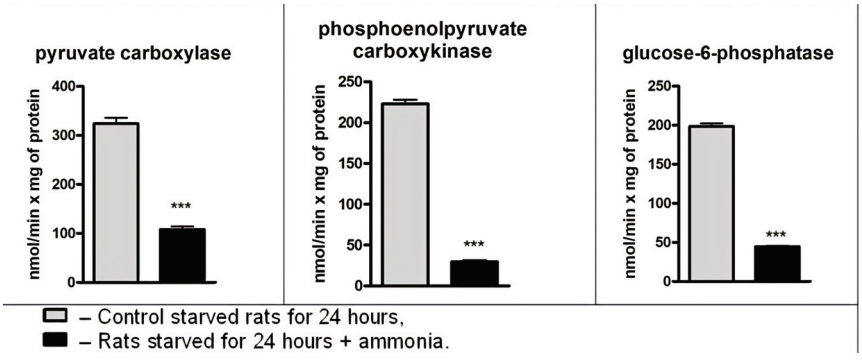


**Figure 4.** Effect of ammonia on the activity of pyruvate carboxylase (PC), Phosphoenolpyruvate Carboxykinase (PEPCK) and Glucose-6-Phosphatase (G6Pase) in the liver of starved rats. The activities of PC, PEPCK and G6P-ase in the liver mitochondria, cytosol, and microsomes are expressed in nmol/min per 1 mg of protein of the corresponding fraction. Values are the means ± SEM ($n$ = 8). The significant differences in the values in the group "24 h starvation" are estimated by the Student's $t$-test: *** $p < 0.001$.

### *3.7. Effect of Ammonia on Enzyme Activities of the HMG-CoA Pathway and Acetoacetate Production in the Liver Mitochondria of Starved Rats*

In some pathological conditions, there is a correlation between gluconeogenesis inhibition and reduced total blood ketone body concentrations, suggesting that inhibition of gluconeogenesis may decrease ketogenesis [57], so we next examined whether hepatic ketogenic capacity in starved animals would actually be altered by ammonia. As can be seen in Figure 5, its injection produced a significant decrease in the specific activities of acetoacetyl-CoA thiolase (45%), HMG-CoA synthase (42%), HMG-CoA lyase (51%), and HBDH (49%). Additionally, the acetoacetate formation rate decreased twice as much as control (Figure 5), simultaneously with its complete disappearance from the blood (Figure 2).

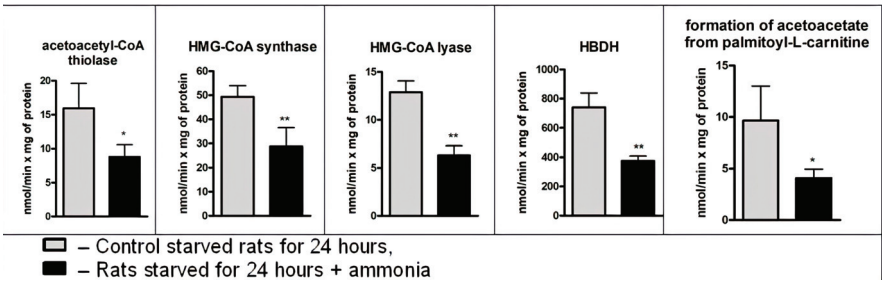


**Figure 5.** Activities of the enzymes of the HMG-CoA pathway and rate of acetoacetate formation in the mitochondria of control and hyperammonemic rats. The activity of enzymes and the rate of acetoacetate formation are expressed in nmol/min per 1 mg of mitochondrial protein. Values are the means ± SEM ($n$ = 8). Significant differences in the values in the group "24 h starvation" are estimated by the Student's $t$-test: * $p < 0.05$; ** $p < 0.01$.

To assess how fluctuations in glucose and ketone bodies in the blood could affect brain bioenergetics and coma development, we studied changes in concentrations of ketone bodies and glucose in the liver, blood, and brain after the first 15 min of loading with ammonia.

Concentrations of acetoacetate and 3-hydroxybutyrate decreased within the first 5 min by 28% and 50% in the liver, 24% and 37% in the blood (Figure 6-1A and Figure 6-2A; Figure 6-1B and Figure 6-2B),

with no change in the brain (Figure 6-1C and Figure 6-2C). Within the next 5 min (10 min after injection), the concentration of ketone bodies continued to decrease in the liver, blood, and finally, in the brain (Figure 6-1C and Figure 6-2C).

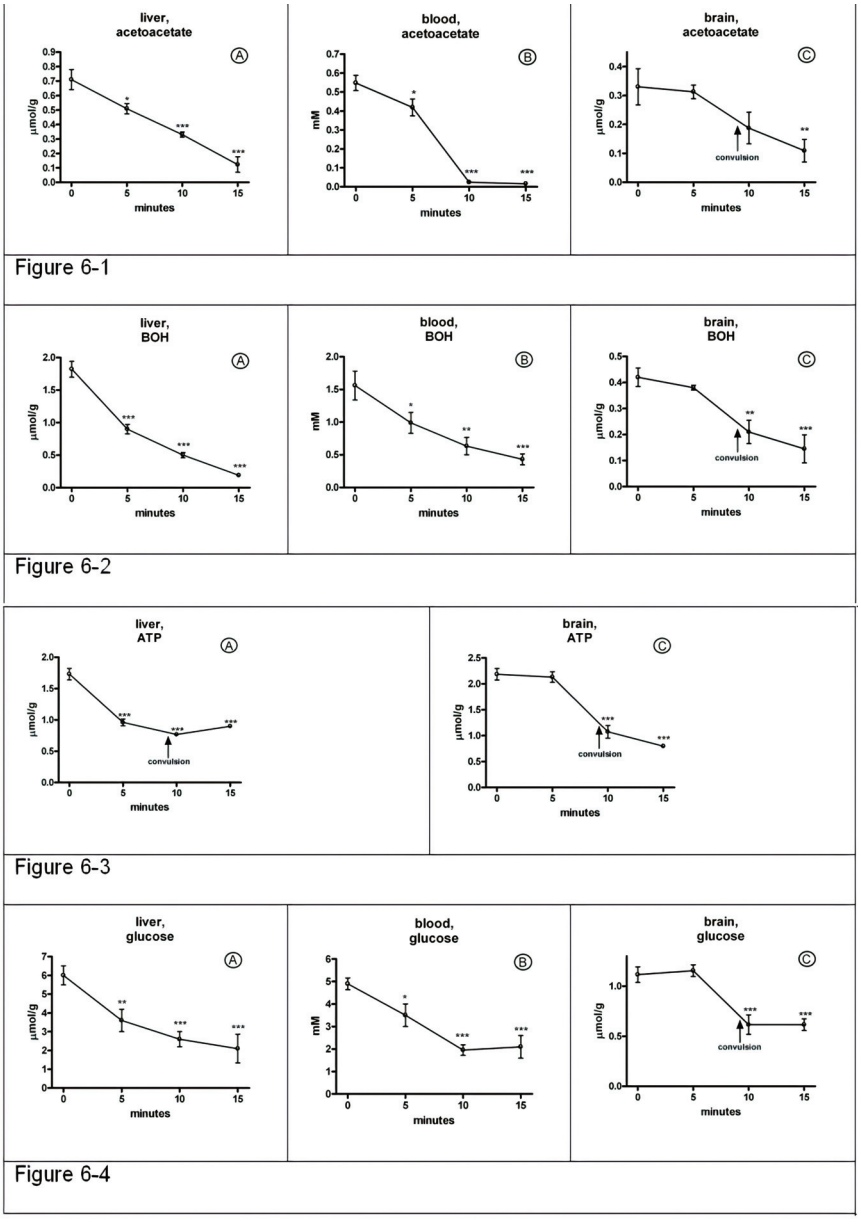


**Figure 6.** Time course of changes in acetoacetate, 3-hydroxybutyrate, ATP, and glucose in the liver, blood, and brain in acute ammonia intoxication. Values are the means ± SEM ($n$ = 8). Significant differences between the values with respect to the time point of 0 min were estimated by ANOVA analysis and corrected with Bonferroni: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Similar changes in the concentration of ATP (Figure 6-3A,C), the total content of adenine nucleotides, as well as the energy charge of adenylate (data not shown) and glucose (Figure 6-4A,B,C) occurred in the brain; concentrations of all metabolites remained unchanged within the first 5 min after injection of ammonia, but sharply decreased just before seizures (by the time point of 10 min), which suggests a time-dependent development of metabolic events—first in the liver, then in the blood and, finally, in the brain.

Thus, these results suggest that there is a relationship between the inhibition of hepatic gluconeogenesis, and ketogenesis, resulting in decreased availability of cerebral glucose and ketone bodies to the brain and bioenergetic collapse of the brain, accompanied by a cascade of pathological reactions [58], leading to irreversible changes in the brain and development of coma as a terminal event in the toxicity of ammonia.

## 4. Discussion

Although the relationship between liver damage and neuropathology has been known for over a century, the genesis of encephalopathy under impaired liver function is not yet completely resolved.

Nonetheless, more recent studies have provided evidence for a paramount role of the liver, reporting the main cause of HE as ammonia accumulation in the blood and brain due to impaired detoxification function of the liver or by portosystemic shunt [3].

In fact, the liver performs a myriad of functions, and in addition to filtering toxins, plays a unique role in the regulation of blood glucose homeostasis, crucial for normal cerebral function, since the brain neither synthesizes nor stores the required amount of glucose [59]. As a result, unstable blood glucose and oxygen delivery to the brain, for even a short period, causes brain damage, while chronic deficiency in these substrates leads to irreversible brain injury, thereby triggering cognitive impairment, coma development, and death [20]. Additionally, when glucose is not readily available, the liver can produce ketone bodies, which are used by the brain as an alternative energy source.

We previously showed that acute hyperammonemia leads to rapid and significant decrease in both acetoacetate and β-hydroxybutyrate levels in the liver and blood of non-starved rats. This suggests that hepatic ketogenesis can be inhibited immediately after increasing ammonia levels in the liver [60], highlighting the role of deterioration of homeostatic liver functions in the occurrence of a brain energy crisis [20].

In light of the notable lack of studies evaluating the effect of ketogenesis and gluconeogenesis in the liver on brain energy metabolism, our study focused on hepatic gluconeogenesis and ketogenesis, including oxidative phosphorylation in mitochondria and the oxidative ability of fatty acids in animal models of acute ammonia intoxication. In this model, ammonia concentrations in the blood quickly increase to the level found in idiopathic hyperammonemia associated with many human pathologies [29,30] and lead to rapid encephalopathy, which underlines the importance of our study.

We revealed that oxidative phosphorylation in rat liver mitochondria was rapidly and progressively suppressed by high mitochondrial ammonia levels.

While ammonium acetate 10 min after administration to the starved rats induced a decrease in state 3 rate and RCI, state 4 respiration remained unchanged during oxidation of lipid and non-lipid substrates (Table 1). These effects of ammonia were reproduced in liver [61] and non-synaptic brain mitochondria [9] of non-starved rats and according to the known action of the uncouplers [62], it was suggested that ammonia seems to have an inhibitory but not uncoupling effect on state 3 respiration and on the electron transfer along the respiratory chain. Finally, deficiency in cytochrome oxidase [63] and degradation of cytochrome-C in liver mitochondria of hyperammonemic rats [64] lead to the same type of oxidative phosphorylation disorders. These findings could explain why ammonium-induced dysfunction of liver mitochondria leads to a sharp drop in the ATP levels in these organelles.

Another consequence of ammonia accumulation is the NO-dependent accelerated formation of superoxide radicals and a simultaneous decrease in the activity of antioxidant enzymes in liver

mitochondria [13]. We therefore hypothesized that NO-dependent oxidative stress would be involved in the mitochondrial toxicity of ammonia.

Indeed, we found that despite increased blood glucagon and intramitochondrial levels of acetyl-CoA (essential activators of PC) [60] in starved rats, ammonia loading enhanced the NAD/NADH ratio (Figure 3); ADP level in mitochondria, a potent PC inhibitor [54], together with ATP deficiency led to a significant decrease in PC activity (65%, $p < 0.001$).

Since there is a causal relationship between PC activity and the whole gluconeogenesis pathway [65], a decrease in activity of other gluconeogenic rate-limiting enzymes was expected. Further studies (Figure 4) showed progressive decrease in liver PEPCK (87%) and G-6-Pase (76%) activity 10 min after ammonia injection.

Thus, we have shown for the first time that in acute hyperammonemia, hepatic gluconeogenesis is inhibited at the level of at least three key enzymes. Accordingly, liver and blood glucose levels began to decline within 5 min (42% and 29%, respectively, compared to the zero time (Figure 6-4A,B)) after ammonia injection, but brain glucose (Figure 6-4C) and ATP levels (Figure 6-3C) were unchanged and behavior of the animals did not differ from control for the same time period. Later on, however, before the seizures, when blood glucose level fell below 2 mM, brain glucose and ATP concentration decreased by 45% and 52%, respectively, and no further significant changes were observed after repeated seizures and coma (Figure 6-4C, Figure 6-3C). Therefore, ammonia-induced acute hypoglycemia resulting from the gluconeogenesis inhibition may precede the onset of seizures and can lead to coma and culminate in neuronal death.

Whereas the levels of ketone bodies in the liver and blood changed in a similar way to glucose levels after ammonia administration (Figure 6-1B, Figure 6-2B), suppression of ketogenesis was predictable.

Indeed, ammonium intoxication caused a significant (approximately twofold) decrease in activity of acetoacetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA lyase, and HBDH (Figure 5). As a result, the acetoacetate formation rate significantly reduced in mitochondria of ammonia-treated animals (Figure 5). We found that the reversal of ketogenesis of starvation occurred due to ammonia within 5 min and became more pronounced just before the first seizure, with no change after the following seizures and coma (Figure 6).

Taken together, these findings indicate that high ammonia level in vivo has a dramatic effect on liver mitochondria as a prooxidant and redox stressor. More importantly, liver mitochondria dysfunction is an early and critical stage in development of ammonia-induced hepatotoxicity, preceding encephalopathy. Given the time-dependent link between abnormalities in the liver and the development of seizures, we hypothesize that HE is a state of the body, in which there is a discrepancy between demand in the brain for energy substrates produced by the liver and inability of the liver to generate these substrates. Our study, thus, provides a framework to further explore the relationship between hepatic dysfunction and progression of brain energy crisis in hepatic encephalopathy.

**Author Contributions:** E.K., L.T., and C.M. provided the original conception and designed the study; E.K., L.T., and G.A. analyzed data and discussed the results. The manuscript was written by E.K. and approved by all authors. All authors have read and agreed to the published version of the manuscript.

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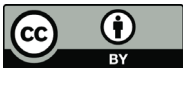

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# Minimal Hepatic Encephalopathy Affects Daily Life of Cirrhotic Patients: A Viewpoint on Clinical Consequences and Therapeutic Opportunities

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**Abstract:** Minimal hepatic encephalopathy (MHE) is a frequent complication of hepatic encephalopathy (HE) and can affect up to 80% of patients with liver cirrhosis. It is characterized by the lack of obvious clinical signs and the presence of alterations detectable using psychometric or electrophysiological testing focused on attention, working memory, psychomotor speed and visuospatial ability. Ideally, each patient should be tested for this condition because, despite the absence of symptoms, it has severe repercussions on daily life activities. It may be responsible for an inability to drive, sleep disturbances, risk of falls and inability to work. Some studies have highlighted its prognostically unfavorable role on mortality and risk of "overt" HE (OHE). Finally, MHE severely affects the lives of patients and caregivers, altering their quality of life and their socioeconomic status. Several treatments have been proposed for MHE treatment, including non-absorbable disaccharides, poorly absorbable antibiotics, such as rifaximin, probiotics and branched-chain amino acids, with promising results. For this reason, early diagnosis and intervention with appropriate measures is essential, with the aim of improving both performance on psychometric tests, as well as clinical aspects related to this condition.

**Keywords:** minimal hepatic encephalopathy; cirrhosis; quality of life; sleep disorders; therapy; non-absorbable disaccharides; rifaximin

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## 1. Introduction

Hepatic encephalopathy (HE) is a neurocognitive disorder associated with both acute and chronic liver damage in which brain function is impaired.

It is a frequent complication and one of the most debilitating clinical manifestations of liver disease, associated with decreased survival and high risk of recurrence.

It is defined as a brain dysfunction related to liver failure and/or the presence of portosystemic shunts, and it is characterized by a wide spectrum of neurological and psychiatric changes, ranging from subclinical alteration to more severe forms that may begin with hepatic coma.

The prevalence of HE is generally 10–14% at the time of diagnosis of cirrhosis, 16–21% in those with decompensated cirrhosis, and 10–50% in patients with transjugular intrahepatic portosystemic shunt (TIPS). The risk of a first episode of HE in the first 5 years after diagnosis of cirrhosis is 5–25%, while in those with previous episodes of overt HE (OHE), the risk of one year recurrence is 40% [1].

Its occurrence causes a worsening of prognosis; in fact, patients with cirrhosis and HE have double the one year mortality risk of patients with cirrhosis without HE [2].

Patients with a higher risk of HE are those with decompensated cirrhosis, spontaneous or iatrogenic shunts, previous OHE, minimal HE (MHE) and muscle changes, such as sarcopenia [3].

Patients with OHE exhibit temporo-spatial disorientation and inappropriate behaviors; appear agitated or, conversely, drowsy; and may progress to deep coma.

Clinical severity is defined according to the West Haven scale. In grade II, patients are disoriented in time; in grade III they are disoriented in space and time with Glasgow coma scale (GCS) > 8; in grade IV, GCS is < 8 and patients do not respond to painful stimuli [2].

Diagnosis is based on excluding other causes of altered mental status because symptoms may resemble those of other neuropsychiatric conditions.

So, it is critical to confirm the presence of cirrhosis and to assess its severity using appropriate scores (i.e., Child–Pugh, MELD score). Plasma ammonium dosage may be useful to rule out the diagnosis.

The second step is to exclude other causes of neuro-psychiatric symptoms, such as acute alcohol withdrawal, hydro-electrolyte imbalances, drug abuse and psychiatric disorders with appropriate tests [1].

Brain imaging is necessary when clinical presentation is unusual, in case of diagnostic doubt or in case of treatment failure; in fact, if symptoms respond to therapies that reduce ammonium levels, it is likely to be HE [2,4].

The treatment of HE is based on adopting general measures for patients with altered consciousness, identifying and treating alternative or coexisting causes of altered mental status and any precipitating factors, and initiating empirical treatments to reduce ammonium levels. The main drugs used for this purpose are non-absorbable disaccharides, such as lactulose, and non-absorbable antibiotics, such as rifaximin. Other possible medications or strategies include polyethylene glycol, branched chain amino acids, probiotics and fecal microbiota transplantation [2].

## 2. Minimal Hepatic Encephalopathy

MHE is the mildest form of HE and is characterized by cognitive and psychomotor deficits without clinically recognizable symptoms of HE [5]. This condition was first described in 1970 by Zeegen et al. who noted that 38% of patients subjected to portal decompression surgery had abnormal "trail making test" scores [6]. Eight years later, the term subclinical HE was introduced and several definitions have followed since then. The latest classification combines MHE with grade I HE (according to West Haven criteria) under the term "covert" HE [7].

The incidence of MHE is 20–80%, and several studies have suggested that most patients with cirrhosis develop HE at some point during the course of the disease. This wide variability is related to different diagnostic criteria and different tests used for its recognition.

Risk factors for MHE include advanced age, alcoholic etiology, previous HE, a more severe disease according to Child–Pugh and the presence of portosystemic shunts [8,9]. In addition, some etiologies increase the risk of cognitive decline, such as hepatitis C virus (HCV) infection [10] and diabetes mellitus, likely due to the negative effect on gastrointestinal motility [11].

Theories about pathogenesis of HE are numerous, but their validity has not yet been fully established. However, there is a general consensus about the impairment of blood neurotoxins detoxification due to liver failure and/or the presence of portosystemic shunts, resulting in changes in brain neurotransmission. Among the neurotoxins implicated in the pathogenesis of HE, the most important is the ammonia NH3/ammonium NH4+ system.

Ammonium derives from the intestinal deamination of glutamine and bacteria metabolism of nitrogenous substances; under normal conditions, it is metabolized into urea through the liver urea cycle. In patients with cirrhosis, the enzyme activities are reduced and blood ammonium levels increase.

Skeletal muscles may play a compensatory role in these patients because muscle glutamine synthase can metabolize ammonia into glutamine. Consequently, muscle depletion may favor ammonia accumulation and HE development [1].

Ammonia induces modifications of astrocytes structure, called type II astrocytosis, in which astrocytes assume a swollen shape with a nucleus broad and pale, prominent nucleolus and chromatin margination.

The consequences of hyperammonemia can be associated with those of other neurotoxic substances of intestinal origin, such as mercaptans, derived from intestinal methionine metabolism, which are able to increase brain ammonium level; indoles, derived from tryptophan metabolism; short-chain fatty acids from anaerobic intestinal fermentation; and substances similar to benzodiazepines and manganese [12,13].

The effects of MHE depend on specific deficits, such as those in attention, vigilance and orientation, which can cause impairments in learning and working memory. In contrast, these deficits do not involve the verbal and communicative aspects, so patients are unaware of their symptoms.

Despite the absence of clinical symptoms, MHE is considered clinically relevant for at least three reasons. Firstly, it impairs everyday activities and has a negative effect on health-related quality of life (HRQOL) not only for patients but also for caregivers, resulting in increased use of health care resources [14]. Secondly, it predicts the risk of developing OHE, and finally it is associated with a worse prognosis [8].

Despite this, a 2007 survey by the American Association for the Study of Liver Diseases (AASLD) showed that most physicians believed that MHE was a significant problem while also remaining under-investigated. In fact, only 50% of physicians evaluated cirrhotic patients for MHE and as many as 38% had never performed a psychometric evaluation of these patients [15].

Therefore, taking these factors together, early diagnosis and eventual treatment are crucial.

## 3. Diagnosis of Minimal Hepatic Encephalopathy

Each patient should be tested for MHE at the time of diagnosis and later during follow-up because it constitutes a serious health problem and, despite its "minimal" expression, is associated with poor prognosis and quality of life.

The diagnosis of MHE can be made with psychometric (computerized and non-computerized) and electrophysiological tests.

Electrophysiological tests are based on standard methodology, require sophisticated equipment and have lower sensitivity than psychometric tests. Computerized tests, on the other hand, are generally based on repeating a large number of tests and give more accurate results than "paper-pencil" tests.

Tests used for diagnosing MHE are:

- Animal naming test (ANT): This is useful as a screening test. Patients have to list as many animals as possible in one minute and a number of animals <15 is indicative of MHE; it is conditioned by education level (<8 years) and age (>80 years);
- Psychometric hepatic encephalopathy score (PHES): This is considered the gold standard for MHE diagnosis (Figure 1). It includes a battery of "paper-pencil" tests for the assessment of psychomotor speed and skill, set shifting, attention, visuospatial orientation, concentration and memory; it lasts about 15 min and a score <−4 is indicative of MHE;
- Critical flicker frequency (CFF): he has to press a button as soon as the impression of fused light switches to oscillating light; it takes about 10 min and it is useful for the evaluation of visual apparatus and cerebral cortex;
- Continuous reaction time test (CRT): The patient has to press a button in response to one-hundred 500 Hz tones presented at 90 dB in random intervals. CRT-index expresses the variability of response times and state of alertness.
- Inhibitory control test (ICT): Several letters are presented at 500 msec intervals, with X and Y interspersed within these letters; patients have to respond only when X and Y are alterning (targets) and not when X and Y are non-altering (lures). This test assesses attention and inhibition (Figure 2);
- Stroop test: In the OFF state, the patient sees a neutral stimulus and has to respond as soon as possible by touching the matching colour of the stimulus to the colour displayed at the bottom of the screen. In the ON phase, the patient sees discordant stimuli and has to touch the colour of the word presented, which is the name of the colour in discordant colouring. This test assesses attention;

- EEG (electroencephalogram): This is useful for studying the cortical activity. In patients with OHE, cerebral activity is slower and three-phase waves are observed [16]. In patients with MHE, the quantative EEG (q-EEG) shows an increase in theta band and a decrease in the MDF (mean dominant frequency) in the posterior derivations, and changes in MDF during sleep represent early markers of brain disfunction. The q-EEG analysis shows alterations in slow oscillatory activity, with an increase in the frequency of dominant delta-rhythm. Evoked potentials: P300 wave (elicited by decision making) has lower amplitude and frequency in MHE [2];
- Magnetic resonance imaging (MRI), MR spectroscopy (MRS) and artificial intelligence: The use of MRI with voxel-based morphometry analysis in patients with liver cirrhosis for the assessment of brain density reveals a reduction in both white and gray matter, mainly in patients with alcoholic etiology, previous OHE and MHE. Such alterations seem to persist even after liver transplantation [17].
- MRS allows the measurement of different metabolites in the brain. In patients with HE, this analysis showed a reduced concentration of myoinositol, increased level of glutamine and decreased choline peak intensities. The elevation of cerebral glutamine concentration is probably do to hyperammonemia, while the lower concentration of myoinositol suggests an important osmoregulatory activity within the astrocyte. These alterations seem to correlate with the grade of HE, as well as with the performance in neuropsychological tests [18,19]. Recently, artificial intelligence has been introduced into many areas of medicine and clinical practice. Several studies have been performed regarding its role in hepatology and MHE diagnosis, mostly using brain magnetic resonance imaging but only in research settings. Machine learning-based approaches role is to examine functional magnetic resonance imaging (fMRI) data in a multivariate manner and extract features predictive of group membership. In particular, using information regarding microstructural integrity and water movement through cell membranes of white matter and the total grey matter volume; machine learning can discriminate cirrhotic patients with and without MHE. However, the costs associated with these technologies are high and currently not sustainable in clinical practice [20].

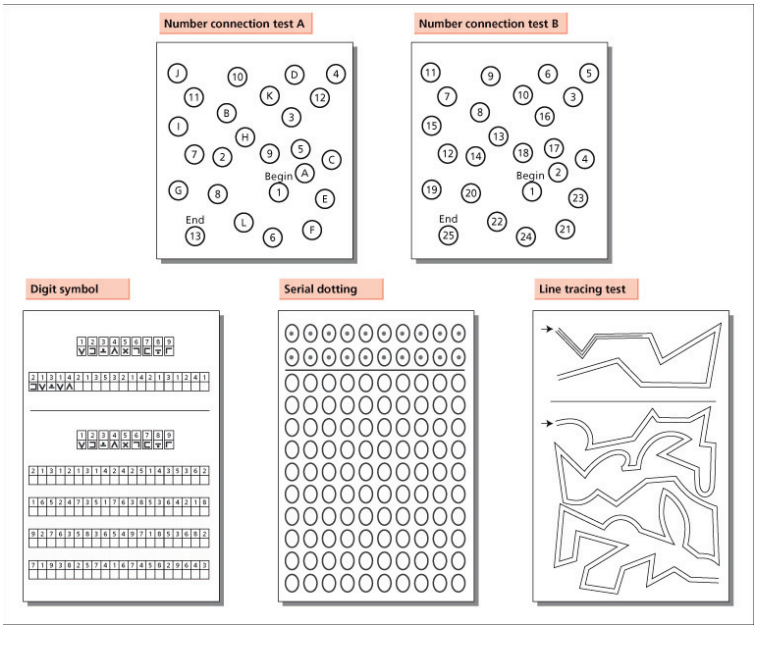


**Figure 1.** The psychometric hepatic encephalopathy score (PHES).

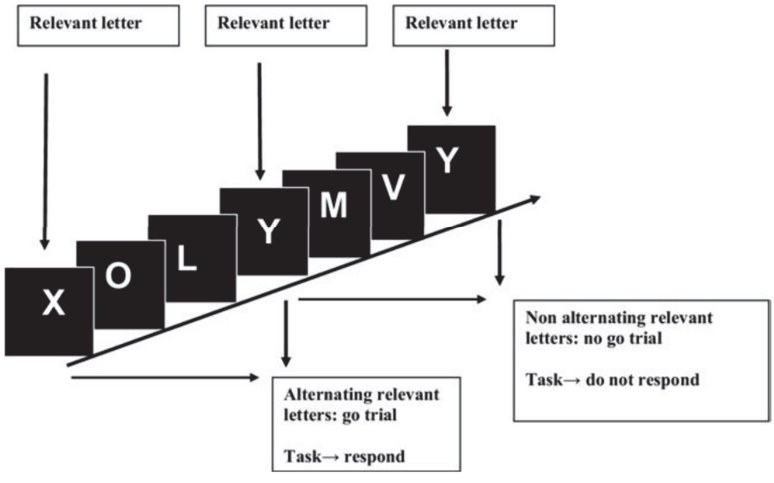


**Figure 2.** The inhibitory control test (ICT).

Unfortunately, there is no single optimal diagnostic method because each method explores different brain functions and none of them cover all aspects of HE. So, as MHE may affect different cognitive domains in different patients, a useful approach might be to use a combination of tests covering the major aspects involved. A possible strategy for MHE diagnosis is to screen cirrhotic patients with rapid and highly sensitive computerized psychometric tests, and then use PHES for further validation [2].

Supplementary Table S1 describes the main tests used for MHE diagnosis and their characteristics.

## 4. Minimal Hepatic Encephalopathy and Quality of Life

Quality of life is a multidimensional concept, affecting aspects of human well-being and encompassing physical and cognitive abilities, functional behavior, emotional state and psychosocial regulation [9].

In patients with liver cirrhosis, it is related to a potentially treatable factor; so, it is a key component in evaluating the effectiveness of various therapeutic interventions.

Quality of life assessment can be performed using questionnaires:

- Sickness impact profile (SIP): this questionnaire is based on 136 items grouped into 12 scales (sleep and rest, eating, work, housekeeping, recreation and hobbies, walking, mobility, body care and movement, social interaction, vigilance, emotional behavior and communication) and provides a total score ranging from 0, corresponding to the best emotional state, to 100, corresponding to the worst one [21];
- Chronic liver disease questionnaire (CLDQ): This contains twenty-nine items grouped into six domains that include abdominal symptoms (three items), fatigue (five items), systemic symptoms (five items), activity (three items), emotional function (eight items) and worry (five items). For each question, patients use a 7-point scale; higher scores indicate better quality of life [22];
- Short Form-36 (SF-36): This is a paper–pencil test adjusted for age and educational level. This test measures eight domains; four related to "physical health" (physical functioning, physical limitation, physical pain and general health) and four related to "mental health" (vitality, mental health and social functioning). Each domain has a score from 0 to 100 and higher scores indicate better quality of life. However, it has only been validated in the Italian population [23,24];
- Nottingham Health Profile (NHP).

Several studies have shown that liver disease affect health-related quality of life (HRQOL).

Activities most affected are those that require attention, information processing and psychomotor skills, such as driving a car or planning a trip. In contrast, everyday activities, such as personal hygiene, dressing or shopping, are preserved [8].

Several mechanisms can be responsible for the impairment of HRQOL in cirrhotic patients. Patients with advanced disease have to limit their daily activities due to impaired physical performance. Similarly, complications of cirrhosis, such as ascites and bleeding, impair HRQOL and social interactions.

Marchesini et al., using SF-36 and NHP, showed that cirrhosis etiology and disease duration had no effect on HRQOL, while symptoms, such as itching and muscle cramps, strongly affected it [25]. Hospitalizations, coexistence of sleep disturbances and disease severity also have a role in determining quality of life [26,27]. Finally, the study by Nardelli et al. showed that symptoms, such as depression, alexithymia and anxiety, were frequent among cirrhotic patients and were among the major determinants of HRQOL [28].

There is convincing evidence on the role of the cognitive decline on patients' daily activities, well-being and HRQOL.

Groenweg et al. showed that patients with MHE had significant reduction in all 12 SIP scales, psychosocial sub score, physical sub score and total score, compared with patients without MHE. On multivariate analysis, the presence of MHE was correlated with reduction in SIP scale, mostly in the area of vigilance, social interactions, recreation and work [29].

A study published by the same group, showed that the SIP statements predictive for the presence of MHE were: vigilance (forgetfulness, confusion, alertness), sleep and rest (sleeping, dozing during the day), fine motor activities and work. Regarding this last item, 50% of patients with MHE reported not having regular employment, compared with 15% of patients without MHE [29], similarly to Schomerus et al. who showed that nearly half of patients with MHE were unable to work [30].

Mina et al., regarding the role of MHE on patients' HRQOL, explored for the first time the role of appetite in patients with MHE [31]. They found that patients with MHE had reduced appetite, and Child–Pugh score was a risk factor for loss of appetite and reduced HRQOL. So, this study showed that both MHE and loss of appetite have a negative effect on HRQOL in patients with decompensated liver cirrhosis [31].

These findings increase interest in demonstrating whether specific treatment of MHE could result in improvement of HRQOL [25,29,32]. Minimal hepatic encephalopathy and daily life functioning

### *4.1. Sleep Disorders*

Sleep is a complex and highly regulated process, fundamental for human health and well-being. Increasingly, sleep–wake cycle disorders seem to be implicated in the pathogenesis of chronic liver disease.

Sleep abnormalities have a prevalence of 26–70% among cirrhotic patients, with substantial repercussions on quality of life and physical health [33,34]. Moreover, sleep abnormalities can worsen patient's liver function and cognitive status [35].

Patients with sleep disorders may complain of several types of symptoms:

- Insomnia, difficulty getting to sleep or difficulty staying asleep;
- Hypersomnia or excessive sleepiness;
- Unusual events associated with sleep, such as apnea or abnormal movements [36].

Sleep assessment can be performed subjectively or objectively. Subjective assessment is based on the use of diaries or questionnaires:

- Pittsburgh Sleep Quality Index (PSQI): This is the gold standard for the assessment of the previous month's sleep, and it lasts about 15 min. It includes 19 items grouped into seven groups (sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, sleep medication and diurnal dysfunction). For each group is assigned a score from 0 to 3 and their sum results in a total score between 0 and 21. A score > 5 indicates poor sleep quality;

- Sleep Timing and Sleep Quality Screening Questionnaire (STSQS): This is a simplified and faster form of the previous one, taking only 2 min. It provides information on sleep quality and time, such as the time you go to bed, latency, night awakenings and wake up in the morning;
- The Epworth Sleepiness Scale (ESS): this assesses daytime sleepiness in eight different situations (while reading, in front of TV, sitting in a public place, passenger in a car, while stopped in traffic, afternoon rest, while talking to someone and sitting after a meal).

Objective assessment is performed with special tools:

- Polysomnography (PSG): this represents the gold standard because it assesses brain electrogenesis, eye and skeletal muscle movements, blood oxygen level and respiratory rhythms during sleep. However, it is expensive and time consuming, so it is generally used for research purposes.
- Actigraphy: this is a semi-quantitative technique that uses an actigraph, which is a three-dimensional sensor placed on a wrist that records patients' movements. Actigraphy assesses periods of quiet and movement over one or more days, considering that if a patient is awake there are movements, while if he is asleep, they are absent. Information obtained are related to total sleep duration, sleep latency and number of nocturnal awakenings [37].

Mechanisms responsible for sleep alterations in patients with liver cirrhosis are numerous.

The clearance of melatonin is reduced because of its hepatic metabolism, and this results in excessive daytime plasma levels. In addition, the alteration of the circadian rhythm of melatonin would seem to be involved with a delay in its nocturnal plasma peak, probably due to reduced sensitivity to light [36]. Additional mechanisms are neuromuscular and thermoregulatory alterations.

Although there are numerous studies on sleep disorders in patients with liver cirrhosis, those related to HE are scarce.

The earliest evidences in favor of a link between HE and sleep disorders came from Sherlock et al. who noted that sleep–wake cycle inversion, restless nights and excessive daytime sleepiness represented early symptoms of OHE [38].

Moreover, the evidence that sleep disturbances may occur after TIPS (transjugular intrahepatic portosystemic shunt) placement may suggest a common pathogenetic mechanism between HE and sleep alterations [39].

Confirming this hypothesis, sleep disturbances seem to develop in line with changes in ammonium plasma levels, which have a key role in the pathogenesis of HE [40].

Finally, some electroencephalographic changes observed during episodes of HE, such as the anteriorization of the alpha rhythm, are similar to those observed during the transition from wakefulness to sleep [36].

Insomnia is the most frequent sleep disorder, even during disease compensation; however, some studies did not show significant differences between cirrhotic patients with and without HE.

In the study by Montagnese et al., cirrhotic patients presented less restorative sleep than healthy controls, without significant differences between sleep indices and the presence/grade of HE, although demonstrating a negative impact on HRQOL [34].

On the contrary, other studies demonstrated a positive association between sleep disturbances and HE. The absence of excessive daytime sleepiness seem to have a negative predictive value on the risk of hospitalization for HE [41], while its occurrence could be responsible for lack of restorative sleep [36].

In this regard, in the study by Samanta et al. in which 100 cirrhotic patients were enrolled and divided between those with MHE ($n$ = 46) and those without ($n$ = 54), 60% were "poor sleepers" according to the PSQI, while 38% demonstrated excessive daytime sleepiness, as measured by the ESS. In terms of multivariate analysis, MHE resulted in a strong correlation with nocturnal sleep disturbances and excessive daytime sleepiness, and

both of these conditions resulted in associations with impaired HRQOL. In fact, MHE was found in 87% of "poor sleepers" and in 6% of "good sleepers" [33].

Finally, in a study conducted in India, both excessive daytime sleepiness and poor nocturnal sleep quality were found more frequently in cirrhotic patients with MHE than those without MHE [42].

MHE seems to cause alterations in sleep architecture. Sleep architecture refers to the repetition of REM (rapid eye movements) and NRME (non-REM) sleep cycles; the latter is further divided into four stages. In patients with normal sleep architecture, the length of stage 1, stage 2, slow waves sleep (SWS) and REM is 5–10%, 50%, 20%, and 20–25% of sleep duration, respectively. The NREM sleep component increases attention, logical thinking ability, language and foresight, and it can increase adaptive capacity and flexibility in response to environmental changes. The REM sleep component is strongly correlated to memory because it can promote plasticity.

Bajaj et al. firstly demonstrated altered sleep architecture among cirrhotic patients with MHE, compared to healthy controls. Specifically, they observed the absence of SWS in 80% and the increase in REM sleep time (19% vs. 7%, $p = 0.02$) in patients with MHE [43].

Similarly, the study by Liu et al. proved a longer duration of stage 1 and 2 of NREM sleep, longer sleep latency, shorter REM sleep latency and higher frequency of nocturnal micro-waking. Finally, the rate of sleep maintenance and sleep quality were lower in patients with MHE than in the healthy controls, indicating a condition of dyssomnia and lower sleep quality [44].

Since sleep architecture is regulated by several cerebral centers, such as the hypothalamus, thalamus and pre-optic region, changes to this architecture could reflect the presence of MHE-induced neuronal damage [44].

Thus, in light of these data, the routine assessment of sleep quality and quantity and excessive daytime sleepiness is essential and this last condition should encourage research for HE [36].

### *4.2. Falls*

Patients with liver cirrhosis have increased risk of falls [45]. Possible causes include endocrine alterations, such as hypogonadism, sleep problems, use of medications such as antidepressants, malnutrition and altered body composition, such as sarcopenia and myosteatosis, and cognitive decline.

Such falls often cause fractures, hospitalizations, increased health care costs and worsening HRQOL. The consequences of falls can be relevant in cirrhotic patients because of coagulopathy and operative risk.

Fall risk assessment can be performed by timed up and go test (TUG). During this test, the time taken to start from a sitting position, walk three meters, turn around and return to the original sitting position is measured. A value greater than 14 s was found to be indicative of a high risk of falls.

In the prospective study by Soriano et al., patients with cognitive dysfunction had a higher incidence of falls (40.4% vs. 6.2%, $p < 0.001$) and a higher severity of falls with more frequent hospitalization (9.5% vs. 0%, $p = 0.01$). In the same study, cognitive dysfunction identified by PHES was predictive of falls at multivariate analysis (OR = 10.2, $p < 0.001$) [46].

Similarly, in the retrospective study by Roman et al., falls were more frequent in patients with MHE than controls and cirrhotic patients without MHE (40% vs. 12.9%, $p > 0.001$), with greater demand for medical care (8.8% vs. 0%, $p = 0.004$). Confirming this evidence, MHE (OR 2.91, $p = 0.02$), previous HE and antidepressant therapy resulted in associations with falls at multivariate analysis [47].

In previous studies, the risk of falls has always been related to cognitive decline and MHE.

As is known, there is a close association between altered body composition and HE.

Up to 40–70% of cirrhotic patients present sarcopenia, which is a wasting of skeletal muscle mass [48]; they may also develop an increase in intramuscular and intermuscular fat, a condition known as myosteatosis.

These two conditions can coexist, albeit infrequently, and both are associated with increased risk of OHE and reduced survival [49].

The link between muscle changes and cognitive decline is the metabolism and trafficking of ammonium. This product has a key role in the pathogenesis of cognitive decline in cirrhotic patients due to the inability to remove ammonia through urea synthesis. In these cases, skeletal muscles can compensate for this defect by serving as alternative sites for urea clearance through glutamine synthesis. Thus, when a qualitative or quantitative change in skeletal muscle is present, this compensatory mechanism fails and HE is promoted [50].

So, it is possible that both cognitive and muscular alterations may increase the risk of falls.

The study by Nardelli et al. aimed to understand whether these two conditions, MHE and muscular alterations, had a similar impact on a cirrhotic patient's risk of falls. In this study, the prevalence of MHE was significantly higher in patients with previous falls and high risk for falls, according to a TUG test value > 14 s. The prevalence of myosteatosis was also higher in these patients. In fact, PHES, beta blockers use and muscle attenuation resulted in a significant correlation with risk of falls. The incidence of falls during follow-up was significantly higher in patients with myosteatosis, but not in those with sarcopenia, and was higher, but did not reach statistical significance, in those with MHE. Thus, muscle alterations seem to play a greater role on risk of falls than altered cognitive status [51].

MHE may increase the risk of falls because it can induce movement disorders, which are still poorly studied in cirrhotic patients.

The study by Urios et al. showed that patients with MHE have increased risk of falls due to impaired postural control, reduced stability, increased reaction time and delayed onset of movements [52]. Data suggest that this last characteristic may be the major cause of bradykinesia in MHE [53].

In a recently published cross-sectional study comparing cirrhotic patients with and without MHE by San Martín-Valenzuela et al., a biomechanical assessment of gait, balance, hand strength and speed in manual actions, was performed. Cirrhotic patients with MHE were slower, with poorer balance, a longer support phase and less pushing force from the ground before the swing phase. They also had greater variability in motor reaction time and lower efficiency in manual activities [54].

Thus, this evidence seems to support the direct relationship between MHE and falls, involving muscle changes and movement disorders. So, health care providers, including nurses and physical therapists, need to be aware of the increased risk of fractures in these patients, and both therapeutic and preventive strategies need to be implemented, especially in those awaiting liver transplantation.

### *4.3. Ability to Work and Wages*

Because MHE impairs many cognitive functions, it may have a negative effect on patient's ability to work, depending on the type of work performed.

So-called "white collar" workers are those workers with intellectual functions, not directly involved in productive activity. In contrast, "blue collar" workers correspond to those workers who perform more manual activities.

The cognitive impairment induced by MHE, causes an imbalance in the work ability of cirrhotic patients. In fact, the "blue collars", who are mainly employed in activities that require vigilance and manual dexterity, have a disadvantage compared to those who perform predominantly intellectual activity.

Shomerus et al. analyzed 110 cirrhotic outpatients who were previously judged fit for work by an expert. Despite this, 44% of patients were judged unfit for work, with a clear difference between "blue collar" and "white collar" (60% vs. 20%). No significant

differences were found between employed and unemployed in terms of severity of illness, while a significant difference was found about psychometric function [30].

Reduced work capacity has a significant socio-economic impact on patients and caregivers. Indeed, reduced work performance and lost wages increase the indirect costs associated with this condition [55]. Finally, reduced work efficiency represents a potential danger for patients and colleagues.

### 4.4. Driving Skills

Driving is a complex and potentially dangerous function, involving the integration of visuomotor coordination, orientation and selective attention. Visuomotor coordination is given by all the information and stimuli coming from traffic, road signs and traffic lights. Orientation is an executive function involving planning, decision making, calculation of potential errors and inhibitory response.

Patients with MHE may be at risk of road accidents because they have impaired attention and delayed reaction times, conditions that may predispose them to the difficulty of controlling a vehicle.

Several studies have been carried out with the aim of studying this relationship.

Assessment of driving ability can be performed using several methods:

- Neuropsychological assessment of cognitive domains involved in driving activity;
- Virtual simulators: i.e., SIMUVEG driving simulator, STISIM simulator. During the simulation, which lasts > 10 min, several variables related to roads, times, distances, actions and decisions are considered to evaluate the driver's driving ability. Two essential aspects of driving performance are longitudinal and lateral control of the vehicle. The former is related to average speed, while lateral control is related to angular speed, wheel movement, distance and time-to-line crossing [56,57];
- Road tests: The assessment is performed by a professional driving instructor who is unaware of the subjects' diagnosis and test results. The assessment is based on the evaluation of four driving categories: car handling, adaptation to traffic situations, caution and vehicle maneuverings. The driving instructor uses a point rating scale to judge driving competence for each category and gave a final score for the overall impression [58].

In the prospective study by Wein et al., the assessment of driving ability by road test showed that the overall driving score of cirrhotic patients with MHE was significantly lower than that of controls or patients without MHE. The most impaired categories were car handling, adaptation, maneuverings and prudence. In addition, the instructor had to intervene more frequently to avoid an accident [58].

It was not known whether this impairment of driving skills increased the risk of traffic violations or accidents.

The study by Bajaj et al. showed that cirrhotic patients have more traffic accidents and commit more traffic violations, or both, than controls of the same age and level of education within 1 and 5 years of questionnaire administration. On multivariate analysis, the presence of MHE was predictive for their occurrence [59].

The same author, in a subsequent prospective study, including only cirrhotic patients, confirmed the previous finding by showing that those with MHE had higher rates of traffic accidents in the previous and subsequent year (22% vs. 7%, $p = 0.03$). Both MHE identified by ICT and accidents/traffic tickets in the past year were predictive of future accidents (OR = 4.51 and OR = 2.96, respectively) [60].

Patients are often unaware of this, tending to overestimate their driving skills.

The study by Kircheis et al. showed that among cirrhotic patients with MHE and Grade I HE, 48% and 39%, respectively, were able to drive according to the driving instructor's judgment, compared with 79% and 89% of cirrhotic patients without HE and healthy controls. Therefore, having MHE correlated with an increased risk of impaired driving ability. In this study, the agreement between instructor judgment and psychometric test results was poor, equal to 70%, with more severe evaluations by the instructor. Another interesting

result of this study was that when subjects were asked to judge their driving ability, 100%, 97% and 90% of those without HE, with MHE and with grade I HE, respectively, rated it as good, thus demonstrating a tendency to underestimate the problem [61].

The study by Bajaj et al. also confirmed this finding, showing that patients with MHE judge themselves as capable as cirrhotic patients without MHE and healthy controls, despite having significantly worse driving performance [57].

In addition, recent evidence has proved that cirrhotic patients have worse outcomes after traffic accidents, with higher in-hospital mortality and longer hospitalization times, as well as higher healthcare costs [62].

Therefore, the determination of driving impairment requires ongoing investigation and definition as traffic accidents are important causes of morbidity and mortality. Because these patients are asymptomatic and often even unaware, it is critical to investigate the history of traffic accidents and traffic violations, collaborate with family members and educate patients as part of the medical interview to increase the likelihood of implementing targeted therapy.

## 5. Development of "Overt" Hepatic Encephalopathy and Prognosis

MHE is a recognized risk factor for progression to OHE and mortality [63]. More than 50% of patients with MHE develop episodes of overt hepatic encephalopathy (OHE) within three years [9].

The relationship between MHE diagnosis and development of OHE is extremely important because the first episode of HE is associated with a reduction in survival (23% at 3 years) [64].

In the prospective study by Hartmann et al., during a mean follow-up of 29 months, patients with MHE presented a 3.7-fold increased risk of developing OHE ($p = 0.002$) and more episodes of OHE during follow-up (56% vs. 8%, $p < 0.001$), compared to patients without cognitive decline. However, Child–Pugh score had a greater impact than MHE on OHE development during follow-up (RR 19.3%, $p < 0.001$). Conversely, no differences were found in mortality, which was mainly determined by Child–Pugh score (RR = 13.95%, $p = 0.003$) [65].

The predictive role of MHE on OHE development was confirmed by subsequent studies in which a higher prevalence of MHE was found among patients who developed OHE during follow-up, with higher ammonium level and greater disease severity identified by MELD [66,67].

In fact, the natural history of MHE is worse in patients with more impaired liver function. Among patients with MHE, the development of OHE is greater in those with advanced cirrhosis and Child–Pugh scores > 7 [9].

However, the clinical relevance of MHE is not only related to the risk of developing major complications but also to its impact on patient survival. There is a great variability in the literature on this topic in light of the different diagnostic tools used to define the presence of MHE.

In this regard, Dhiman et al. defined cut-off values for both the Child–Pugh score and PHES score based on their relationship with survival. In fact, a PHES < −6 and a Child–Pugh score > 7 were associated with a significant increase in mortality [68].

Amodio et al. also showed that MHE, at multivariate analysis, had a prognostic value on survival during the first year of follow-up, together with disease severity identified by Child–Pugh score (HR = 2.4 for Scan test, $p = 0.035$) [69].

Thus, although MELD and Child–Pugh scores are currently used to assess the prognosis of the cirrhotic patients, it is clear that cognitive function also plays a determining role. Therefore, it is desirable for the future that new, more accurate staging systems will be introduced to consider this parameter as well.

## 6. Therapy of Minimal Hepatic Encephalopathy

Since the proposed pathogenetic mechanism of MHE is similar to that of OHE, the therapeutic strategies used for MHE are the same as those used for the treatment and prophylaxis of OHE.

Strategies for the treatment of MHE should include both reduction in ammonium formation, such as through adequate energy and protein intake (30–45 kcal/kg/day and 1.2–1.5 g protein/kg/day, respectively), and enhancement of ammonium detoxification/elimination (fiber supplementation, nonabsorbable disaccharides and antibiotics).

The main treatments for HE include nonabsorbable disaccharides, of which lactulose is the most widely used, and rifaximin.

Lactulose is fermented and metabolized into acetic acid and lactic acid; thus, the intestinal environment becomes acid and ammonia (NH3) and is converted into ammonium ions (NH4+), which cannot be absorbed through the intestinal barrier. This mechanism is also enhanced by its cathartic effect, which facilitates intestinal excretion of nitrogen.

Several studies have evaluated the effect of lactulose on MHE, considering improved performance on psychometric tests as main endpoints.

The study by Watanabe et al. showed that lactulose treatment for 8 weeks resolved MHE in 50% of the 20 treated patients and persisted in 85% of the 13 untreated patients [70].

Similarly, Dhiman et al., after 12 weeks of lactulose therapy, demonstrated psychometric improvement in 80% of patients and in none of those who had not received such therapy [71].

Other studies came to the same conclusions regarding the efficacy of lactulose therapy [72]. Luo et al. found that lactulose therapy was superior to a placebo in all outcomes considered; in fact, it reduced the mean number of abnormal neuropsychological tests, time taken to complete the NCT-A (number connection test A), ammonium levels and risk of developing OHE, while improving quality of life [73].

Rifaximin is the second most widely used drug for HE. It is a non-synthetic and broad-spectrum intestinal antibiotic, which can modulate the intestinal production of ammonium and other toxins.

The study by Zhang et al. showed that short-term therapy with rifaximin (one week) was able to resolve MHE based on psychometric test results, as well as to improve SIBO and plasma ammonium levels [74].

In a study of 20 cirrhotic patients with MHE by Bajaj et al., rifaximin significantly improved all but one of the psychometric tests used (NCT-A and B, DST, LTT, LTE, SDT) [75].

A comparison study between lactulose (30–120 mL/day) and rifaximin (400 mg three times a day) administered for 12 weeks published by Sidhu et al. demonstrated the effect of both drugs on psychometric test results, resulting in the disappearance of MHE in 69.1% of patients in the lactulose group and 73.7% of patients in the rifaximin group. However, the study did not demonstrate the non-inferiority of rifaximin to lactulose [76].

The combination of both drugs, after 8 weeks of therapy, significantly increased PHES score and reduced brain oedema in the study by Rai et al. [77].

Some studies have been performed on probiotics, which, by reducing the intestinal activity of bacterial ureases, decrease the absorption of ammonium and other toxins potentially involved in the pathogenesis of MHE.

Unfortunately, these studies have not demonstrated an advantage of probiotics alone or in combination with other drugs for the disappearance of MHE [78,79].

Although the relevance of these studies, one limitation is that they do not consider the clinical implications of the psychometric test and the effects of this therapy on major complications of MHE.

Regarding the benefits of therapy on quality of life, Prasad et al. demonstrated that lactulose treatment in patients with MHE improved both cognitive performance and HRQOL and also that improvement of quality of life was related to a better performance on psychometric tests [8]. Similarly, Sidhu et al. demonstrated that rifaximin therapy improved

the SIP score and resolved MHE in 75% of patients, with a positive correlation between improved performance on psychometric tests and HRQOL, compared with a placebo [80].

Quality of life also improved after administration of probiotics or LOLA (L-ornithine L-aspartate), in line with improvements in MHE [81]. Despite this, no significant differences in improvement in HRQOL, MHE, hospitalization, or risk of developing OHE were found when probiotic therapy was compared to lactulose [82].

Regarding the impaired driving skills of cirrhotic patients, Bajaj et al. showed that rifaximin therapy administered for 8 weeks versus placebo reduced errors in simulated driving (31% vs. 76%, $p = 0.013$), speeding (33% vs. 81%, $p = 0.005$) and illegal activities (19% vs. 62%, $p = 0.01$) while improving cognitive performance and quality of life in the psychosocial sphere [83].

In contrast to rifaximin, however, lactulose would be more cost-effective in reducing health care costs due to traffic accidents [84].

A number of studies have also been conducted regarding the role of therapy on risk of falls.

Roman et al. studied the therapeutic effect of probiotics; their use for 12 weeks in patients with MHE improved PHES and TUG test score and walking speed, as well as reducing inflammatory cytokines (PCR and TNF-alpha) and markers of intestinal permeability (FABP-6 and urinary claudin-3) [85].

Regarding sleep impairment, the administration of lactulose for 12 weeks determined the improvement of some parameters, such as total sleep time, sleep efficiency and latency, and waking time in patients with MHE [42].

However, there is no robust evidence that treatment of MHE reduces the risk of OHE.

A study focusing on the use of probiotics vs. placebo for the treatment of patients with MHE showed that such therapy resulted in both the regression of MHE, with improvement on paper–pencil psychometric tests (71% vs. 0%, $p = 0.003$), and lower OHE during follow-up (0% vs. 25%), although this was not statistically significant [86]. Probiotics act by depriving substrates to potentially pathogenic bacteria and providing fermentation end products to beneficial ones.

Recently there has been growing interest in the role of albumin in patients with MHE.

Fagan et al. recently published a randomized versus placebo clinical trial on the role of albumin on MHE and HRQOL in patients with prior HE and already on standard secondary prophylaxis. Albumin administration determined MHE reversal and improved quality of life, probably through improved endothelial dysfunction [87]. Finally, given the beneficial effects of therapies on MHE and as suggested by the most recent EASL guidelines, patients with MHE should be treated with nonabsorbable disaccharides and/or rifaximin, and, if effective, this may be an ex adiuvantibus criterion for diagnosis [7]. Supplementary Table S2 summarizes the published study on the treatment of MHE [8,42,70,71,73,74,76,80–83,85–95].

## 7. Conclusions and Future Perspective

MHE represents the earliest and mildest form of HE and it is often under-recognized and under-diagnosed.

Although there is no gold standard for diagnosis, one or a combination of several psychometric and/or electrophysiological tests can be helpful for this purpose.

These tests, of which some are very simple for both examiner and patient, should be performed not only for research purposes but also at the time of diagnosis and then periodically during follow-up in all cirrhotic patients. Early diagnosis is crucial because an association with worse clinical outcomes has been demonstrated in patients with MHE. There is no gold standard for diagnosis of MHE; some tests are better than others, such as PHES, and so they serve as a reference for validating new tests.

Moreover, a diagnostic tool able to identify the presence of MHE and assess its fluctuation over the time should be investigated. A diagnostic tool that, like a thermometer for a fever, can allow the clinician to follow the patient and monitor the effects of a proposed

intervention(s), appears to be needed. Such a tool must be inexpensive, reproducible in results, easily accessible, non-invasive and safe.

However, despite the clinical implications of this condition, also due to the lack of a univocal diagnostic test, MHE is often missed. Therefore, it is crucial to develop effective diagnostic algorithms to progress screening and treatment. Moreover, multicenter studies are needed to explore the predictive value of these tests and the effects of associated co-morbidities.

There are different options for treatment, such as non-absorbable disaccharides, rifaximin and probiotics. The management of MHE should also include a diet with adequate fiber, preferably plant-based protein, probiotics and physical exercise, if tolerated. The aim of these measures is to prevent malnutrition and sarcopenia and, therefore, to ameliorate ammonia catabolism, and thus improve the cognitive impairment that is typical of MHE.

To date, the treatment of MHE is recommended by the recent EASL guidelines, and the two proven effective drugs are lactulose and rifaximin. There are numerous studies showing their effect not only on psychometric tests but also on the main clinical complications of MHE and quality of life.

It is critical to conduct additional randomized clinical trials focused on robust endpoints to determine the best treatment strategies and their optimal duration, as well as to conduct a cost–benefit analysis of the various available therapies.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11237246/s1, Supplementary Table S1: diagnostic tests for MHE; Supplementary Table S2: published studies on treatment of minimal hepatic encephalopathy.

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# Nutrition Assessment and Management in Patients with Cirrhosis and Cognitive Impairment: A Comprehensive Review of Literature

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**Abstract:** Hepatic encephalopathy (HE) represents a common complication of liver cirrhosis. Protein-calorie malnutrition is frequently encountered in the cirrhotic patient and its most obvious clinical manifestation is sarcopenia. This condition represents a risk factor for HE occurrence because skeletal muscle acts as an alternative site for ammonium detoxification. Preventive intervention through an adequate assessment of nutritional status should be carried out at early stages of the disease and in a multidisciplinary team using both non-instrumental methods (food diary, anthropometric measurements, blood chemistry tests) and instrumental methods (bioimpedance testing, DEXA, CT, indirect calorimetry, dynamometry). Dietary recommendations for patients with HE do not differ from those for cirrhotic patient without HE. Daily caloric intake in the non-obese patient should be 30–40 Kcal/Kg/day with a protein intake of 1–1.5 g/Kg/day, especially of vegetable origin, through 4–6 meals daily. In patients with HE, it is also essential to monitor electrolyte balance, supplementing any micronutrient deficiencies such as sodium and zinc, as well as vitamin deficiencies because they can cause neurological symptoms similar to those of HE. In light of the critical role of nutritional status, this aspect should not be underestimated and should be included in the diagnostic–therapeutic algorithm of patients with HE.

**Keywords:** hepatic encephalopathy; protein caloric-malnutrition; sarcopenia; dietary intervention; cirrhosis; mortality

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## 1. Introduction: Hepatic Encephalopathy

Hepatica encephalopathy (HE) represents one of the most frequent complications of liver cirrhosis and certainly one of the most debilitating, with a negative impact on quality of life, morbidity and mortality.

It affects 30–45% of patients with liver cirrhosis and up to 50% of patients with TIPS. It is characterized by an altered cognitive status and neuromuscular function, and its onset depends on the degree of hepatocellular failure and the presence of porto-systemic shunts.

HE must be qualified by the context in which it occurs, severity, precipitating factors and response to treatments.

Regarding the severity of the episode, the new classification suggested by the AASLD/EASL guidelines identify two forms of HE and introduces the term "overt" to refer to asymptomatic or only mildly symptomatic patients.

In particular, "covert" EE includes two forms, "minimal" HE (MHE) and HE of grade I according to West Haven criteria; patients with MHE, despite the absence of clinical evidence of abnormalities in cognitive status, present neuropsychological alterations in tests that explore psychomotor speed and cognitive functions.

Patients with grade I HE, despite normal spatiotemporal orientation, present some degree of cognitive/behavioural impairment compared to normal habits.

The "overt" HE (OHE) includes patients with temporo-spatial disorientation, inappropriate behaviours, agitation or drowsy up to coma [1,2].

Sleep disturbances are common in cirrhotic patients with a prevalence varying between 27 to 70%. The most common sleep disorders are: sleep onset insomnia, fragmented sleep, difficulty falling asleep after nocturnal awakenings, shortened sleep duration and poor sleep quality.

Patients with HE may have hypersomnia, somnolence, excessive and inappropriately timed sleepiness and long daytime naps up to sleep–wake inversion in severe OHE. A study by Singh et al. showed that excessive day time sleepiness and impaired sleep quality were common in patients with MHE and correlated with neuropsychiatric impairment; in addition, the improvement of MHE with lactulose also lead to improvement in sleep disturbances and HRQOL [3].

The pathogenesis is multifactorial. It has been shown that hyperammonaemia, through adenosine, correlates with daytime sleepiness and disruption of sleep architecture. Furthermore, abnormalities in the circadian rhythm of melatonin of both central and peripheral origin may play a role. Finally, disturbances in the 24-h rhythm of skin temperature have been recently reported in patients with cirrhosis, with impaired thermoregulation.

Sleep disturbances can be assessed with diaries and questionnaires, polysomnography and actigraphy [4].

The diagnosis of HE is a challenge for the clinician. Difficulties arise primarily in the presence of "covert" forms of HE or in cases where there are no true precipitating factors.

Precipitating factors include classic ones such as infections, digestive hemorrhage, medications, constipation, dehydration and electrolyte imbalances and "new precipitants" such as sarcopenia and spontaneous or iatrogenic porto-systemic shunts.

The diagnosis of OHE is generally a diagnosis of exclusion and is based primarily on the objective examination and judgment of the clinician. The approach that can be used is the "two-step" approach.

The first step is to determine whether the patient has a known history of hepatopathy and whether the latter is severe enough to warrant an episode of OHE.

The second step is to rule out other causes of neuro-psychiatric symptoms such as acute alcohol withdrawal, hydro-electrolyte imbalances, drug abuse and psychiatric disorders. Brain imaging is necessary when the clinical presentation is unusual or sudden, when there are diagnostic doubts, or when the patient does not respond to the usual therapies.

In contrast, the diagnosis of MHE can be made with psychometric testing, both computerized and non-computerized, and electrophysiologic testing, such as electroencephalography (EEG) and evoked related potentials (ERPs) [1].

The main goals of treating HE are to manage the acute event, reduce its duration and prevent complications and hospitalizations for recurrence.

The treatment of type C HE is based on four basic principles: adopt general measures for patients with altered consciousness, identify and treat alternative or coexisting causes of altered consciousness, identify and correct precipitating factors and initiate empirical treatments to reduce ammonium levels.

The most common drugs used to reduce ammonium levels are nonabsorbable disaccharides, such as lactulose, and nonabsorbable antibiotics, such as rifaximin.

Lactulose is considered the standard-of-care for treating HE and preventing recurrent episodes because it reduces ammonium levels through several mechanisms:

- Laxative effect: by creating a hyperosmolar intestinal environment, lactulose accelerates intestinal transit and prevents ammonium absorption in the colon;
- Ammonium ionization: acidification of intestinal contents results in ionization of ammonium, which in this form can no longer diffuse freely across cell membranes;
- Bacterial uptake of ammonium and the beneficial effect on intestinal microbiota: volatile fatty acids released because of lactulose metabolism, are used by bacteria as an energy substrate for proliferation, while ammonium trapped in the colon is used as a source of nitrogen for protein synthesis;

- Reduction of the intestinal production of ammonium: lactulose inhibits the activity of the enzyme glutaminase and the intestinal uptake of glutamine, blocking the subsequent conversion into ammonium;

Among the non-absorbable antibiotics, Rifaximin is the most widely used in this field. It is a semi-synthetic and non-absorbable antibiotic whose mechanism of action consists in modulating the function and composition of the intestinal microbiota by acting on gram+ and gram– enterobacteria. It also presents anti-inflammatory and eubiotic effects. It is generally well tolerated and interactions with other drugs are negligible. It is used for acute treatment and for secondary prophylaxis in case of lactulose failure.

Other possible medications or strategies include polyethylene glycol, branched chain amino acids, probiotics and faecal microbiota transplantation [5].

## 2. Metabolic Alterations in Liver Cirrhosis

The liver is the principal organ responsible for distribution, storage and detoxification of nutrients absorbed from the gastrointestinal tract.

One of its critical roles includes the metabolism and storage of glycogen as an energy source during fasting periods to maintain glucose homeostasis. Therefore, this organ provides glucose during fasting states and energy to cell types that heavily consume glucose, such as neurons, red blood cells and kidney cells.

Conversely, when blood glucose level rises, the liver increases glycogen synthesis and suppresses hepatic glycogenolysis and gluconeogenesis [6].

Another function of the liver is the production of bile acids; they promote lipid digestion in the small intestine and indirectly participate to lipid and glucose metabolism.

In addition, the liver is involved in the storage of micronutrients and in the detoxification of products derived from the gastrointestinal tract, primarily ammonia, which is converted to urea through the urea cycle [6].

When liver function fails, these functions are severely compromised.

In cirrhosis, which is the final stage of all chronic liver diseases, major metabolic alterations are observed, and they cause high mortality and reduced quality of life. Several metabolic mechanisms are compromised in patients with liver cirrhosis:

Carbohydrate metabolism: as a major consequence of cirrhosis, the hepatic glycogen storage capacity becomes significantly reduced and this causes abnormalities in energy production.

Because of the decreased ability to utilize glucose, lipid oxidation and gluconeogenesis are accelerated, leading to a higher incidence of post-prandial hyperglycaemia [6]. This condition is also due to insulin resistance; in fact, a significantly lower insulin sensitivity index after oral tolerance testing and increased pancreatic insulin synthesis have been demonstrated in several studies to compensate the lack of glucose clearance [7]. Up to 70% of cirrhotic patients have some degree of glucose intolerance or insulin resistance, whereas 14–46% have type II diabetes mellitus [8].

Moreover, hyper catabolism and hyperglucagonemia disproportionate to insulin level are observed in cirrhotic patients, with an increased glucagon to insulin ratio [9].

Protein metabolism: protein turnover is also enhanced; in fact, amino acids are used as alternative substrates for hepatic gluconeogenesis. Therefore, the body becomes highly catabolic to support the increased protein demand.

In addition, the use of branched-chain amino acids (BCAAs) such as leucine, isoleucine and valine obtained from muscle catabolism for glutamine synthesis and ammonium clearance, cause an imbalance in plasma amino acid concentrations with predominance of aromatic amino acids (AAA), i.e., tryptophan, phenylalanine and tyrosine [6].

A low BCAA to AAA ratio has been shown to be associated with a worse prognosis and risk of developing HE [10]. When this ratio is <3, AAAs cross the blood–brain barrier and act as substrates for the synthesis of false neurotransmitters such as octopamine, tyramine and phenylethanolamine, which displace major neurotransmitters such as dopamine and norepinephrine [7].

Unlike healthy subjects in whom these responses to fasting are observed after 2 or 3 days, in cirrhotic patients these changes already occur during the night; in fact, a high catabolic activity of the organism has been demonstrated during this period [11]. This adaptation mechanism is related to the scarcity of glycogen reserves [12].

Lipid metabolism: in cirrhotic patients are observed an increased lipolysis and oxidation of non-esterified fatty acids with the aim of obtaining energy from other sources [9].

Other alterations: additional metabolic alterations observed in these patients include alteration in appetite-regulating hormones and, in patients with cholestasis, reduction in the pool of bile salts resulting in malabsorption of fats and fat-soluble vitamins [13].

These metabolic changes may have an influence on basal metabolism, although some studies have shown contradictory results. In fact, some studies have shown that up to 34% of patients have a rest energy expenditure (REE) of 120% above the expected value [14], while in others this value is normal in most patients [15].

## 3. Protein-Calorie Malnutrition in Liver Cirrhosis

In addition to known complications such as HE, digestive haemorrhage and ascites, liver cirrhosis is associated with altered nutritional status that can cause severe complications and reduced life expectancy.

Some consider malnutrition as the most frequent complication of liver cirrhosis.

Malnutrition is defined as a state of continuous and inadequate oral intake that results in altered nutritional status with significant loss of weight and muscle mass [6].

The best term to define malnutrition in cirrhotic patients is protein-calorie malnutrition, in which both muscle and fat mass are depleted. However, the predominant and later loss of muscle mass in cirrhotic patient suggests that sarcopenia represents the major nutritional deficiency [16].

Protein-calorie malnutrition affects 25 to 56% of patients with liver cirrhosis. Although it is more frequent in patients with advanced disease, surprisingly it is present in up to 16% of Child-Pugh class A patients [7]. Thus, even patients with modest disease are at risk of malnutrition.

However, malnutrition cannot be considered only a consequence of liver disease, because it can accelerate its natural history and negatively affect the patient's prognosis exacerbating the vicious cycle of anorexia-cachexia [17].

Anorexia is defined as pathological lack of appetite that is not conscious refusal of food, but loss of sense of hunger and desire to eat with a persistent sense of fullness.

In liver cirrhosis, this condition may be due to various factors such as systemic inflammation, polypharmacotherapy, dyspepsia, maldigestion, malabsorption and social or psychiatric factors.

Anorexia in turn can cause pre-cachexia and cachexia, which is the loss of weight and fat mass, and sarcopenia, which is the loss of muscle mass and function.

However, cachexia and sarcopenia do not always coexist in liver cirrhosis. For example, inactivity can cause an increase in weight and fat mass and simultaneously a reduction in muscle mass. This condition is known as sarcopenic obesity [15].

The presence of protein-calorie malnutrition is associated with increased complications of liver cirrhosis, such as digestive variceal haemorrhage, HE, hepatorenal syndrome, impaired liver function and regeneration capacity and post-surgical morbidity and mortality. Malnutrition is also an independent predictor of mortality in cirrhotic patients [8].

The causes of malnutrition are diverse and include nausea, loss of appetite, alterations in gustatory receptors, reduced oral energy and protein intake, dysgeusia due to zinc or magnesium deficiency, increased basal metabolic rate, unappealing hypoprotein diets, also often restricted in sodium and fluid content, gastroparesis and increased circulating leptin levels [18]. Certain medications, such as diuretics and lactulose, may also be the cause of nutritional deficiencies. Equally, alcohol abuse can cause loss of appetite [16].

In cirrhotic patients, portal hypertension can cause malabsorption that results in changes in intestinal mucosa, such as increased intestinal permeability and consequent

protein loss [16]. Finally, in patient with HE, frequent hospitalizations, confusion or excessive sleepiness, may cause a worsening of nutritional status [18].

## 4. Beyond BMI: Sarcopenia, Myosteatosis and Sarcopenic Obesity in Liver Cirrhosis

In cirrhotic patients, the assessment of nutritional status based on BMI may not be reliable because it can be influenced by water retention. In addition, several studies have now ascertained the presence of alterations in body composition, such as sarcopenia and myosteatosis.

Sarcopenia affects 40 to 70% of patients and the pathogenesis appears to be multifactorial. Possible causes include increased plasma levels of myostatin, reduced caloric and protein intake, intestinal malabsorption of nutrients and increased pro-inflammatory cytokines [6].

The presence of sarcopenia, which is also associated with impaired muscle function, increases the risk of sepsis and mortality by two- to three-fold [17].

Some evidence has shown that patients with cirrhosis can develop both loss of skeletal muscle and increase in intermuscular and intramuscular fat, a condition called 'myosteatosis', characterized by a reduction in radiodensity on CT scans.

This complication is independent of dietary lipid introduction because pathological fat deposition can also be observed in non-obese or even in underweight patients.

Mechanisms of pathological lipid storage within the muscle in cirrhosis have not been identified but may be linked to metabolic aberrations associated with hepatic dysfunction. In fact, elevated skeletal muscle ammonia uptake can promote skeletal muscle mitochondrial dysfunction via diminished lipid oxidation, which results in the accumulation of lipid mediators.

Sarcopenia and myosteatosis can coexist in cirrhotic patients, although in the study by Ebadi et al. this coexistence affected only 17% of the patient cohort [19].

Several studies have shown that sarcopenia and myosteatosis are associated with poor prognosis and with different complications including HE.

In fact, the study by Bhanji et al. demonstrated that sarcopenia and myosteatosis were independently associated with OHE risk in patients with liver cirrhosis [20], while in the study by Montano-Loza et al., sarcopenia (HR = 2.00) and myosteatosis (HR = 1.42) were significantly associated with an increased risk of mortality [21].

Patients in whom these two conditions coexist, would appear to have the worst prognosis.

A study was recently conducted on the prognostic role of myostatosis in transplanted patients. In this study, the probability of graft and patient survival at five years was significantly worse in the presence of myosteatosis. These patients showed significantly higher all-cause mortality, mostly due to respiratory and septic complications. Surprisingly, sarcopenia was not significantly associated with graft and patient survival [22].

In this context, it must be considered that in recent years the number of overweight or obese cirrhotic patients has significantly increased. In spite of what one may think, these patients can be malnourished in the same way as normal weight or overweight patients; therefore, even and especially for them, an early dietary intervention is indicated.

In fact, a lot of patients with liver cirrhosis tend to be presenting with obesity these days due to changes in lifestyle.

"Obesity paradox" is a phenomenon in which obese patients seem to have a reduced risk of death compared to people with standard weight; this is because while obesity is associated with increased adipose tissue mass, it is difficult to accurately assess adipose tissue mass with BMI, which includes muscle mass and bone mass in addition to fat. In fact, there are cases of excess visceral fat even when BMI is within the normal range [23].

Sarcopenia and obesity are closely related, with a prevalence from 2 to 46%; Montano-Loza et al. reported that patients with sarcopenic obesity had significantly poorer survival compared to the control group (median OS, 22 months) [21] and this condition could compromise the outcome of surgery. In fact, Kobayashi et al. reported that sarcopenic

obesity was an independent risk factor for mortality (HR = 2.504, $p = 0.005$) and HCC recurrence (HR = 2.031, $p = 0.006$) after hepatic resection for hepatocellular carcinoma [24].

It is therefore important to look at the emerging clinical determinants of cirrhosis, such as muscle alterations, from a different perspective, in which new factors could add prognostic value to the oldest and most well-established ones, such as MELD.

Indeed, not only the presence of a severe liver disease, or a previous history of minimal/covert HE, or iatrogenic portosystemic shunts, but also sarcopenia, nutritional deficit or spontaneous portosystemic shunts could play a major role.

The attention to muscular alterations could also have a therapeutic implication. In fact, current therapies for HE aim to lower ammonium levels, since its pathogenetic role in cognitive alterations in cirrhotic patients is well known.

However, the improvement of nutritional status and body composition should be considered an important endpoint in the management of these patients because it could have a preventive and prognostically favourable role.

## 5. Malnutrition in Patients with Hepatic Encephalopathy

The role of nutrition in the pathophysiology of HE has long been hypothesized. Indeed, because HE is a late complication of advanced liver cirrhosis, it is not surprising that cirrhotic patients are susceptible to nutritional disorders.

Nutrition and HE may influence each other for several reasons [25].

Ammonium metabolism is probably the most studied mechanism of nutritional effects on HE, because its production is strongly influenced by diet. Indeed, because most protein substrates for bacterial fermentation originate from dietary protein, it is safe to assume that dietary interventions that affect proteins can have a significant therapeutic impact [6].

Skeletal muscle may play a compensatory role in ammonia clearance through glutamine synthase, which metabolizes ammonia into glutamine. Consequently, muscle depletion may favour ammonia accumulation and HE development.

In fact, approximately 75% of patients who develop HE have moderate-to-severe malnutrition and this condition is associated with an increased risk of mortality.

In fact, as we recently demonstrated with our group, the presence of sarcopenia (29% vs. 7%, $p < 0.0001$), previous HE (28% vs. 6%, $p < 0.001$) or HE during follow-up (25% vs. 9%, $p = 0.005$) were associated with a higher mortality rate. The co-presence of previous HE and sarcopenia were independently associated with mortality (HR 2.56, $p = 0.0056$, 95% CI 1.3–5) [26].

For this reason, improving nutritional status must be considered a key goal to improve cognitive decline in these patients. So, dietary modulation should be considered a valid option when trying to prevent episodes of HE in patients with liver cirrhosis.

The existence of a relationship between HE and nitrogen-containing foods has long been claimed.

The initial evidence in support of this hypothesis derives from animal experiments conducted in the late 1800s; in particular, in portocaval derived dogs, a meat-based diet caused neurological symptoms, while if dogs were fed with bread and milk or did not lose weight, no neurological complications were observed [12].

Based on this evidence, Sherlock et al. first described that, in cirrhotic patients, symptoms of HE could be controlled by a low-protein diet.

For this reason, the first published studies on HE treatment suggested protein restriction as an effective strategy to reduce plasma ammonium levels and improve HE symptoms and has been the cornerstone treatment of HE since the 1950s. However, clinical benefits on neurological symptoms have not always been observed.

In fact, an hypoproteic diet in patients with reduced oral nutrient intake and in a hypercatabolic state, can worsen the patient's nutritional status and prognosis.

## 6. Assessment of Nutritional Status

In 2019, the European Association for the Study of the Liver (EASL) published new guidelines regarding nutrition in patients with chronic liver disease. The aim was to answer a number of questions related to this topic, such as how to recognize nutritional problems and which patients need evaluation, what are the consequences of malnutrition and how to intervene for its correction and prevention.

These guidelines consider the problem of malnutrition in several areas, including that of obesity and hepatic encephalopathy, and focus attention on sarcopenia and how to intervene to prevent it [1].

Acquiring a diagnosis of malnutrition in the early stages of liver cirrhosis can be complicated and there is no consensus on the best method to quantify and classify this condition. Since malnutrition and loss of muscle mass are frequently observed in cirrhosis, a preliminary step in the evaluation of cirrhotic patients is the definition of nutritional status [12]. In fact, it represents the first step to define the pattern of tissue loss and to establish the appropriate treatment strategies.

The knowledge on the pathophysiology of HE and the evidence of the prognostic impact of sarcopenia, makes the assessment of muscle mass and function a key element in the evaluation of cirrhotic patients. Of these, the most complex techniques for assessing nutritional status require patient collaboration, often high costs and trained personnel, but they are useful for confirming patient's bedside measurements.

Screening should be performed in all patients, but especially in those at higher risk of malnutrition such as those with advanced cirrhosis (Child-Pugh class C) and underweight (Body mass index, BMI, <18.5) [27].

However, there are some limitations to the definition of nutritional status in cirrhotic patients [6]:

- There are gender differences in body composition and tissue loss characteristics that limit the usefulness of instruments measuring muscle mass and function in women. In fact, the study by Riggio et al. showed that body composition is different between men and women. In particular, in women, fat reserves were more deficient with maintenance of muscle mass. In contrast, in men, the loss of muscle tissue was more evident as observed under stress conditions [28].
- There is no standardized approach to diagnosing and classifying malnutrition;
- Prevalence is affected by aetiology of cirrhosis, being very high in hospitalized patients with alcoholic aetiology;
- Hydrosaline retention makes body weight and body mass index unreliable;
- The value of biochemical markers, such as albumin, are affected by plasma dilution and altered hepatic synthesis;
- More accurate measurements, such as DEXA and dilution techniques, have high costs, are not always available and require specialized personnel.

There are several tools used in clinical practice to assess nutritional status, including those that combine objective and subjective data.

### *6.1. Non-Instrumental Methods*

- Food diary: represents a simple tool to obtain information regarding daily food intake [8];
- Objective examination: allows recognition of signs of nutritional deficiency such as loss of muscle mass, loss of subcutaneous fat, dry skin, hair loss and signs of vitamin and micronutrient deficiencies;
- Biochemical parameters: parameters such as albumin, pre-albumin and retinol-binding protein are influenced by the residual capacity of hepatic synthesis, so they are not reliable for the assessment of nutritional status in cirrhotic patients; therefore, the level of total plasma proteins correlates more with severity of hepatopathy than with nutritional status [8].

- Micronutrient dosage: zinc deficiency is extremely common in cirrhotic patients and has a prevalence of 84–96%; it may be due to reduced intestinal absorption or excessive diuretic use; symptoms of deficiency include anorexia, immune system dysfunction and dysgeusia [8]; in addition, because zinc participates in urea detoxification, it may increase the risk of HE.

As known, hyponatremia can cause cognitive decline by acting on astrocyte swelling. Both hypovolaemic and hypervolaemic hyponatremia can occur in patients with cirrhosis. The second one is characterized by an expansion of extracellular fluid volume, with ascites and oedema.

Splanchnic vasodilation and arterial underfilling play a major role in development of this type of hyponatremia: the opening of porto-systemic collaterals and the synthesis of circulating vasodilators causes the reduction in vascular resistance predominantly in the splanchnic arterial circulation; this condition leads to a decrease in the effective circulatory volume and, in order to restore the effective circulatory volume, the sodium-retaining neurohumoral mechanisms, such as the renin-angiotensin-aldosterone system, sympathetic nervous system and ADH, are activated leading to maximal retention of sodium and water [1,29].

In addition, patients with cirrhosis and ascites usually follow a sodium-deficient diet, which may precipitate neurological symptoms in addition to water retention.

On the contrary, hypovolaemic hyponatremia is characterized by the frequent absence of ascites and oedema as a result of a prolonged negative sodium balance with marked loss of extracellular fluid due to excessive diuretic therapy [1].

- Plasma vitamin dosage: among possible vitamin deficiencies, thiamine deficiency is often encountered, especially in alcoholic aetiology, because of reduced intake, reduced storage and reduced intestinal absorption [7]. Its deficiency causes Wernicke's encephalopathy, which must be placed in differential diagnosis with HE, so it must always be supplemented.

Vitamin D may also be deficient because of reduced dietary intake, reduced exposure to sunlight, reduced intestinal absorption, deficiency of binding proteins and impaired protein hydroxylation. Rarely, it causes osteomalacia.

A study by Vidot et al. demonstrated that there is a significant correlation between 25-OH vitamin D deficiency and the presence of HE; in fact, with equal disease severity, episodes of HE were more frequent in patients with low vitamin D levels [30].

Vitamin B12 and folic acid deficiencies may develop primarily from a loss of hepatic storage in advanced stages of disease. Normal plasma values may not reflect actual tissue levels of them. Symptoms of deficiency include anaemia, glossitis and neuropsychiatric manifestations [8].

Retinol deficiency may be caused by reduced intestinal absorption and hepatic clearance of vitamins. Vitamin A is stored in stellate cells and its deficiency may promote collagen release and fibrosis [31]. Vitamin A deficiency can cause night blindness, photophobia and increased risk of neoplastic complications. In addition, a level <78 umol/L in cirrhotic patients is associated with an increased risk of mortality. However, because high doses of vitamin A are hepatotoxic, supplementation should be conducted with caution [32,33]. In patients with cholestasis or alcoholic aetiology, a deficiency of fat-soluble vitamins may be found [8]

- Creatinine-to-weight ratio: if patient's renal function is normal, this ratio can be used to estimate muscle mass. In fact, creatinine is almost entirely contained in skeletal and smooth muscle and reduced urinary excretion may be due either to impaired renal function or to reduced muscle mass, but not to impaired hepatic function [33];
- Anthropometric measurements: these are objective methods for assessing patient's nutritional status. They are rapid, non-invasive and low-cost techniques specifically designed to assess somatic characteristics. However, even these have limitations when applied to cirrhotic patients, especially with HE [25].

Among anthropometric measures, certainly the best known is BMI. Its value can be altered by electrolyte alterations, renal insufficiency and presence of ascites or oedema; therefore, it has a low sensitivity and poor reliability for measuring nutritional status in these subjects [8].

The measurement of triceps skin fold thickness (TSF) and mid-arm muscle circumference (MAMC) are less affected by water retention than BMI, although they can be influenced by sex and oedema in the case of upper limb localization [8]. In fact, while the value of MAMC seems to be more compromised in men, the opposite is true for TSF in women. Moreover, obesity may compromise a correct assessment and both measures are subject to some inter-observer variability [25].

However, these measurements are easily performed at patient's bedside, provide an immediate assessment of fat and muscle mass, respectively [6], and are considered one of the best methods for indirect assessment of patient's nutritional status.

Several studies have been conducted on these methods.

A study by Fiore et al. confirmed the reliability of these methods and demonstrated that the percentage of fat mass identified with the skinfold method differed by less than 5% from that obtained with DEXA [34].

A study by Alberino et al. proposed that TSF and MAMC could be included in the Child-Pugh classification to improve its predictive value, although the prognostic accuracy of TSF is lower than that of MAMC [35].

Regarding the association with HE, the study by Merli et al. confirmed that the prevalence of this complication among hospitalized patients was higher in patients with low muscle mass, as measured by TSF and MAMC, as well as in those with reduced muscle strength [36].

- Subjective global assessment questionnaire (SGA): this is one of the most widely used methods for hospitalized patients. It is a questionnaire that uses several components of nutritional assessment including objective examination, dietary and clinical history (weight, dietary intake, gastrointestinal symptoms, functional capacity, nutritional demands and metabolic demands), to classify patients according to their degree of malnutrition. It is quick and easy to administer and takes approximately 15 min; it has been validated for nutritional assessment in cirrhotic patient and may provide prognostic information [8].

However, this method has some methodological limitations in patient with HE. In fact, personal information is required, and they are difficult to obtain in patients with impaired cognitive status; moreover, the only anthropometric measure used is body weight, which is often affected by the presence of ascites or oedema; finally, it is poorly sensitive in detecting early stages of malnutrition [6] and in fact the ISHEN guidelines state the possibility of underestimating malnutrition with this method [27].

### *6.2. Instrumental Methods*

- Bioimpedance testing: it is a tricompartmental model technique as it identifies the muscle body mass and the non-fat body mass, which is divided into extracellular body mass and cellular body mass or metabolically active tissue.

This is based on the principle that different types of tissue express a specific electrical conductivity, that make them recognizable. In particular, electrical conduction is faster through water and slower through adipose tissue, due to the resistance imposed by fat deposits.

It is a non-invasive, safe, simple to perform, inexpensive and sensitive method to obtain information about the subject's body composition and also prognostic information. However, it has some limitations in patients with electrolyte disturbances [8].

The measurement is performed by placing a pair of electrodes on the back of the hand and another pair on the back of the subject's foot [tetra-polar hand–foot technique), which are then connected to the measuring instrument. Then an imperceptible alternating

current of very low intensity (800 μA) and high frequency (50 KHz) is passed through the electrodes. The software then transforms the detected electrical measurements into clinical data, based on algorithms that take into account the reference values, the anthropometric measurements (weight and height), his age and sex.

The values obtained from these measurements are phase angle (PA) and body cell mass (BCM).

The latter estimates the body cellular elements and has been considered one of the best nutritional parameters to assess metabolic pathways such as protein turnover and energy expenditure [25]. Phase angle (PA) represents a measure of strength and muscle mass, and this value is reduced in advanced liver cirrhosis.

BCM identifies muscle body mass, and this is reduced in protein-calorie malnutrition and advanced stages of cirrhosis [25].

- Handgrip strength: this is an instrument used to assess muscle strength. It is one of the most sensitive methods for measuring nutritional status and has been shown to predict prognosis in patients with advanced liver disease [6].

In the study by Alvares-da-Silva et al., the ability of handgrip to detect malnutrition was superior to SGA; moreover, this method was the only one able to predict the incidence of significant complications of cirrhosis at one year in malnourished cirrhotic patients [37].

Regarding the association with HE, in the study by Merli et al., the prevalence of HE, both minimal and overt, was higher in patients with impaired muscle strength measured by this method, suggesting a correlation between muscle function and HE [36].

However, this method has some limitations, especially in relation to the different body composition between men and women.

In fact, according to the ISHEN consensus, muscle function is associated with muscle mass only in males; therefore, dynamometry would not be a reliable tool for measuring nutritional status in women [27].

- DEXA: this examination has received particular attention because it is widely used to validate body composition results obtained with other methods. The procedure is based on measuring body composition according to a model that divides body elements into bone, fat, muscle mass and body free mass based on the passage of photon. In addition, compared to other techniques, radiation exposure is minimal [25].

Thus, DEXA allows the assessment of muscle mass and fat mass, with good correlation with both bioimpedance and anthropometric measurements [25].

With DEXA we can obtain the muscle mass to height squared ratio, called fat-free mass index (FFMI). In the study by Kalaitzakis et al. in cirrhotic patients awaiting liver transplantation, this index was found to be an independent predictor of risk of HE [38].

DEXA can also be used to calculate the appendicular muscle mass index (AMMI), which is obtained by dividing the appendicular muscle mass of the four limbs (free of fat and bone tissue) by height squared [25]. This index provides a more accurate estimate of muscle mass because it does not use bone density, which is affected by age, ethnicity and medications. It also excludes the trunk, which is often involved in water retention in cirrhotic patients [25].

One of the major advantages of DEXA is the high reproducibility of measurements. In addition, it also allows measurement of bone density, and this is an important finding to obtain, because it is often reduced in cirrhotic patients and can increase the risk of fractures [25].

Nevertheless, a possible disadvantage is the water imbalance that can alter the passage of X-rays [25].

- Indirect calorimetry: is used to define REE by measuring oxygen consumption and carbon dioxide production. The patient is considered hypermetabolic if the REE is 10–20% higher than the reference value. However, this method is expensive, available only in some centres and may be affected by the presence of ascites [8].
- Computed tomography scan: recently there has been a growing interest in the use of CT scan for the assessment of muscle mass loss and the presence of porto-systemic shunts, that may favour the development of HE in cirrhotic patients.

Measurements are made in a single image at the level of the intervertebral disc between the third and the fourth lumbar vertebra, discriminating muscle tissue from other tissues by density limits. The 35 Hounsfield (HU) limit is used to discriminate muscle tissue from fat, whereas the 150 HU limit is used to discriminate it from bone [25].

The skeletal muscle index (SMI) is obtained from muscle area to height squared ratio; sarcopenia is defined when this value is <39 $cm^2m^2$ and 50 $cm^2/m^2$ in women and men, respectively [25].

Sarcopenia, defined according to this method, is significantly correlated with mortality [30]. The limitations of this method are certainly the high cost and the exposure to ionizing radiation [25].

- Assessment of global physical performance: up-and-go test, six minutes' walk test.

As evident, for reasons of sensitivity, specificity, availability and cost, there is no ideal method for nutritional assessment of cirrhotic patients, especially with HE, since the degree of HE may condition the use of certain methods and compliance of patients.

Probably the best approach, as also recommended by the European Society for Enteral and Parenteral Nutrition (ESPEN) guidelines, is the multiparametric one. Thus, as an initial assessment, the use of indirect measures such as the SGA scale and anthropometric assessments is recommended, since they are sensitive and adequate to identify subjects at risk of malnutrition. After identifying malnourished subjects, the use of quantitative methods, such as BIA, is recommended as they are more accurate in classifying patients [39].

Figure 1 illustrates the diagnostic algorithm for evaluating the nutritional status in cirrhotic patients.

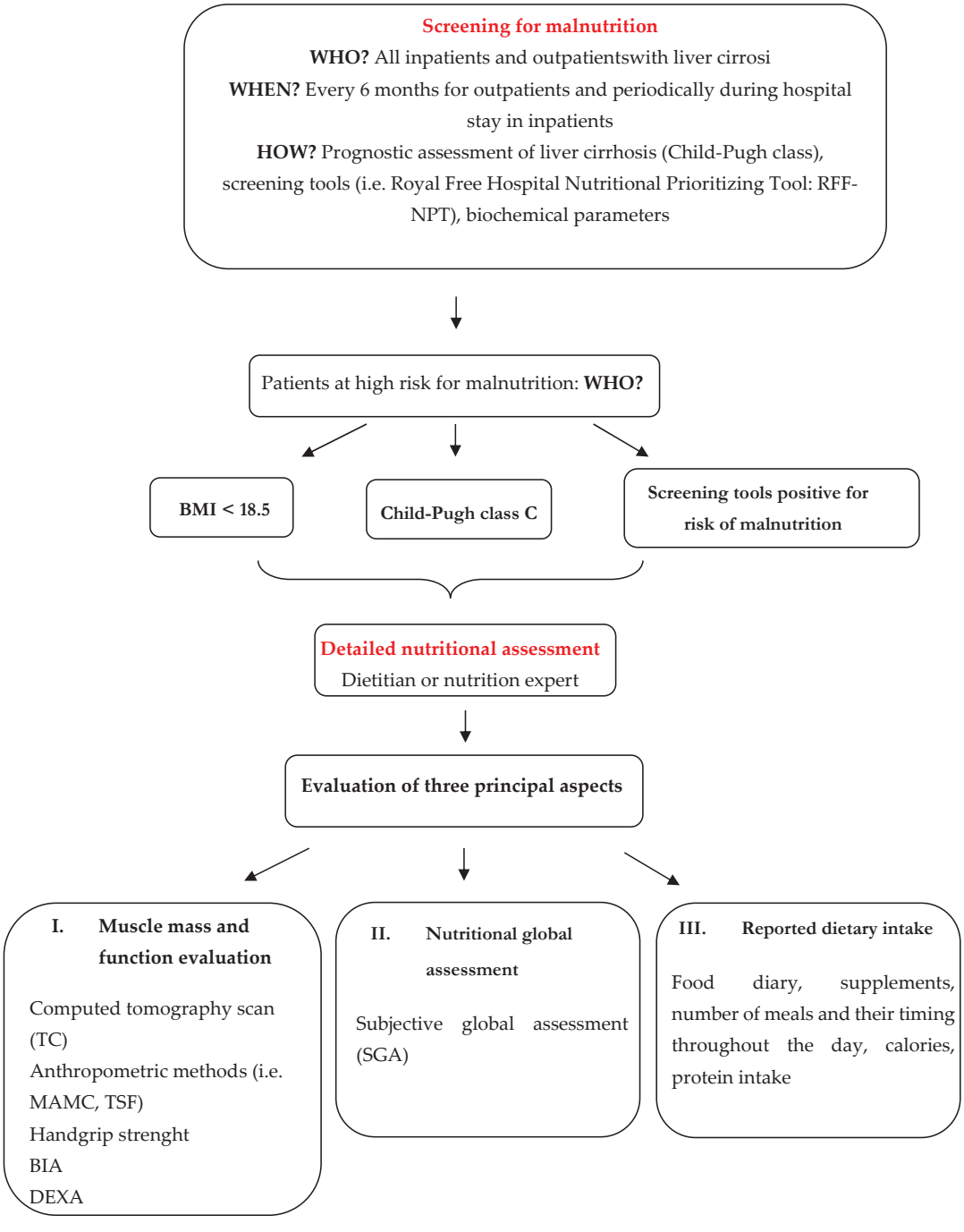


**Figure 1.** Diagnostic algorithm for nutritional status evaluation in patients with liver cirrhosis.

## 7. Optimization of Nutritional Status

The quality of evidence supporting dietary interventions in liver cirrhosis is poor and limited and those conducted in patients with HE are even more limited. Moreover, studies conducted in this field are often short-lived.

The multidisciplinary approach is fundamental. In fact, dieticians are not only trained in the aspect of nutritional assessment, but also have the ability to identify nutritional problems and provide appropriate dietary counselling tailored to patients [6]. In contrast, few physicians receive significant training or have the time to perform a comprehensive nutritional assessment; so, they risk underestimating this aspect [6].

Diet, although a key aspect in clinical management of cirrhotic patients, is often underestimated. In fact, in a retrospective study by Huynh et al., only 57% of hospitalized patients received a formal nutritional assessment at time of admission and of these, 56% were judged malnourished [40].

The importance of nutritional aspect in cirrhotic patients is confirmed by the study of Iwasa et al. who demonstrated that an early dietary intervention performed with a multidisciplinary approach in patients with cirrhosis was able to improve survival and quality of life [41].

Therefore, it seems essential to take care of nutritional aspect of cirrhotic patients as for any other complication of cirrhosis [27].

A relevant problem concerns the obesity and metabolic comorbidities of patients with post-NASH cirrhosis, in which patients often appear overweight or obese and in which it is necessary to recommend low-calorie diet and weight loss.

However, in these patients, in the absence of counselling and a clear-cut specialist, the risk of incurring in sarcopenia is high, despite the underlying obesity. A study by Berzigotti et al., showed that a low-calorie diet, high protein content and moderate physical activity were safe and effective in weight loss if adequately monitored [42].

The goals of nutritional intervention are to improve protein-calorie malnutrition, ensure adequate nutrient intake, achieve a positive nitrogen balance and avoid hepatotoxic agents and muscle loss [8]. Other goals are to avoid progression of liver failure and to manage complications arising from the disease [6].

As suggested by the ISHEN guidelines, the feeding of cirrhotic patients with HE should not be different from that of cirrhotic patients without HE. In fact, the recommendations are equivalent [27]. What should be avoided are dietary restrictions, which appear harmful, counterproductive and can worsen the protein breakdown.

Strategies that can be implemented to improve the nutritional status of the cirrhotic patient involve several areas.

Table 1 summarizes the dietary recommendations for cirrhotic patients, while Table 2 illustrates the main studies published on this topic.

**Table 1.** Dietary recommendations for patients with cirrhosis.

| | **Normal** | | **Moderate Malnutrition** | | **Severe Malnutrition** | |
|---|---|---|---|---|---|---|
| BMI | <30 | >30 | <30 | >30 | <30 | >30 |
| Caloric intake (kcal/die) | 35–40 | 20–35 | 35–40 | 20–35 | 35–40 | 20–35 |
| Carbohydrate intake (%) | 50–60% | | | | | |
| Protein intake (g/die) | 1.2–1.5 | 1–1.5 | 1.2–1.5 | | | |
| Number of meals/die | 4–6 meals | | | | | |
| Bedtime snacks | High in calories (at least 50 g of complex carbohydrate) | | | | | |
| Protein source | Vegetables and dairy products | | | | | |
| Fibre (g/die) | 25–45 g | | | | | |
| Vitamin and micronutrients | Correction of deficiency as good clinical practice | | | | | |

**Table 2.** Published studies on dietary interventions and exercise in patients with liver cirrhosis.

| Target | Author/Year | Study Design | N. Patients | Intervention | Comparison | Duration | Main Results |
|---|---|---|---|---|---|---|---|
| Number of meals/late snack | Swart et al., 1989 [42] | Randomised crossover | *n* = 9 cirrhotic patients | 4–6 meals/die | Three meals/die | 2 periods of consecutive five days. | 4–6 meals/die resulted in more positive nitrogen balances than three meals/die. |
| | Plank et al., 2008 [43] | Randomized | *n* = 103 cirrhotic patients | Night-time supplementary nutrition (*n* = 52) | Daytime supplementary nutrition (*n* = 51) | 12 months | A night-time snack resulted in a total body protein accretion sustained over 12 months (equivalent to about 2 kg of lean tissue). |
| | Verboeket-van de Venne, 1995 [11] | Randomized crossover | *n* = 8 cirrhotic patients and 23 healthy subjects (controls) | 4–7 meals/die ("nibbling pattern") | 2 large meals ("gorging pattern") | Two periods of 2 consecutive days. | The "gorging pattern" had greater fluctuations in respiratory quotient and higher nocturnal protein oxidation than in the daytime in both groups, reflecting a higher oxidation ratio of fat to carbohydrate compatible with a more catabolic state. |
| | Nakaya et al., 2007 [44] | Randomized | *n* = 48 cirrhotic patients | Late-evening supplementation with BCAA-enriched nutrient mixture (= 25) | Late-evening supplementation with ordinary food (*n* = 23) | 3 months | BCAA supplementation significantly improved serum albumin level, nitrogen balance and respiratory quotient than ordinary food. |
| Caloric and protein intake | Manguso et al., 2005 [45] | Randomized | *n* = 90 cirrhotic patients | Controlled diet (*n* = 45) | Spontaneous diet (*n* = 45) | 3 months | The controlled diet caused an increase in MAMC, serum albumin and creatinine-height index |
| | Maharshi et al., 2016 [46] | Randomized | *n* = 120 cirrhotic patients with MHE | Nutritional therapy (30–35 kcal/kg/die and 1.0–1.5 g vegetable protein/kg/die) | No nutritional therapy | 6 months | A higher proportion of patients in the nutritional therapy group reversed MHE; nutritional therapy increased PHES and HRQOL and reduced OHE incidence. |

**Table 2.** *Cont.*

| Target | Author/Year | Study Design | N. Patients | Intervention | Comparison | Duration | Main Results |
|---|---|---|---|---|---|---|---|
| | Kato et al., 2013 [47] | Prospective | *n* = 19 cirrhotic patients with MHE | Nutritional consultation (30–35 Kcal/Kg/die and 1–1.5 g/Kg/die of protein) | - | 8 weeks | The MHE scores significantly improved at 8 weeks. |
| | Gheorghe et al., 2005 [48] | Prospective | *n* = 153 cirrhotic patients with OHE | High caloric and high protein diet (vegetable and dairy products) | - | 1 year | Almost 80% of patients improved their mental status; high protein diet significantly reduced ammonia level. |
| | Hirsch et al., 1993 [49] | Randomized | *n* = 51 patients with decompensated alcoholic cirrhosis | Oral nutrition support (1000 Kcal, 34 g protein) (*n* = 26) | One placebo capsule (*n* = 25) | | Oral nutrition support significantly improved nutritional status, MAMC, serum albumin and handgrip strength than placebo. |
| | Cordoba et al., 2004 [50] | Randomized | *n* = 30 cirrhotic patients with acute HE | Normal protein diet (*n* = 10) | Low protein diet (*n* = 10) | 14 days | The outcome of HE was not significantly different between both groups. |
| Protein source/ type of protein | Bianchi et al., 1993 [51] | Crossover randomized | *n* = 8 cirrhotic patients with chronic HE in therapy with lactulose | Diet containing vegetal proteins (50 g) | Isocaloric, isonitrogenous diets containing animal protein (50 g) | 2 consecutive periods of 7 days | The vegetable protein diet improved ammonia level, insulin and nitrogen balance, and clinical grading of HE. Psycometric tests improved significantly but remained abnormal |
| | Ruiz-Margáin et al., 2017 [52] | Randomized | *n* = 72 Cirrhotic patients | High protein and high-fibre diet with BCAA (protein: 1.2 g/Kg/die, fibre 30 g, BCAA 110 g) (*n* = 37) | High protein, high-fibre diet and no BCAA (*n* = 35) | 6 months | BCAA supplementation increased muscle mass. No significant changes in PHES or CFF score resulted in both groups (no development of HE). |
| | Horst et al., 1984 [53] | Randomized | *n* = 37 cirrhotic patients with recurrent HE | 20 g of dietary protein for 1 week, after which BCAA were added weekly to obtain a protein intake of 80 g/die (*n* = 14) | 20 g of dietary protein for 1 week, after which 20 g of proteins were added weekly to obtain a protein intake of 80 g/die (*n* = 12) | | BCAA supplementation significantly reduced HE recurrence and improved mental status grade and asterixis. |

**Table 2.** *Cont.*

| Target | Author/Year | Study Design | N. Patients | Intervention | Comparison | Duration | Main Results |
|---|---|---|---|---|---|---|---|
| | Uribe et al., 1982 [54] | Crossover randomized single blind | $n = 10$ cirrhotic patients with chronic HE | 40 g/die of vegetable protein (high fibre diet, low methionine and low aromatic amino acids) and 80 g/die of vegetable protein (rich in BCAA and fibre, with same amount of sulfurated amino acids). | 40 g/die of meat protein plus neomycin-milk of magnesia | 3 consecutive periods of 2 weeks | After 2 weeks, patients on vegetarian diets performed the NCT more quickly than meat diet. Patients treated with the 80 g/day vegetable diet improved EEG. |
| | De Brujin et al., 1983 [55] | Randomized crossover | $n = 8$ cirrhotic patients with MHE | 60 g/die of vegetable diet (second and fourth week) | 60 g/die of a mix diet with 1:1 ratio of vegetable and meat diet (first, third and fifth week) | 5 consecutive periods of 1 week. | During the vegetable diet, the nitrogen balance tended to be more positive, but without changes in neurological status or ammonia level. |
| | Keshavarzian et al., 1984 [56] | Crossover randomized | $n = 6$ cirrhotic patients with chronic HE on lactulose therapy | 80 g vegetable-supplemented diet (3:5 ratio of animal and vegetable protein) | 40 g protein conventional diet (3:1 ratio of animal and vegetable protein) | 2 consecutive periods of 5 days. | After 10 days, patients treated with a vegetable diet showed clinical improvement and amelioration of EEG. |
| Physical exercise | Zenith et al., 2014 [57] | Randomized | $n = 20$ | exercise training ($n = 10$) | usual care ($n = 10$) | 8 weeks | Aerobic exercise increased peak VO2 and muscle mass and reduced fatigue in cirrhotic patients. |

### 7.1. Caloric Requirement

Cirrhotic patients who are not malnourished and in the absence of stressful conditions should have 30 Kcal/Kg/day; however, energy demand should be gradually increased to 35–40 Kcal/Kg/day under stressful conditions (e.g., bleeding, infections or surgery) or in malnourished subjects to achieve the state of anabolism [7].

The study by Maharshi et al. conducted in India demonstrated that an appropriate nutritional regimen with adequate calorie intake for 6 months in patients with minimal HE (MHE) was effective in treating this condition and especially in preventing progression to overt HE [46].

Obviously, caloric excess should also be avoided because it has a detrimental effect on lipiogenesis and thus on residual liver function [8].

These recommendations do not apply to obese patients. In fact, in this case the energy intake should be moderately reduced to 20–25 Kcal/Kg/day, avoiding excessive restriction that could be responsible for loss of muscle mass. Energy reduction should concern lipids and carbohydrates, while protein intake should be preserved. In addition to diet, moderate physical activity should be recommended [12].

In these patients, the increase in number of daily meals to four–six should also be encouraged, introducing snacks in the mid-morning, mid-afternoon and before bedtime to prevent gluconeogenesis, since this can cause sarcopenia, increased ammonium production and therefore HE.

Confirming this, the study by Swart et al. showed that consuming four–six meals per day resulted in a positive nitrogen balance, compared to three meals per day [41].

The evening snack should contain complex carbohydrates to ensure slow glucose absorption and should be high in calories (at least 50 g of carbohydrate) [58].

Recently, an evening snack containing BCAAs has been shown to be effective in preserving muscle mass, nutritional status and preventing HE episodes, infections and mortality [39]. Indeed, while overnight BCAAs are preferentially used for protein synthesis, during the day they are primarily used as an energy resource [8].

### 7.2. Protein

In cirrhotic and HE patients, the goal of maintaining adequate protein intake is to improve nitrogen balance and prevent sarcopenia.

Prevention of sarcopenia may be advantageous especially in patients with HE, since muscle is known to represent an alternative site for ammonium detoxification.

In fact, several studies have examined the effect of protein diet on changes in cognitive status in patients with HE, confirming its undoubtedly beneficial role [17]. In contrast, even a transient protein restriction has not shown any benefit in patients with HE.

Contrary to what was originally thought about the role of protein in encephalopathic patients, in 1997 the ESPEN published new guidelines recommending an adequate protein intake in patients with liver disease, possibly around 1–1.5 g/kg/day, depending on the degree of decompensation and accordingly with renal function.

In case of intolerance to dietary protein, the same guidelines suggested to administer 0.5 g/Kg/day of protein and to supplement the rest with BCAAs to ensure the achievement of an adequate daily protein intake [39]. This dosage is supported by numerous studies that confirm the achievement of positive nitrogen balance by supplementation.

In support of this evidence, the study of Cordoba et al. showed that a diet with normal protein content, being metabolically more adequate, could be safely administered to patients with HE while a low protein content diet did not confer any benefit [50].

The effect of protein feeding is related not only to the amount of protein intake, but also to the timing of intake. In fact, night-time supplementation is associated with greater gains in muscle mass than daytime supplementation [6].

There is also a strong debate about the origin of dietary protein intake.

Some studies showed a significant decrease in ammonium levels by replacing meat with dairy products [6].

Among proteins, plant proteins contained in fruits, vegetables, cereals and legumes seem to be a good choice to increase dietary protein intake [59], also because they are often better tolerated than animal proteins [60].

In this regard, the study by Bianchi et al. demonstrated the superiority of plant proteins over animal proteins in association with lactulose in reducing ammonium levels and improving neurological symptoms in a small group of patients with overt HE [51].

In fact, ISHEN guidelines recommend, in the cirrhotic patient, to prefer vegetable and dairy proteins over meat and fish [27].

The reasons why diet rich in vegetable protein and low in animal protein results in improved HE is not clearly known. There are a few possibilities:

- The lower ratio of sulphur amino acids, such as methionine and cysteine, to BCAAs [59];
- The reduced formation of mercaptans from sulphur amino acids fermentation, which appear to be involved in the genesis of HE along with ammonium [13];
- The significant increase in fermentable fibre. In fact, levels of protein fermentation end products, such as ammonium and phenols, are significantly reduced when dietary fibre intake is increased. This occurs because increased fermentation of carbohydrates by colonic bacteria results in increased nitrogen utilization by bacteria and in pH reduction, which favours excretion of ammonium over its absorption [61].
- The increased clearance and reduced intestinal absorption of nitrogen products as a result of reduced intestinal transit time due to the mass-forming effect of fibres [17].
- The modulation of intestinal microbiota [62]. In liver cirrhosis, the prevalence of potentially pathogenic bacteria such as Enterobacteriaceae and Streptococcaceae, and the deficiency of beneficial populations such as Lachnospiraceae, may impact the prognosis of patients with cirrhosis [63].

The microbiota is also modified in patients with HE. In fact, high levels of Alcaligenaceae, Enterobacteriaceae and Fusobacteriaceae and low levels of Ruminococcaceae and Lachnospiraceae have been found in these patients compared with healthy controls and cirrhotic patients without HE [64].

In light of this evidence, it could be thought that the beneficial effect of vegetable proteins is actually related mainly to the high fibre content.

The effect of fermentable fibre, could in this case recall that of lactulose that, once fermented, traps ammonium in the nonabsorbable form NH4 and allows its elimination with the stool [6].

Therefore, a rational dietary intervention might be to manipulate the fermentable carbohydrate/protein ratio. This would provide an effect similar to that of lactulose while still ensuring adequate protein intake [6].

It is clear that an adequate nutritional intervention cannot be generalized, but must be adapted case by case according to patient, his nutritional status and gastrointestinal tolerance to fermentable fibres [6].

### *7.3. Protein Supplementation*

The availability of BCAAs in cirrhotic patients is reduced and this can impair the ammonium conversion to glutamine in skeletal muscle, with deleterious effects on its elimination.

A review of 16 randomized trials that compared BCAAs, administered orally or intravenous, vs. placebo, no intervention, diet, neomycin or lactulose, revealed that BCAAs have beneficial effects on symptoms and signs of HE, without affecting mortality or quality of life [64].

For oral and long-term supplementation with BCAAs, the recommended daily dosage is 0.25 g/Kg/day, which demonstrated beneficial effects on nutrition and reduction in recurrence and symptoms of HE [65].

However, some studies have not demonstrated these clear benefits, so ESPEN guidelines do not currently recommend its regular use [39].

On nutritional basis, BCAAs have been shown to increase energy and protein intake, reduce anorexia and improve both albumin levels and nitrogen balance. In addiction, they improve the nutritional status of cirrhotic patients by counteracting protein loss and promoting protein synthesis; finally, BCAAs can enhance the innate and adaptive immune response [66].

However, a barrier to this supplementation remains patient compliance, which is often reduced due to the poor palatability of the products, the frequency of daily intake and possible associated gastrointestinal symptoms such as abdominal distension and diarrhoea [28]. A viable alternative could be to obtain the same benefits from BCAA-rich foods.

### 7.4. Carbohydrates and Lipids

The literature regarding the role of carbohydrates and lipids in the management of malnutrition is less than that of proteins; so, there are no particular recommendations in cirrhotic patients with HE.

It is known that complex carbohydrates are useful in delaying the switch to the fasting phase of metabolism, which causes increased amino acid utilization and renal ammoniagenesis [12].

The main concern with carbohydrate intake relates to hyperinsulinemia and diabetes, which are seen in approximately 40% of cirrhotic patients.

However, from the available data, a diet rich in carbohydrates, especially if complex and with low glycaemic index, would not seem to worsen glycaemic control in cirrhotic patients and indeed it would represent an additional source of energy [6].

In this regard, the study by Schulte-Frohlinde et al. showed that a diet rich in proteins and carbohydrates resulted in a greater increase in plasma insulin levels than one rich in protein. However, when a low-carbohydrate diet of 20% of daily calories was subsequently instituted, no changes in plasma insulin levels, blood glucose or BCAA/AAAs were observed compared with a high-carbohydrate diet of 60% [67].

In general, carbohydrates should therefore represent the mainstay of the diet in the cirrhotic, amounting to approximately 50–60% of non-protein energy requirements [8].

Relative to lipids, these can be freely administered to cirrhotic patients. Indeed, nuts such as almonds, hazelnuts and peanuts are highly caloric and rich in lipids and therefore may be useful in cachectic patients with reduced appetite.

Conversely, lipid reduction should be recommended in obese patients [12].

### 7.5. Fibres, Vitamins and Micronutrients

As previously hypothesized, the beneficial effect of vegetables in patients with liver cirrhosis may actually be due to their high fibre content.

Therefore, in light of their prebiotic and laxative effect, consumption of fibre from fruits and vegetables should be encouraged in cirrhotic patients with HE, amounting to a daily intake of 25–45 g/day, where tolerated. In obese patients, increased fibre consumption may also be beneficial to achieve early satiety.

Likewise, cirrhotic patients with HE should be encouraged to consume milk-derived products, such as yogurt, in light of their probiotic effect and modulation of the intestinal microbiota [12].

Confirming this evidence, the study by Gheorghe et al. showed that a high-calorie, high-protein diet rich in vegetables and dairy products administered for 14 days to patients with HE in addition to standard therapy was able to determine an improvement in cognitive status in up to 80% of subjects [48].

Relative to vitamins, their deficiency may be responsible for neuropsychiatric symptoms that may overlap with HE symptoms or make their differential diagnosis difficult. Therefore, brief vitamin supplementation is recommended in patients with HE, paying attention to any deficiencies that may develop during follow up [12].

Regarding vitamin D supplementation, in cirrhotic patients it is indicated for levels <30 ng/mL by administration of 5000 IU/day of vitamin D3 or 50,000 IU/week of vitamin D2 or D3 for three months. This would also seem to have a favourable impact on survival by enhancing immune defences against viruses and bacteria [7].

Finally, in patients with cirrhosis and HE, the hydro-electrolyte balance should always be monitored. Prompt recognition of micronutrient deficiency is crucial because the use of nutritional supplements has been shown to be associated with a reduction in infection risk and in-hospital mortality, as well as with an improvement in liver function [8].

Relative to zinc deficiency, which is extremely frequent in cirrhotic patients, the beneficial effects of supplementation on neurologic symptoms are still debated and would appear to be limited. In any case, the deficiency should always be corrected, as good clinical practice, at an oral dosage of 600 mg/day during the treatment of underlying HE [7].

Another deficiency that may be encountered in the patient with cirrhosis and HE is sodium deficiency. Management of hyponatremia is challenging for both physicians and patients.

Hypovolemic hyponatremia should be treated with fluid resuscitation to restore the circulatory volume and withdrawal of the precipitating factor, usually diuretic therapy.

On the contrary, hypervolemic hyponatremia in cirrhosis is ideally managed with fluid restriction and measures to enhance the renal solute-free water excretion to a level sufficient to induce a negative water balance. Hypertonic saline is indicated in symptomatic patients who are intolerant or unresponsive to free water restriction and in those with profound hyponatremia (<110 mEq/L). In this case, correction of hyponatremia should be conducted slowly to prevent central pontine myelinosis. In general, although a low-sodium diet (<2 g) is recommended in cirrhotic patients with ascites, daily sodium intake should be >60 mmol [1.38 g) to prevent palatability problems and consequent reduction in dietary intake [27]. However, compliance with fluid restriction is usually poor in these patients and conventional therapy is frequently inefficacious because the progression of cirrhosis and ascites leads to impairment of the kidneys to eliminate solute-free water and, while fluid restriction is helpful in preventing a further decrease in serum sodium concentration, it is rarely effective in improving it.

One option could be the use of drugs that act on the hormonal mechanisms underlying hyponatremia; vaptans selectively block arginine-vasopressin hormone V2 receptors in major collector duct cells; however, their use is currently recommended only in an experimental setting [1].

Finally, the pragmatic approach in cirrhotic patients, suggested by the ESPEN guidelines, is a brief supplementation of vitamins and micronutrients during the first two weeks of nutritional support, because assessment of deficiency of each micronutrient would require high costs and delays in initiating supplementation [39].

### *7.6. Parenteral Nutrition*

This represents a second choice to enteral nutrition, but should be initiated when a patient cannot be fed orally or parenterally or when an adequate caloric goal is not achieved. The use of parenteral nutrition, possibly enriched with BCAAs, is indicated in patients with HE who appear comatose because of risk of inhalation, or when enteral nutrition is not possible. It should be initiated in cases of non-functioning gastrointestinal tract, intestinal obstruction, unprotected airway, intolerance to enteral nutrition or when the fasting period exceeds 72 h [8].

The recommended caloric requirement is 35 Kcal/Kg/day with 1.2 g/Kg/day of protein, which can be increased to 1.5 in severely malnourished patients or those under severe stress [55].

However, enteral nutrition is always preferable to parenteral nutrition because of risk of infection, water overload and cholestasis [7].

### 7.7. Physical Exercise

One thing to keep in mind is the role of physical exercise. In fact, several studies have shown that a combination of diet, dietary supplements and exercise can lead to improved muscle mass in cirrhotic patients.

In fact, exercise is known to have a beneficial effect on muscle mass in patients with chronic disease [62].

The prospective study by Zenith et al. showed that eight weeks of controlled aerobic exercise in patients with Child-Pugh Class A and B liver cirrhosis, was able to increase muscle mass and reduce fatigue, without experiencing any adverse events [57].

As previously mentioned, the improvement of muscle mass can have a positive effect on risk of developing HE.

## 8. Conclusions

HE is a frequent complication and one of the most debilitating clinical manifestations of liver disease, associated with decreased survival and a high risk of recurrence.

Nitrogen metabolism plays a key role in the pathogenesis of HE in patients with liver cirrhosis and therefore its modulation may play a key role in the treatment of this complication.

One of the first steps in the evaluation of patients with liver cirrhosis, especially with HE, is the assessment of nutritional status. However, this is often problematic because it is conditioned by different body composition between men and women, the cost and availability of some methods, the lack of a standardized evaluation criterion and the need to have the cooperation of patients. This last point is essential in some evaluations, but in patients with altered cognitive status this goal cannot always be achieved.

Dietary control represents a valid tool to improve the nutritional status and prognosis. In particular, it can be useful to prevent sarcopenia, which is included among the "new precipitants" of HE; in fact, skeletal muscle is involved in the detoxification of ammonium, activating the enzyme glutamine synthase that catalyses the condensation of ammonium with glutamate to form glutamine.

Despite the impact of nutritional aspects on patient's prognosis, this field is often neglected or addressed only in stages in which malnutrition is frankly evident.

Therefore, it would be essential to manage patients in a multidisciplinary team involving a specialized dietician and address this aspect even in the early stages of the disease, in order to prevent the appearance of sarcopenia and potentially other complications of liver cirrhosis such as HE.

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# Ammonia and the Muscle: An Emerging Point of View on Hepatic Encephalopathy

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**Abstract:** In the last years the link between the presence of muscular alterations and hepatic encephalopathy (HE), both minimal and overt, has been deeply studied. The pathophysiological background supporting the relationship between muscle depletion, and HE is characterized by an imbalance between the capacity of muscle in ammonia metabolism and trafficking and the inability of the liver in removing ammonia through urea synthesis due to liver failure and/or the presence of porto-systemic shunts. This review will focus on the clinical burden, the physio pathological mechanisms understanding the liver muscle axis and principles of management of muscular alterations in cirrhosis.

**Keywords:** cirrhosis; sarcopenia; myosteatosis; hepatic encephalopathy

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## 1. Introduction

Hepatic encephalopathy (HE) is defined as a spectrum of neuro/psychiatric alterations caused by liver failure and/or porto-systemic shunts with different clinical conditions ranging from subclinical alterations to coma [1]. It's one of the most frequent complication of liver cirrhosis affecting up to 30–40% of cirrhotic patients. It can be divided into overt hepatic encephalopathy (OHE), that is clinically evident and minimal hepatic encephalopathy (MHE), a condition characterized by subclinical alterations detectable only with psychometric tests or electroencephalography. More recently the term "covert HE" has been coined to combining MHE and Grade I of overt HE. The aim is to unify the terminology, make the diagnosis clearer and allow the design of more inclusive and uniform clinical studies. The term "covert" refers to a condition that is not unapparent, but also not overt. Covert HE, if investigated, can reach up to 80% of cirrhotic patients [1]. It is well known that HE worsens the prognosis of patients with cirrhosis and it is one of the main causes of hospitalization [2]; in particular MHE has been associated with falls [3,4] and car accidents [5–7], a reduction in quality of life and the socio-economic status and has a major impact on economic health costs [8–10].

Another common complication of liver cirrhosis is malnutrition; it correlates with the severity of liver disease and disease complications, including hepatic encephalopathy [11]. Sarcopenia, the generalized loss of muscle mass and function, is the major component of malnutrition [12]. Skeletal muscle is the main protein reserve in the body, it is maintained thanks to a continuous balance between protein synthesis and catabolism (proteostasis), and represents up to half of the entire protein turnover of the body [13]. Although it is a pathophysiological mechanism that correlates with the advancement of age (primitive sarcopenia), some chronic pathologies can accelerate this process (secondary sarcopenia) [13].

Sarcopenia is present between 30% and 70% of cirrhotic patients, with an increasing trend depending on the degree of liver disease [14]. Sarcopenia is a part of the frailty

complex in cirrhotic patients, with a decreased reserve and resistance to stressors. Furthermore, it has been demonstrated that sarcopenia increases mortality in cirrhotic patients. In recently published metanalysis by Tantai et al. [15], authors conclude that sarcopenia was highly and independently associated with higher risk of mortality in patients with cirrhosis.

It has been suggested that considering muscle alterations in addition to prognostic scores improves the prediction of death in patients with cirrhosis [16]. Despite this, nutrition is often overlooked as nutritional assessment could be complex in cirrhotic patients [17]. Moreover, muscular alterations not only affect muscle mass but also its quality and function, making the picture more complex.

The methods for the diagnosis of sarcopenia are not uniform. This makes the overall evaluation of the studies difficult. They can be based on the different aspects of sarcopenia, that are the assessment of muscle mass, muscle performance and muscle strength. The gold standard for assessing sarcopenia is CT-scan, which allows to evaluate the muscle mass at the level of the L3 section [17].

The link between muscle alterations and liver disease is not well defined; it is multifactorial and includes hormonal alterations [18], hyperammonemia [19,20] and endotoxinemia [21]. Portal hypertension could play a role regardless of liver function in sarcopenia genesis [22], although this link needs to be better investigated. Therapies that act by reducing portal hypertension, such as transjugular intrahepatic portosystemic shunt, seem to improve muscle structure [23,24].

Among the etiological causes one of the most important roles is certainly given by hyperammonemia, which is probably the most evident expression of the close link that existing between liver and muscle. For this reason, cirrhotic patients should never be evaluated without considering the nutritional aspect and muscular alterations that are often present.

## 2. Muscle Alterations and Hepatic Encephalopathy in Liver Cirrhosis

During the natural history of chronic liver disease, muscle alterations may appear and these include muscle wasting, sarcopenia and myosteatosis. Sarcopenia is the most prevalent muscle abnormality, with a generalized reduction in muscle mass and function. It is defined as a muscle mass two standard deviations below the healthy young adult mean [25]. Myosteatosis is a fat infiltration of the skeletal muscle mass with an increased proportion of intramuscular and intermuscular fat that could impact muscle function and lead to a systemic inflammation [26].

The presence of myosteatosis and sarcopenia has been associated with a poor prognosis in patients with liver cirrhosis [27]. Moreover, these conditions are associated to several complications of liver cirrhosis, such as ascites [28], Spontaneous bacterial peritonitis (SBP) [29], variceal bleeding [30], hepatocellular carcinoma [31] and infections [32,33].

One of the closest and well-known relationship between muscle alterations and complications of liver disease is between sarcopenia and hepatic encephalopathy [34] (Table 1).

### *2.1. Sarcopenia and Cognitive Impairment*

From as far back as 1964 it has been known that malnutrition could impact the prognosis of patients with liver cirrhosis in specific settings [35]. This link was confirmed by a large Italian multicentric prospective study conducted in 1996 [36]. Since that moment, several studies were conducted to investigate the link between malnutrition and liver complications. Among these, it was immediately clear that patients with malnutrition were at higher risk to develop cognitive impairment.

Soros et al. in 2008 analyzed 223 cirrhotic patients' muscle mass (assessed by bioelectrical impedance analysis): parameters of fat and fat-free mass were found to be similar in patients with and without HE [37].

Despite this, more recent studies demonstrated different results. Using handgrip strength (HGS) in 84 cirrhotic patients in 2011, Huisman et al. [28] found that muscle strength was an independent predictor of complications (including HE) after correcting for

comorbidities, age and Child Pugh score. In a large cohort of patients (675 cirrhotic patients enrolled from 2000 to 2014) [38], sarcopenia was found to be associated with a higher risk of overt hepatic encephalopathy (OHE). In this study, sarcopenia was evaluated with CT scan, in particular cross-sectional areas were obtained from transverse CT images at the level of L3 of each patient and adjusted for height to calculate the Skeletal Muscle Index (SMI). In this study there was a strong correlation between sarcopenia and mortality, after adjusting for multiple confounding factors.

More recently, a Japanese group retrospectively analyzed nearly 300 patients that have a HGS measurement [39]. They found that HGS was able to stratify patients at high risk to develop OHE. Despite the retrospective nature of this study, it demonstrated that a simple and economical measurement bedside of the patient can reliably discriminate patients at risk for this complication.

In 2013 Merli et al. [40] enrolled 300 cirrhotic patients and at multivariate logistic regression analysis, muscle depletion, evaluated with BMI, mid-arm-muscle-circumference (MAMC), triceps skinfold-thickness (TSF) and HGS, was found as an independent risk factor for OHE during hospitalization. Moreover, this study was one of the first study that investigated the relationship between muscle depletion and minimal hepatic encephalopathy (MHE). MHE is a subclinical condition in which cognitive impairment isn't detectable with physical examination, but only with psychometric tests [41,42], electrophysiological and other functional brain measures [43,44]. In this study [41] MHE was evaluated with psychometric tests and the reduction in muscle mass and muscle function were significantly associated not only with overt HE but also with MHE. Although the relationship between muscular alterations and MHE was directly researched for the first time in this study, in 2007 it has been demonstrated that patients with malnutrition (assessed by anthropometry and estimation of recent weight change) and patients with diabetes mellitus were at higher risk of cognitive impairment [45].

In a retrospective study conducted by Hanai et al. in 2017 [46], appendicular skeletal muscle mass (ASM) using bio-impedance analysis and HGS were performed to investigate the presence of sarcopenia. In this cohort of patients (120) sarcopenia was strongly associated with the presence of MHE. In addition to the retrospective and single-center nature of this study, another limit is that MHE was investigated with number connection test-A (NCT-A), number connection test-B (NCT-B), digit symbol test (DST) and block design test (BDT), a combination easier and quicker than the gold-standard PHES (psychometric hepatic encephalopathy score) [11].

However, all these results have been recently confirmed in a prospective study of 64 patients with liver cirrhosis [34]. The muscle assessment was investigated with CT-scan using Carey's cut-off of the SMI for determination of sarcopenia [47]. Thirty-two patients (50%) had MHE at the time of enrollment, of whom 84% had sarcopenia; only 31% of patients without MHE had sarcopenia. In the multivariate analysis, only sarcopenia, myosteatosis and previous episodes of HE, were independently associated to the presence of MHE.

### *2.2. Myosteatosis and Cognitive Impairment*

It is well known that the reduction in muscle mass is not the only muscle alteration that can be associated with chronic liver disease. The infiltration of muscle mass by intermuscular and intramuscular fat was first described in 1983 because of ageing [48] and metabolic abnormalities [49] and later defined as myosteatosis [50]. It is associated with poorer muscle strength and physical performance in older persons. As in sarcopenia, it has been demonstrated that this condition can appears also in younger people with chronic disease [51].

Montano-Loza et al. [27] have demonstrated that sarcopenia and myosteatosis increase the risk of mortality by 1.5-to twofold compared with patients without muscular abnormalities. Whereas these are very frequent alterations in cirrhotic patients, it's important to investigate them. In this study, however, they have considered mainly patients with

advanced liver cirrhosis (Child-Pugh B-C) and the percent of hepatocellular carcinoma was quite high.

Few studies have analyzed the relationship between myosteatosis and HE. Bhanji et al. have studied a large cohort of cirrhotic patients with an available CT-scan in a retrospective analysis [38]. At multivariable regression analysis, myosteatosis was independently associated with a higher risk of HE. Patients with HE and myosteatosis had worse survival (15 ± 8 months), in comparison to those without these conditions (58 ± 14 months; $p = 0.001$) or with only HE or myosteatosis (31 ± 6 months; $p = 0.02$). Nardelli et al. investigated for the first time the link between myosteatosis and MHE [34], demonstrating that myosteatosis was strongly associated not only with OHE but also with MHE.

**Table 1.** Studies evaluating the relationship between muscle alterations and hepatic encephalopathy in cirrhosis.

| First Author (Year) | Number of Patients | Methods to Identify Sarcopenia and/or Myosteatosis | Prevalence of Sarcopenia and/or Myosteatosis | Results |
|---|---|---|---|---|
| Merli et al. (2013) [40] | 300 hospitalized cirrhotics | Anthropometric measurements (MAMC) and handgrip strenght (HGS) | 48% | Overt HE in 30% with sarcopenia vs. 15% without sarcopenia ($p = 0.003$) Minimal HE in 49% with sarcopenia vs. 30% without sarcopenia ($p = 0.001$) |
| Hanai et al. (2017) [46] | 120 cirrhotics | Bio-impedance Analysis (BIA), handgrip strenght | 27% | Sarcopenia and serum branched-chain amino acids levels were associated with MHE in the multivariate analysis ($p = 0.02$ and $p = 0.03$ respectively). |
| Miwa et al. (2021) [39] | 270 cirrhotics | Handgrip strength | 38% | Multivariate analysis showed that reduced HGS was associated with a higher prevalence of CHE and higher risk for developing OHE |
| Nardelli et al. (2017) [52] | 46 cirrhotics submitted to TIPS | CT scan to evaluate sarcopenia with Skeletal Muscle Index (SMI) | 57% | Twenty-one patients (46%) developed overt HE after TIPS placement; all of these patients were sarcopenic. At multivariate analysis, only MELD score ($p = 0.043$) and sarcopenia ($p < 0.001$) were independently associated with the development of HE after TIPS placement. |
| Kalaitzakis et al. (2007) [45] | 128 cirrhotic patients | BMI, weight loss, MAMC and triceps skinfold | 40% | HE in 46% with malnutrition vs. 27% without malnutrition ($p = 0.03$) |
| Huisman et al. (2011) [30] | 84 cirrhotic patients | Handgrip strength | 67% | Increased complications in cirrhotic patients with lower muscle function, including HE (18% vs. 48%, $p = 0.007$) |
| Nardelli et al. (2019) [34] | 64 cirrhotic patients | CT scan to evaluate sarcopenia and myosteatosis | Sarcopenia 58% Myosteatosis 38% | Both myosteatosis and sarcopenia were more frequent in patients who developed overt HE. On multivariate analysis, only sarcopenia ($p = 0.005$) and myosteatosis ($p = 0.002$) were independently associated to the development of overt HE. |
| Bhanji et al. (2018) [38] | 675 cirrhotic patients | CT scan to evaluate sarcopenia and myosteatosis | Sarcopenia 36% Myosteatosis 52% | Both myosteatosis (70 vs. 45%, $p < 0.001$) and sarcopenia (53 vs. 32%, $p < 0.001$) were more frequent in patients with hepatic encephalopathy. By multivariable regression analysis, both myosteatosis and sarcopenia were associated with a higher risk of hepatic encephalopathy, independent of the MELD score. |

### 3. Liver-Muscle Axis and Hyperammonemia: A Link to Explore

It has long been known that liver cirrhosis is a systemic disease which, especially in the advanced stages, affects different organs and systems. Although suspected for a long time [53], it has only recently become evident that the relationship between liver and muscle is very close, where one affects the other. Data collected until now show that both muscle synthesis and lysis can be altered in cirrhosis.

Regarding the synthesis of muscle, several factors reduce the potential of the organism to synthesize muscle mass. First, cirrhotic patients are known to have a reduced calories intake; the presence of ascites can lead to early satiety due to increase in abdominal pressure. In turn sarcopenia contributes to fatigue and limits exercise tolerance, reduces performance status and activities of daily living; reduced physical activity obviously contributes to reduced anabolic stimulation [54], which is already altered in the patient with liver cirrhosis [55]. Notably, contrary to what is always claimed, the low-sodium diet could be counterproductive in cirrhotic patients with ascites, making food less palatable and therefore leading the patient to take less calories. Moreover, cirrhotic patients have different causes of malabsorption, such as reduced bile flow with malabsorption of fat-soluble vitamins and fats, pancreatic insufficiency in alcoholic related liver disease with alteration in absorption of fats, bacterial overgrowth due to impaired intestinal motility and portal hypertension [56]. Second, testosterone is an anabolic hormone that increases muscle mass by improving levels of insulin-like growth factor-1 (IGF-1), also called mechano-growth factor [57]. Through IGF-1, testosterone is able to activate mammalian target of rapamycin (mTOR), which is a crucial point in the activation of muscle synthesis. As is well know, cirrhotic patients have low testosterone levels [58]. Finally, leucine-enriched BCAAs have an important role in the synthesis of muscle mass [59]. All these mechanisms, through the stimulation of mTOR, lead to the activation of satellite muscle cells. These cells live in a state of quiescence and when stimulated allow the restoration of muscle mass [60].

On the other hand, proteolysis is upregulated in cirrhotic patients. Above all, cirrhosis is a hypermetabolic condition due to proinflammatory state. In this way, the organism uses gluconeogenesis to compensate for glycogen deficiencies, already altered in the cirrhotic, by consuming proteins and muscle mass. In this perspective, prolonged fasting should be avoided. Chronic inflammation induces autophagy by activating the ubiquitin-proteasome system [61]. Finally, a fundamental regulator of proteostasis is myostatin, a TGFB super-family member that induces muscle loss. This regulation is due to the inhibition of the mammalian target of rapamycin complex 1 (mTORC1) [55].

Within this complex system of regulations, hyperammonemia has one of the most important roles. Ammonia is a compound of nitrogen and hydrogen, mostly a gut-derived toxin produced by bacterial metabolism of urea from proteins that are consumed in the human diet (urease-producing bacterial organisms). With the progression of liver disease, the microbiome enters a dysbiosis state, leading to greater inflammation and cholestasis. So, the composition of gut microbiota becomes altered and plays an important role in the pathogenesis of HE [62]. Moreover ammonia is generated from the continuous amino acid catabolism and purine turnover. From the catabolism inside the enterocytes and from the bacterial production, ammonia enters the portal system and then reaches the liver. Within the liver, ammonia passes through two filter systems, periportal hepatocytes and perivenous hepatocytes. Ammonia is used in the first system as a substrate of the urea cycle. However, in the perivenous hepatocytes there is a strong expression of the enzyme glutamine synthetase which removes the remaining part of toxin to prevent it from entering the systemic circulation [63]. When the liver is damaged and subverted by chronic injury and/or when collateral circles are established that pass the liver filter, ammonia passes directly into the systemic circulation and produces its cytotoxic effects at the level of the central nervous system. At this point the mechanism leading to hepatic encephalopathy is established. What is the role of the muscle within this liver-brain link? Skeletal muscle also expresses glutamine synthetase, although its activity is very low. However, considering the entire muscle extension, it is possible that the muscle is a good

buffer system to dispose of excess circulating ammonia [64]. From this, if the skeletal muscle is reduced it has a lower ability to absorb circulating ammonia and therefore the risk of hepatic encephalopathy increases. Merli et al. have demonstrated that venous blood ammonia levels were significantly higher in patients with muscle depletion and in patients with a decreased muscle strength [40].

In this context, hyperammonemia has a direct negative effect on muscle turn-over. Its action is polyhedral and acts both on synthesis and on muscle lysis. Moreover, hyperammonemia induces a cellular stress response and mimes cell responses activated by amino acid deficiency. In particular, it inhibits the translation of mRNA and protein synthesis into skeletal muscle through activation of general control non-depressed 2 (GCN2) and inhibition of mTORC1 with unknown mechanisms [65]. In addition, the disturbance of the tricarboxylic acid cycle, linked to the loss of alpha-KG (necessary for the conversion of ammonia into glutamate) leads to loss of ATP, mitochondrial dysfunction, reduction of contractile function and finally to sarcopenia. Ammonia also can potentially cause post-translational modifications, including protein nitration and oxidative stress-induced carbonylation of contractile proteins with impaired actomyosin interactions. That is why ammonia-mediated nitration is a potential molecular mechanism of impaired contractile function [20].

It has also been widely demonstrated that hyperammonemia leads to increased activation of myostatin in cirrhotic patients [66], inhibiting protein synthesis. Nishikawa et al. [67] have demonstrated that higher levels of myostatin is associated with hyperammonemia and muscle loss in cirrhotic patients; moreover, patients with increased myostatin had worse prognosis, suggesting the importance of muscle in the prognostic overview of the patient with liver cirrhosis. This concept echoes the above-mentioned idea of considering sarcopenia in the predictive mortality model for cirrhotic patients in liver transplant evaluation [16].

Finally, it has been demonstrated that hyperammonemia increases autophagy in cirrhotic patients with unclear mechanisms [19].

Regarding myosteatosis, the physiopathological association with hyperammonemia and HE is more complex and partially unknown. Myosteatosis seems to derive from a complex mechanism involving the metabolism of fatty acids and glycogen; the pivotal point of this process is mediated by the proinflammatory state that is present and that leads to muscle depletion [26]. Nardelli et al. have demonstrated the association between myosteatosis and HE, hypothezing that fat infiltration, by reducing the fat-free mass, may contribute to the reduction of leads to partial loss of function of glutamate synthetase, expressed in the muscle cell. The significant correlation between ammonia, SMI, and muscle attenuation seems to support this hypothesis [34].

In summary, the set of these mechanisms activates a vicious circle in which cirrhosis induces muscular depletion with multiple mechanisms; the muscular deficiency on the other hand reduces the capacities of absorption of circulating ammonia. Hyperammonemia in turn alters proteostasis and induces further muscle loss. It is now known that muscle depletion reduces survival in cirrhotic patients. The biochemical link of the liver-muscle axis is probably more complex than said and given its prognostic importance it must be further investigated to find therapy targets that can block this vicious circle.

## 4. Clinical and Therapeutic Management

Although it has long been known that muscular alterations impaired survival and quality of life of patients with cirrhosis, definite and effective therapeutic approaches are not yet available. Several therapeutic alternatives have been explored, but extensive evidence on the effectiveness of these approaches is still lacking. Surely the cirrhotic patient at risk of malnutrition must be managed in a multidisciplinary way and carefully followed up also from a nutritional point of view given the strong bidirectional link between muscle and liver. Thus, the management of malnutrition and muscular alterations is also important for the prevention of complications of liver disease, especially HE. If the skeletal muscular system

has a good function of disposing of excess circulating ammonia, acting on muscle mass and function could be a target therapy for HE. To date, different therapeutic approaches for the management of muscular alterations have been investigated (Table 2).

It has been proven that moderate physical activity increases muscle mass and function in patients with cirrhosis [68,69]. However, it is not known how much this impacts on survival and long-term complications. Another important approach consists of increase calorie intake and prefer small and frequent meals with night snacks before going to sleep as suggested by international guidelines [12] to compensate the condition of hypermetabolism frequent in cirrhotic patients [70].

Therapies aimed at managing either HE or muscle mass could play a role in both situations, having muscle and HE a very close bond. By improving muscle mass, it is possible to increase its ability to dispose of circulating levels of ammonia, due to the pathophysiological mechanisms described above. Different therapeutic possibilities have been proposed, such as testosterone, IGF1 [71] and inhibitors of myostatin (follistatin) [72,73]. However, there are few evidence, especially in human models. The use of testosterone has been more studied. Fifteen studies were analyzed in a recent systematic review [74], nine of which were interventional. Although both observational and interventional studies have shown that a low level of testosterone in cirrhotic correlates with sarcopenia, disease decompensation and death, testosterone supplementation cannot improve survival, the risk of decompensation and hepatic encephalopathy, despite the increase in muscle mass [74].

The cataplerosis (loss of critical TCA cycle intermediate and alfa-KG) that is present in cirrhotic patients, could be reversed by oral supplementation of BCAA. They are deaminated to provide carbon skeleton for TCA cycle. Lower levels of BCAA in plasma and muscle have been prompted by hyperammonemia [75,76]. Miwa et al. have demonstrated that low serum BCAA levels can be the predictor of MHE in patients with cirrhosis [39]. Although the integration of BCAA would appear to increase ammonia plasma levels in the short term by increasing glutamine synthesis, prolonged administration would lead to the reduction of hyperammonemia [77]. Among these, isoleucine and its active metabolite β-hydroxy-β-methylbutyrate (HMB) were widely studied for the anti-catabolic effects in skeletal muscle. Lattanzi et al. [78] have demonstrated that adequate dietary intake and physical activity counseling alongside HMB supplementation for 12 weeks improve muscle performance without modification in cognitive status evaluated by the PHES test; notably, none of the 24 patients had cognitive alterations at the baseline.

Ammonia-lowering therapy could be useful in improving muscle mass, by inhibiting autophagy and the expression of myostatin. It has been demonstrated on animal models that combined therapy of LOLA (L-ornithine L-aspartate) plus rifaximin increases strength and muscle mass [79]. In an old study in cirrhotic patients the use of LOLA compared to placebo improved protein synthesis rates measured directly in muscle biopsies [80]. The action of LOLA in this context is manifold. It acts both on the muscle and on the liver. In the periportal hepatocytes LOLA induces the conversion of ammonia into urea. It also ensures the production of glutamate that is exploited by GS to convert ammonia to glutamine in the muscle [81]. Finally, it seems to have anti-oxidant power on the liver and hepatic-protective functions, thus reducing liver dysfunction and hyperammonemia [82].

Finally, TIPS involves substantial changes in the setting of hyperammonemia and muscle state. TIPS is known to worsen hyperammonemia by increasing the shunt of portal blood that bypasses the liver. Sarcopenia has been shown to be a negative prognostic factor for the risk of MHE and OHE after TIPS placement [52]. Probably a poor muscle reserve reduces the muscle's ability to buffer the hyperammonemia. On the other hand, it seems that the positioning of TIPS can improve muscle mass despite the increase of ammonemia [24]. This is probably linked to the reduction of portal hypertension which plays an independent role in the genesis of muscular alterations. It has been shown that even in the absence of significant liver alterations the presence of portal hypertension may be associated with the development of sarcopenia [24].

**Table 2.** Studies evaluating the different interventions tried for improving sarcopenia in cirrhosis.

| First Author (Year) | Number of Patients | Type of Treatment and Duration | Method to Explore Muscle Depletion or Nutritional Impairment | Results |
|---|---|---|---|---|
| Muto et al. (2005) [77] | 646 with decompensated cirrhosis | BCAA orally (12 g/day) Vs diet therapy for 2 years | albumin concentration and health-related quality of life (QOL) measured by Short Form-36 questionnaire | The incidence of death and event free survival significantly decreased in the BCAA group ($p$: 0.015). Regarding hyperammonemia, exhibited lower mean blood ammonia levels than the diet group, with no statistical significance. |
| Lattanzi et al. (2021) [78] | 24 cirrhotics | HMB orally 3 gr/die vs. placebo for 12 weeks | anthropometry, electrical bioimpedance analysis (BIA), quadriceps ultrasound, physical performance battery, Liver Frailty Index (LFI), and cognitive tests | Improving in muscle performance without modification in cognitive status evaluated by the PHES test. Nb: none of the 24 patients had cognitive alterations at the baseline |
| Roman et al. (2016) [68] | 23 cirrhotics | 14 patients randomized to an exercise program vs. 9 patients to relaxation program | Anthropometry, Dual-energy X-ray absorptiometry (DEXA), Time Up & Go (TUG) | The exercise group shows a decrease in fat body mass ($p = 0.003$), increase in lean body mass ($p = 0.01$), lean appendicular mass ($p = 0.03$) and lean leg mass ($p= 0.02$). TUG decreased at the end of the study ($p = 0.02$). |
| Gioia et al. (2019) [24] | 27 cirrhotic patients submitted to TIPS | TIPS placement | Skeletal muscle index at CT scan lumbar 3 | SMI significantly improved after TIPS placement ($p = 0.0001$). Patients with improved SMI had reduced number of episodes of OHE and prevalence of MHE ($p = 0.0001$) |
| Deng et al. (2021) [74] | 580 cirrhotic patients | Testosterone | Various | Testosterone supplementation improved appendicular mass and bone mineral density with no results in terms of liver decompensation and death |

## 5. Conclusions and Further Prospectives

The concept that liver and muscle are two different entities has long since passed. The interaction between muscle and liver are numerous and the alterations of one can involve considerable modifications in the other. The task of the clinician is therefore to consider the cirrhotic patient as a whole in his alterations, including the nutritional and muscular state. However, studies demonstrating these links are often small and retrospective; information is often fragmented and does not provide a complete picture. It is therefore necessary in the future to design extensive and prospective studies with epidemiological descriptions and natural history of cirrhotic patients with muscular alterations.

Moreover, the presence of muscle alterations is establishing itself as one of the heaviest factors capable of influencing the prognosis of the cirrhotic patient, identifying a subgroup of individuals at truly "high risk". It would be important that future studies also focus on the possibility of developing prognostic scores capable of considering this variable, together with other emerging ones such as the presence of spontaneous shunts, to identify this subgroup of patients deserving of more intense management.

It will also be necessary to better define the role of some of the most promising therapies and the weight of dietary and lifestyle changes in this group of patients. It is necessary to define better the role that ammonia-lowering therapies can have in improving the altered proteostasis of cirrhotic patients. Finally, it is likely that gender may have a different effect on the muscle-liver axis and the natural history of cirrhotic patients with

muscular alterations; however, little effort has been made on this aspect; gender studies must therefore be developed in the future.

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# Cognitive Impairement in Non-Cirrhotic Portal Hypertension: Highlights on Physiopathology, Diagnosis and Management

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**Abstract:** Hepatic encephalopathy (HE) is one of the most frequent complications of cirrhosis. Several studies and case reports have shown that cognitive impairment may also be a tangible complication of portal hypertension secondary to chronic portal vein thrombosis and to porto-sinusoidal vascular disease (PSVD). In these conditions, representing the main causes of non-cirrhotic portal hypertension (NCPH) in the Western world, both overt and minimal/covert HE occurs in a non-neglectable proportion of patients, even lower than in cirrhosis, and it is mainly sustained by the presence of large porto-systemic shunt. In these patients, the liver function is usually preserved or only mildly altered, and the development of porto-systemic shunt is either spontaneous or iatrogenically frequent; HE is an example of type-B HE. To date, in the absence of strong evidence and large cooperative studies, for the diagnosis and the management of HE in NCPH, the same approach used for HE occurring in cirrhosis is applied. The aim of this paper is to provide an overview of type B hepatic encephalopathy, focusing on its pathophysiology, diagnostic tools and management in patients affected by porto-sinusoidal vascular disease and chronic portal vein thrombosis.

**Keywords:** porto-sinusoidal vascular liver disease; idiopathic non-cirrhotic portal hypertension; portal vein thrombosis; hepatic encephalopathy; porto-systemic shunt

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## 1. Criteria for the Literature's Selection

Clinical studies that assessed the prevalence and incidence of any type of hepatic encephalopathy (HE) in patients affected by chronic portal vein thrombosis and porto-sinusoidal vascular disease (PSVD) were included. Studies that evaluated diagnostic tools for the detection of cognitive impairment in this population or that evaluated the efficacy of treatment strategies were included too. No language, publication date, or publication status restrictions were imposed. The studies were identified by searching electronic databases (PubMed and SCOPUS). The last search was run on 28 October 2021. Reference lists of all studies included in the present review were screened for potential additional eligible studies.

One investigator (SG) searched the electronic databases, combining the following keywords: (hepatic encephalopathy AND non-cirrhotic portal hypertension), (hepatic encephalopathy AND porto-sinusoidal vascular disease), (hepatic encephalopathy and portal vein thrombosis), (type B AND hepatic encephalopathy), (hepatic encephalopathy AND idiopathic non-cirrhotic portal hypertension), (hepatic encephalopathy AND nodular regenerative hyperplasia), (cognitive impairment AND non-cirrhotic portal hypertension). Studies were excluded if the title and/or abstract showed that the articles did not meet the selection criteria of our review. For potentially eligible studies, or if the relevance of an article could not be excluded with certitude, we procured the full text. We defined the following exclusion criteria: (1) studies in which HE developed in patients with cirrhosis; (2) studies unrelated to our topic; and (3) studies in which HE developed in patients with a kind of non-cirrhotic portal hypertension other than portal vein thrombosis (PVT) and PSVD. A total of nineteen papers were finally analyzed.

## 2. Definition

Hepatic encephalopathy is a frequent complication and one of the most debilitating manifestations of liver disease, having a relevant impact on the quality of life of the patients and their caregivers [1]. It represents a brain dysfunction caused by liver insufficiency and/or porto-systemic shunting and is characterized by a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma. According to the underlying disease, HE can be divided into: type A, due to acute liver failure, type B, secondary to porto-systemic bypass or shunting, and type C, resulting from cirrhosis [2].

## 3. Historical Point and Pathophysiology

The pathogenesis of hepatic encephalopathy is still much debated and not completely understood. It represents a multifactorial and complex syndrome in which there is an imbalance between production, metabolism and regulation of several neurotoxins and neurotransmitters [3,4] as a result of an interorgan trafficking. According to the most accredited hypothesis, which has its origins as early as 1954 with the studies conducted by Sherlock et al. [5], substances of a predominantly, but not exclusively, nitrogenous nature (ammonium, glutamine, methionine, mercaptans, phenol, indole, serotonin, GABA, etc.) reach the central nervous system, causing the spectrum of symptoms typical of HE. Many studies recognize ammonium as the key pathogenetic element responsible for the astrocytic swelling, known as "astrocyte swelling" [6,7]. In the cirrhotic patient, this process is made possible both by the inability of the liver to catabolize these substances and by the fact that portal hypertension acts as a stimulus for the creation of porto-systemic venous anastomoses. These anastomoses allow "dirty" blood, coming from the intestine/gut, to bypass the liver and reach the brain through systemic circulation, where these toxic substances cause an alteration of neurotransmission. The role of portosystemic bypasses in the development of type C HE is demonstrated by various pieces of evidence: their presence in 46% to 71% of patients with recurrent or persistent HE [8,9], the disappearance or in any case the reduction in the number of HE episodes in these patients after embolization of the shunt [9,10], and finally the development of HE in 25–45% of patients undergoing transjugular intrahepatic portosystemic shunt (TIPS), with evident improvement after revision of the stent [11]. Similar values are also achieved after porto-systemic surgical anastomoses.

In patients with type B HE, by definition, the liver is normally functioning, so the presence of portosystemic shunts would seem to be the main pathogenetic factor. Some animal models of type B HE have been used, in particular in rats, cats, and dogs, and less frequently in rabbits, in which HE was based on the presence of portal-systemic shunting. Moreover, while the presence of portosystemic shunts in humans is a rather rare vascular anomaly, in dogs, it is much more frequent. A 2003 multicenter study [12] found the presence of congenital shunts in 0.18–3.2% of all dogs evaluated, with higher or lower values depending on the breed. Dogs affected by such shunts show clinical symptoms similar to those of human HE. Finally, other studies showed that rats treated with portal-cava anastomosis were sensitive to ammonia administration, which leads to severe encephalopathy [13,14]. The study of these animal models helped to better understand the pathogenesis of type B HE also in humans [15,16].

Most of the works on type B HE conducted on humans come from the Eastern world, and derive from studies on patients with congenital porto-systemic shunts, consequently not related to portal hypertension. Hepatic encephalopathy linked to this type of congenital vascular anomalies was first described by Raskin et al. [17] in 1964, who published the clinical case of a patient with HE associated with a large spontaneous intrahepatic shunt. In the following years, the interest of the scientific community towards this clinical condition increased, above all due to the development of diagnostic techniques for non-invasive images, through which it was possible to obtain increasingly accurate images of the portal venous system (Doppler Ultrasound, contrast enhanced CT-scan and MRI). The Japanese Society for the Study of Liver Disease is the organization most interested

in bypass HE. Thanks to the results of a national survey, in 2000, Watanabe published a review specifically focused on the subject [18]. From this investigation, it emerged that many patients with shunt-related HE were wrongly diagnosed as being affected by dementia, psychiatric or neurological disorders, or even by cirrhosis or acute liver failure, and therefore were submitted to prolonged hospitalizations and inappropriate medical interventions. Hence the importance, according to Watanabe, of searching for the presence of spontaneous porto-systemic shunts in all patients with typical symptoms and signs of HE even in the absence of an altered liver function. Watanabe identified both congenital vascular anomalies (patent ductus venous duct, absence of portal vein, arteriovenous malformations, rupture of intrahepatic portal varices, Rendu-Osler-Weber disease, etc.) and acquired (post abdominal surgery, trauma, liver biopsy, etc.) as causes of the formation of these collateral vessels. However, since, in most cases, it was not possible to find a specific cause, the author hypothesized that these shunts were due to portal hypertension, which disappeared after the development of the aforementioned anastomoses due to their decompressive action.

Patients affected by non-cirrhotic portal hypertension (NCPH) theoretically represent an ideal model in which to study type B HE, as they maintain preserved liver function for a long time, but have portal hypertension, which is an important stimulus to shunt formation (spontaneous acquired porto-systemic). Therefore, in this review, we focused on the prevalence, the diagnosis and management of HE occurring in patients affected by idiopathic non-cirrhotic portal hypertension (INCPH), recently named as porto-sinusoidal vascular disease (PSVD), and chronic portal vein thrombosis (PVT), which represent the most frequent vascular liver diseases causing NCPH in the Western world [19–24]. In the presence of portal hypertension, the porto-systemic shunts develop both passively, following the reopening of collapsed embryonic vessels and the inversion of flow in pre-existing vessels (in fact, physiologically there are numerous portosystemic anastomoses), and actively thanks to an increase in VEGF levels [25,26].

Moreover, Das et al. showed that patients with PSVD had cerebral alterations typically observed in cirrhotic patients [27]. In greater detail, they confirmed that the majority of cirrhotics have a hyperintense globus pallidus on T1 W MRI images, and they showed that none of the patients with PVT but more than half of the patients with PSVD had similar radiological findings. The diagnosis of PSVD or cirrhosis was the only independent predictor of the presence of these findings. That cerebral alteration has been attributed to the effects of manganese, which is deposited in excess in the brain of cirrhotics [28–32], and that normally, it is mainly cleared by the liver and excreted in bile [33], and its deposition in cirrhotics is probably due to lower biliary clearance secondary to the hepatocellular damage and to porto-systemic bypass. In cirrhosis, both mechanisms are involved, and this makes it hard to define what is responsible for these alterations. The authors speculate that, as with cirrhotics, but unlike PVT, patients with PSVD have an increased fasting arterial ammonia and abnormal ammonia tolerance test [34], making them prompt in developing HE under appropriate stress. Finally, the finding that the studied cerebral changes were not observed in patients with PVT is in opposition to previous results [35].

## 4. Prevalence of HE

The literature on hepatic encephalopathy in patients with portal hypertension due to portal vein thrombosis is mostly based on studies conducted in the Eastern world [36–39], where this clinical condition is more frequent, or in pediatric populations [40–42]. The main results of these studies are summarized in Table 1. Sharma et al. [36] showed that the prevalence of minimal hepatic encephalopathy (MHE) assessed by psychometric tests and critical flicker frequency (CFF) was 35.5% in patients with chronic extrahepatic portal vein obstruction (EHPVO). The same group demonstrated [37], in a cohort of 32 patients with EHPVO followed up for 1 year, that 12 patients were affected by MHE at baseline, that 75% of them continued to have MHE at follow-up, and that one of the patients without MHE developed it later. In the short time of follow-up, none of the patients developed overt HE.

The presence of MHE in these patients was strongly associated with a higher expression of ammonia, pro-inflammatory cytokines, and brain glutamine levels.

**Table 1.** Published studies on hepatic encephalopathy in patients with non-cirrhotic portal hypertension (NCPH): chronic portal vein thrombosis (PVY) or porto-sinusoidal vascular disease (PSVD).

| Author, Year | N° of Patients | Type of NCPH | Prevalence of HE | Type of HE |
|---|---|---|---|---|
| Sharma et al., 2008 [36] | 34 | PVT | 37.3% | MHE |
| Sharma et al., 2012 [38] | 70 | PVT | 43% | MHE |
| Srivastava et al., 2011 [39] | 20 | PVT | 60% | MHE |
| Yadav et al., 2010 [40] | 22 | PVT | 32% | MHE |
| D'Antiga et al., 2014 [41] | 13 | PVT | 45% | MHE |
| Srivastava et al., 2010 [42] | 42 | PVT | 36% | MHE |
| Siramolpiwat et al., 2014 [43] | 84 | PSVD | 7% | OHE |
| Nicoletti et al., 2016 [44] | 51 | PVT and PSVD | 34% (PVT)/25% (PSVD)<br>5.7% (PVT)/12.5% (PSVD) | MHE<br>OHE |
| Bissonnette et al., 2016 [45] | 41 | PSVD | 31% | OHE |
| Liu et al., 2019 [46] | 150 | PSVD | 4.7%<br>32.7% | OHE<br>MHE |
| Lv et al., 2019 [47] | 76 | PSVD | 16% | OHE |

The literature on patients with PSVD is based on several individual case reports [48,49] and some studies, principally conducted in the Western world, describing HE as a tangible complication of the disease, mainly related to the presence of large porto-systemic shunts (Table 1) [43–45].

Evaluating the prevalence of hepatic encephalopathy (minimal and overt) in 51 patients affected by NCPH in comparison with that of a control group of cirrhotic patients [44], Nicoletti et al. showed that, even lower than that observed in cirrhotic patients, a cognitive impairment was detectable in a relevant proportion of patients with non-cirrhotic portal hypertension, with no difference between the patients with chronic portal vein thrombosis and PSVD. The presence of a large portal-systemic shunt (spontaneous or iatrogenic) was considered the main risk factor for HE in these patients, as it was identified in 71% of the patients with cognitive impairment. Another study showed that in patients affected by NCPH, the incidence of OHE was similar, while the prevalence of MHE was lower than that of cirrhosis patients. The authors confirm that together with upper gastrointestinal bleeding and infection, a portosystemic shunt was an independent factor for HE [46].

Additionally, post-TIPS HE is a not infrequent complication of portal hypertension due to PSVD [47]. In a European cohort of 41 patients affected by PSVD and submitted to TIPS as the treatment of portal hypertension-related complications [45], HE was an in-hospital complication of two patients, while at long-term follow-up, overt HE occurred in 31% of the patients, and the one-year rate of overt HE was 24%. In two patients, HE was severe enough to require shunt reduction [45].

## 5. Diagnosis

The tests and the methods used to diagnose hepatic encephalopathy in patients with NCPH are the same currently used for the diagnosis of HE in cirrhotic patients.

### *5.1. Diagnosis of Overt Hepatic Encephalopathy*

The diagnosis of overt HE is mainly based on clinical examination. Some scales are used to stage the severity of the encephalopathy, and with this aim, the most applied is the West Haven scale, which still represents the gold standard. The diagnosis of cognitive dysfunctions is not difficult; less easy is the attribution of them to overt HE, and that is why the exclusion of other causes of mental alteration by laboratory and radiological assessment is often required [2,50].

### 5.2. Diagnosis of Covert Hepatic Encephalopathy

For the diagnosis of minimal/covert HE, the same tools used for cirrhotic patients are used in NCPH patients, and they include paper-pencil tests (the psychometric hepatic encephalopathy score- PHES), computerized tests such as continuous reaction time, the inhibitory control test, the SCAN test and the Stroop test, or neurophysiological tests including the CFF and EEG. Clinicians may use tests for the diagnosis of MHE with which they are familiar that have been validated for use in this patient population [2,50–53].

In the studies exploring the prevalence of minimal HE in patients affected by EHPVO, especially in children, the most used tools for the assessment of HE were psychometric tests and CFF. In the study by Yadav, the superiority of psychometric tests in comparison to CFF was demonstrated. The same observation resulted from a study by Srivastava [42]. In a recent study by Suresh et al., the diagnostic accuracy of the computerized Stroop test for the assessment of MHE in an Indian pediatric cohort of patients with EHPVO was investigated in comparison to other validated tests. The authors observed that the Stroop test can be useful to detect MHE in children and identify a subgroup of patients to be submitted to psychometric tests in clinical care. Nicoletti et al., as previously reported, used two categories of tests to evaluate the presence of minimal/covert HE [2]: the PHES and the Scan battery. The accuracy of the Scan battery and of PHES in the detection of MHE was similar, but some discordance was observed, suggesting that the two tests have different levels of difficulty [54] (the scan test is more complex than PHES), and that they explore different domains of cognitive function.

## 6. Treatment

To date, the treatment of overt hepatic encephalopathy occurring in patients affected by NCPH is the same as that of HE in cirrhotic patients.

The initial management includes a prompt start of care of hospitalized patients with HE, including the identification and the treatment of co-existing causes; the identification and correction of precipitating factors and the start of empirical treatment targeted the reduction in ammonia levels. The cornerstones of medical treatment of overt HE include nonabsorbable disaccharides, such as lactulose, and antibiotics, such as rifaximin, where lactulose is recommended for the prevention of recurrent episodes of HE after the initial episode, and rifaximin as an add-on to lactulose is recommended for the prevention of recurrent episodes of HE after the second episode. Alternative therapies, such as oral branched-chain amino acids, intravenous L-ornithine L-aspartate, and probiotics have been studied and used in cirrhotic patients, but no data in patients with NCPH have been provided [2,53]. Whether applying in patients with NCPH the same therapeutic strategies used in cirrhotic patients is correct is unknown. Although HE is a less frequent complication of a less frequent disease, more cooperative studies are needed to identify the best approach to treat hepatic encephalopathy in patients with NCPH.

As hepatic encephalopathy occurring in patients affected by portal vein thrombosis or idiopathic non cirrhotic portal hypertension is a type B HE, mainly sustained by the presence of large porto-systemic shunts, the radiological occlusion of the shunt may represent a fundamental approach in patients with persistent HE, despite an adequate medical treatment. [43,55] Radiological techniques such as plug-assisted retrograde transvenous obliteration (PARTO) or coil-assisted retrograde transvenous obliteration (CARTO) are currently used to treat recurrent or persistent HE [56–58], as well as gastric varices often present in these patients (Figure 1). Finally, in patients with persistent post-TIPS HE, the reduction in the caliber of the stent or its occlusion must be evaluated.

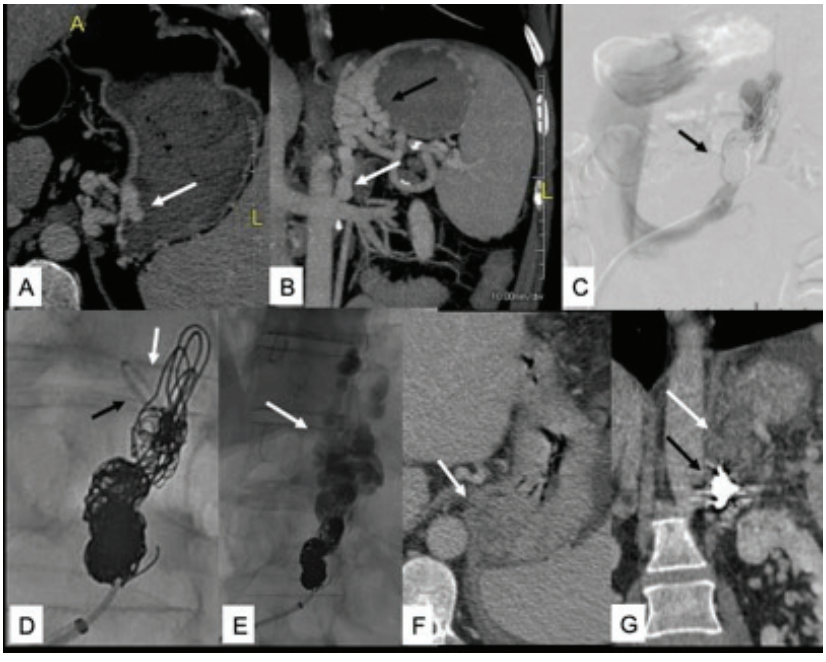


**Figure 1.** (**A**,**B**) Pre-procedural CT axial and coronal reformats show intra- and extramural gastric varices (GV) (white arrow in (**A**) and black arrow in (**B**)) and GRS (white arrow in (**B**,**C**)). Coil embolization of GRS (black arrow) with persistent shunt patency. (**D**) Angiographic catheter distal to coils (black arrow) and coaxial microcatheter looped backward for NBCA injection (white arrow). (**E**) Extensive filling of GV with gelfoam and contrast media (white arrow). (**F**,**G**) Post-procedural CT shows complete thrombosis of GV and GRS (white arrow) and coils in the GRS (black arrow).

Finally, as in cirrhosis, the necessity to treat MHE is still debated. Despite its clinical implications (impairment, poor quality of life, etc.), guidelines state that the treatment of minimal/covert HE in cirrhotic patients is to be evaluated on a case-by-case basis [53,59,60].

However, some studies observed an improvement in psychometric tests in the majority of the EHPVO pediatric patients with MHE after therapy with lactulose, and that such treatment was well-tolerated [61]. These results confirm a previous study by Sharma were lactulose seemed to be effective in the treatment of minimal hepatic encephalopathy in patients with portal vein thrombosis, and that patients with cognitive impairment and with porto-systemical shunts had a better response to lactulose than the patients without any collaterals [38]. In the patients who responded to lactulose, the blood ammonia levels significantly reduced, while in the patients who were non-responders to the treatment, they did not.

## 7. Conclusions and Future Directions

Type B HE can be considered a complex and multidimensional cognitive deficit that is not infrequently found in patients with NCPH and that shares substantial physio-pathological bases with type C HE. Both the presence of shunts per se and the neurotoxic effect of toxins of intestinal origin play a fundamental role in determining and supporting alterations in mental status, even in the absence of hepatocellular damage. Moreover, a multidisciplinary approach for the best management of these patients is often needed. In fact, patients affected by non-cirrhotic portal hypertension may develop all the sequelae of portal hypertension, such as portal hypertensive bleeding or refractory ascites often requiring TIPS placement. However, the development of HE episodes that are also poorly

responsive to medical treatment after this procedure, which could require stent revision with the reduction in its caliber or occlusion, is not rare.

Finally, a thorough understanding of the impact of HE in NCPH patients cannot fail to consider the need for reliable epidemiological data. Therefore, further studies will also be needed to establish the exact prevalence and incidence of cognitive impairments in these patients. In fact, only a thorough knowledge of all the facets of the problem will allow us to promptly identify patients at risk, study the cognitive deficit extensively and undertake appropriate therapies.

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## Abbreviations

HE: hepatic encephalopathy; GABA: gamma-aminobutyric acid; TIPS: transjugular intrahepatic portosystemic shunt; PSVD: porto-sinusoidal vascular liver disease; PVT: portal vein thrombosis; NCPH: non-cirrhotic portal hypertension; INCPH: idiopathic non-cirrhotic portal hypertension; EHPVO: extrahepatic portal vein obstruction; CT: computed tomography; MRI: magnetic resonance imaging; VEGF: vascular-endothelial growth factor; CRT: continuous reaction time; ICT: inhibitory control test; CFF: critical flicker frequency; EEG: electroencephalography; CARTO: coil-assisted retrograde transvenous obliteration; PARTO: plug-assisted retrograde transvenous obliteration.

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# Lights and Shadows in Hepatic Encephalopathy Diagnosis

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**Abstract:** Hepatic encephalopathy (HE) is a form of brain dysfunction that is caused by liver insufficiency and/or portal-systemic shunting. The exact nature of HE is debated; as such, conflicting uses of the term "HE" may cause inconsistencies in its detection and management. This review highlights the meaning of the term "HE" on the basis of its historical origins and current consensus. It also provides criteria for the diagnosis of the condition based on its phenotypes and risk factors for its occurrence. The procedure for differential diagnosis from other conditions which result in similar phenotypes is considered, together with precipitants and confounders. Finally, the current multidimensional approach for the correct clinical reporting of HE episodes is discussed.

**Keywords:** liver failure; encephalopathy; delirium; coma; cirrhosis

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## 1. Introduction

The diagnosis of hepatic encephalopathy (HE) is relevant as it is a marker of poor survival in cirrhosis and acute liver failure (ALF) [1,2], and is a disabling condition resulting in poor quality of life for patients and their caregivers [3]. Further, HE incurs significant direct costs to health service systems [4], as it is the second leading cause of hospitalization in patients with cirrhosis and the primary reason for re-hospitalization [5]. It also results in considerable indirect costs related to loss of work for patients and caregivers [6]. A correct diagnosis is required in order to select proper treatment, prevent further episodes in individual patients, and conduct meaningful prevention/treatment trials.

While the diagnosis of HE may seem simple (and frequently this is the case), the diagnosis can be complex and uncertain. The issue is not trivial and firstly depends on what one considers to be HE; i.e., on its definition.

## 2. The Meaning of the Term "HE"

The recognition of an association between jaundice and behavioral alterations is ancient, while that between cirrhosis and confusion/stupor is some three centuries-old, and the pathophysiological explanation for these associations dates back to end of the nineteenth century [7].

On this basis, the American Association for the Study of Liver Disease (AASLD)/European Association for the Study of the Liver (EASL) practice guidelines for HE define HE as "*Brain dysfunction caused by liver failure and/or portal-systemic shunting (PSS); it manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma*" [8]. The term "hepatic" is used to underline a specific pathophysiological link to liver failure and/or portal-systemic shunting.

However, the habit of calling encephalopathy "*hepatic*" on the basis of a "clinical suspicion" in patients with liver disease remains [9–12]. This is open to criticism, since seldom in medicine is encephalopathy qualified based on the disease in which it occurs. By contrast, it is generally qualified based on the pathophysiological mechanism causing encephalopathy/delirium in order to direct etiological treatment. Thus, the terms

"*lung encephalopathy*" or "*cardiac encephalopathy*" do not exist, while the terms "*hypercapnic encephalopathy*", "*hypoxemic encephalopathy*", and "*cerebral hypoperfusion encephalopathy*" are used.

Thus, if "*hepatic*" does not refer to a specific mechanism, it would be better replaced by a term that does (i.e., "patient with cirrhosis and benzodiazepine intoxication", "opioid overdose", "hyponatremic encephalopathy", "septic encephalopathy", or "hyperammonemic encephalopathy", etc.). This would avoid misinterpretation and mismanagement due to the use of the same treatment for conditions with different underlying types of pathophysiology.

As an analogy, using the term HE in a broad meaning would be the same as calling fever in patients with cirrhosis "*hepatic fever*", regardless of its cause (i.e., pneumonia, urinary tract infection, spontaneous bacterial peritonitis, etc.), and treating it in the same way. The idea that in patients with cirrhosis all encephalopathies should be classified and managed depending on their etiology was clearly formulated by Riddell around 65 years ago [13]: " *. . . among a group of patients with severe liver disease a number of neurological disturbances will be met with; not all of these are the disease known as hepatic coma. Among these other states are the psychoses associated with chronic alcoholism and nicotinic acid deficiency, electrolyte disturbances, septicaemia, increased response to narcotics and subdural haematoma.*"

Considering the definition given by the AASLD/EASL practice guidelines, the following question may arise: *"Which type of brain dysfunction is caused by liver failure and/or PSS?"*. This question implies the detection of a mechanism that *specifically* links these conditions. Such a link between a failing liver and/or PSS and encephalopathy concerns abnormalities in nitrogen metabolism, as the liver has a unique role in the detoxification of ammonia and most other substances coming from the gut. This has been proven by the observation of dogs undergoing portal-caval shunting who (1) developed encephalopathy after the consumption of ammonia salts and nitrogen-containing-foods, (2) reduced their urinary urea excretion, and (3) reduced their capacity to synthetize urea from gastric-infused carbamic acid [14]. Further, the oral administration of ammonium chloride to cirrhotic patients causes coma [15], and the toxicity of ammonia to the human brain has been proven by cognitive defects with respect to attention/executive function and coma in individuals with hereditary defects in urea cycle enzymes [16]. Finally, the creation of large portal-systemic shunts causes hyperammonemia and encephalopathy in humans, and shunt obliteration reduces ammonemia and improves HE [17].

PSS and hepatic failure may cause an increase in any neurotoxic substance originating from the gut that has a high first-pass hepatic metabolism, like ammonia. Research on this has been limited over the past years, after emphasis was given to the topic by Zieve [18]. Substances with the above features which may have a pathophysiological role in HE include: (1) manganese (particularly in the motor disturbances associated with HE), since this heavy metal deposits in the basal ganglia due to its reduced clearance in portal-systemic shunting and cholestasis [19], and (2) indole, that crosses the blood–brain barrier and produces oxindole within the brain, which is a neurotoxic substance [20]. Further, gut dysbiosis, Kuppfer cell dysfunction [21], and portal-systemic shunt may favor systemic inflammation, which also affects brain function [22,23].

It should be emphasized, however, that despite hyperammonemia being a necessary condition for the occurrence of HE (and thus subjects without hyperammonemia should be suspected of having a delirium of alternative origin), hyperammonemia is common in cirrhosis, mainly because of PSS [24], and its occurrence does not imply a phenotypical HE expression. Indeed, ammonia interacts with other factors to disturb brain function [25]. This explains why the clinical expression of HE is only roughly related to ammonia plasma levels, since individual susceptibility to developing a hyperammonemia-related phenotype depends on several factors. Thus, in the same individual, changes in co-factors may change the effect of ammonia, and varying brain susceptibility to the levels of ammonia was proven long ago [15]. More recently, an elegant study by Shawcross and co-authors [26] proved the different effects of ammonia depending on cytokine levels. We ourselves have shown

that same levels of ammonia have different effects on brain electrogenesis depending on sodium levels [27].

This limits the value of using absolute isolated ammonia levels as an index of HE. However, the diagnostic value of plasma ammonia is high, since normal ammonia levels suggest that the degree of liver failure/shunt is insufficient to support a working diagnostic hypothesis of HE. A recent study [11] seems to contradict this view, showing that recovery of normal mental state is the same in patients with delirium and ammonia levels higher or lower than 75 mmol/L (the upper limit of normality) [24]. Of note, 80% of these patients had a history of overt HE. However, the patients with low ammonia levels had a higher rate of infection than the others. In addition, in all patients the precipitating factor of the HE episode was treated, and it is reasonable to expect that this would have resulted in clinical improvement. The use of lactulose in all patients was probably irrelevant to the conclusions of the study, since it is useful in patients with HE and innocuous in those with other kinds of delirium.

HE in acute liver failure is a distinct type of HE [8] that occurs in the context of systemic hemodynamic alteration and multi-organ failure (MOF) [28,29]; thus, it has separate features in which brain swelling due to acute hyperammonemia and intracranial hypertension have a peculiar role.

Recently, it has been observed that HE in acute-on-chronic liver failure also has some peculiar features [30,31]. It frequently occurs in sepsis, cytokine storm, and MOF that reasonably produce overlapping metabolic/hemodynamic encephalopathies. These may deserve to be considered separately and frequently require multitarget treatment in managed intensive care unit (ICU) patients.

In all cases of encephalopathy, especially in the context of ALF or acute-on-chronic liver failure (ACLF), the exclusion of alternative causes is mandatory because an incorrect, missed, or delayed alternative diagnosis (e.g., brain hemorrhage) has profound consequences.

## 3. The Diagnosis of HE

The diagnosis of HE, as with every clinical diagnosis, results from the a priori probability of HE before any observation, and the probability that a clinical finding relates to HE. This should be compared with the probability of alternative conditions.

Thus, the degree of certainty for the diagnosis of HE depends on three key steps: (1) the a priori probability of HE, (2) the recognition of a clinical pattern suggestive of HE, and (3) the consideration of alternative conditions.

In formal Bayesian terms:

> *Odds of HE = a priori odds of HE prevalence × positive likelihood ratio of clinical findings for HE vs. a priori odds of alternative condition prevalence × positive likelihood ratio of clinical findings for alternative conditions.*

However, formal Bayesian estimations cannot be performed, because an exact quantification of the parameters of the equation is not available and varies reasonably depending on the clinical settings. However, the clinician's reasoning is still Bayesian and the related questions are as follows: Is the presentation suggestive of HE? Is it probable that in this patient HE may occur? Are there other conditions that may explain this clinical presentation?

The a priori probability of HE depends on the severity of liver failure [32,33] and/or the extent of PSS [34], in addition to the history of previous episodes of overt HE [27]. Recently, a clinical score was proposed to assess the risk for the first episode of overt HE [11].

Of note, the severity of liver failure and PSS have a fundamental role, and they are associated with high levels of plasma ammonia. This is a known risk factor for HE [35,36].

The a priori probability is increased by the occurrence of precipitating factors for HE, even if their prevalence varies considerably between the studies (Table 1).

**Table 1.** Prevalence (percentage and 95% CI) of precipitating factors for hepatic encephalopathy (HE).

| | Ali et al. [37] *N* = 100 | Cordoba et al. [31] (*N* = 460) | Devrajani et al. [38] (*N* = 87) | Stauss et al. [39] (*N* = 168) |
|---|---|---|---|---|
| Infections | 20 (13–29) | 26 (22–30) | 67 (56–76) | 31 (24–38) |
| Bleeding | 14 (8–22) | 11 (9–14) | 45 (35–55) | 20 (15–27) |
| Constipation | 37 (28–47) | - | 40 (39–60) | |
| DDEI * | 70 (60–78) | 89 (86–91) | 54 (44–64) | 36 (29–44) |
| Acute alcoholism | | 17 (14–20) | - | 2 (1–6) |
| Not recognized | 11 (6–19) | - | 6 (2–13) | 10 (6–15) |

DDEI *: Dehydration, diuretics, electrolyte imbalance; CI: confidence interval. Note: the sum of the percentages may be higher than 100 because of overlap.

*Precipitating factors* can be considered as those conditions which intervene in the pathophysiology of HE by increasing the production of ammonia, reducing its disposal or increasing its neurotoxicity. For instance, gastrointestinal bleeding and constipation increase ammonia production, and inflammation (especially that associated with infections) and hyponatremia increase ammonia toxicity [26,27,40]. Of note, infections, as well as hypothyroidism, increase ammonia production [13,37].

In contrast to precipitants, conditions that alter brain function by a direct mechanism, independent of liver nitrogen metabolism, can be called *confounders* (Figure 1).

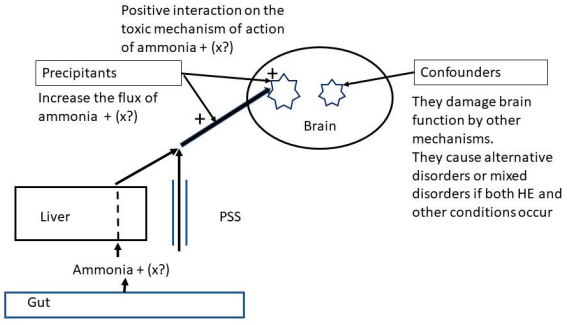


**Figure 1.** Schema showing the different concepts of precipitants and confounders.

It should be considered that the distinction between precipitants and confounders is not always simple or clear. Some factors such as sepsis, hyponatremia, and hypothyroidism, which function as precipitants (because they intervene in the mechanisms of ammonia production or toxicity), can also alter brain function per se, as occurs in patients without liver failure [41–44]. Treatment should be directed at these factors and not only at hyperammonemia [8].

The recognition of the clinical presentation that HE is an obvious pre-requisite for its diagnosis. HE may manifest as coma, delirium of various degrees (mainly sedated, but sometimes agitated) [8], and sometimes with transient focal symptoms [45]. Further, HE may present as an almost continuous cognitive dysfunction, interspersed with more or less severe episodes of delirium [8]. A mild presentation of HE mimics persistent or episodic non-amnestic minimal cognitive dysfunction or disinhibition [46]. Finally, motor signs can be associated or (rarely) dominate the presentation. The most obvious and common is negative myoclonus, which produces the classical finding of asterixis. Other motor findings are extrapyramidal manifestations such as parkinsonism [47], chorea and hemiballismus [48], or spastic paraparesis [49]. These symptoms, generally found in association with large PSS, are called non-Wilsonian hepatocerebral degeneration and hepatic myelopathy [46]. These conditions, however, can be considered subtypes of HE as both are superimposed, and portal-systemic shunting which intervenes in their pathophysiology and overlap with mild confusion is the rule (Table 2).

**Table 2.** Phenotypes of HE.

| Pattern | | Description |
|---|---|---|
| (A) Coma (grade 4 West Haven classification) | | The patient's eyes are closed; they are unresponsive even to painful stimulation. |
| (B) Rapidly developing state of confusion (delirium)(grade 2–3 according to the West Haven classification) | Inhibited | The patient is more or less disoriented in time and/or space and/or identity and is more or less somnolent/stuporous. Asterixis is usually detectable. |
| | Agitated | The patient is disoriented in time and/or space and/or identity and is agitated/angry/restless. Asterixis is usually detectable |
| (C) Almost continuous mild mental dysfunction with interspersed recurrent episodes of confusion(persistent/almost continuous HE with frequent relapses) | | The pattern is dementia-like. Asterixis is usually detectable. |
| (D) Predominant motor disorder with mild/moderate mental dysfunction/confusion(corresponds to the conditions of hepatic parkinsonism and hepatic myelopathy/non-Wilsonian hepatocerebral degeneration) | Extrapyramidal | Parkinsonism, chorea, or athetosis. Asterixis is usually detectable. |
| | Pyramidal | Spastic paraparesis with hyperreflexia. Asterixis is usually detectable. |
| (E) Mild brain dysfunction | | The patient is oriented for time and space and his/her mental activity seems normal/near-normal; however, caregivers or heath personnel may recognize a deterioration of the patient in terms of behavior, irritability, and cognition.Upon psychometric testing, alterations are detectable (related to attention, working memory, cognitive speed, and inhibition). Other signs, associated or independent of psychometrical alterations, include slowed electroencephalographyc activity and/or reduced critical flicker frequency. Dissociations between the techniques are frequent. |

Accordingly, for the differential diagnosis of the clinical findings compatible with HE [50], one should briefly consider the probability of alternative/overlapping causes of the clinical findings: coma, delirium, transient focal attacks, persistent undulating dementia-like mental decay, and motor disorders [51–55]. The occurrence of sudden coma requires brain imaging, since hemorrhagic stroke has an increased prevalence in liver failure [56]. Similarly, the occurrence of persistent/highly recurrent HE may require the search for additional/alternative causes of dementia or neurodegenerative disorders by brain imaging and extensive investigation.

Of note, the occurrence of a finding compatible with HE and a high a priori probability does not exclude the existence of mixed encephalopathy. Indeed, the addition of various factors damaging brain function facilitates the occurrence of symptoms. In the case of a mixed encephalopathy, the treatment of the brain dysfunction caused by HE may result in an improvement, but does not completely revert the clinical picture [57].

A chart that summarizes the diagnostic process is reported in Figure 2.

Additionally, if HE is present and is the only cause of the clinical findings (thus, *what is HE and what is not HE*), the diagnosis of HE requires some additional attributes relating to its type, severity, rate of recurrence, precipitant, and facilitating factors [58]. Thus, a complete diagnosis should be multidimensional (as was first suggested by Ferenci et al. [10] and emphasized in the practice guidelines of the AASLD/EASL [8] and the Italian association for the Study of the Liver AISF [58]).

Of note, recent brain imaging techniques [59–61] and accurate microbiome and metabolomic studies [62] are providing important new insights on HE, but at the moment these techniques are limited to research purposes.

Conflicting opinions concern the diagnosis and quantification of mild forms of HE. In the 1950s Parsons-Smith et al. [63] recognized two important findings: (1) some patients with cirrhosis may have subtle mental changes and psychometrical alterations in the absence on unequivocal neurological signs, and (2) patients without any alterations may have EEG abnormalities. These authors clearly showed that HE can produce (1) mild equivocal mental/cognitive/behavioral alterations, and (2) even neurophysiological alterations without any other evidence. Conn et al. [64] provided a quantification of mental changes in HE, slightly modifying that of Parsons-Smith et al. [63], and classified grade 1 HE as a condition characterized by subtle cognitive/behavioral alterations without disorientation (in time or space). Since then, new techniques of investigation of cognitive investigation

(based on techniques requiring patient cooperation) and brain function (i.e., independent of patient's cooperation) have been developed, with a plethora of definitions and names provided, including latent HE, subclinical HE, minimal HE, and grade 1 HE. Basically, there is an agreement that (1) some patients do not show any clinical symptoms/signs of HE but do have neurophysiological or psychometrical alterations, and (2) other patients have "something wrong" in their awareness, behavior, or attention that can be recognized by skilled clinicians and caregivers. The former condition is preferably called minimal HE, whereas the latter is grade 1 HE. However, since both conditions are not unequivocal (at odds with frank disorientation) and the line between the two depends on the sensitivity of the observer, a proposal was made [8,65] to gather the two conditions under the heading of "covert" HE (mainly because the sound "covert" evokes the antonym of "overt"), considering that this condition requires quantification by objective tests. This proposal aimed to avoid possible inter-observer disagreement and inconsistency in the detection of grade 1 HE. At any rate, possible confusion arises if "covert" is misinterpreted as a synonym of minimal, or if mildly symptomatic HE (grade "1") is considered to overlap with asymptomatic HE (i.e., minimal HE). Thus, conflicting opinions still exist about the use of the terms.

Operative definition for the diagnosis of grade 1 HE was provided by Vilstrup et al. [8]. The diagnosis of minimal HE requires proper testing. This can be neurophysiological, (e.g., quantified electroencephalogram, evoked potentials, etc.), psychophysiological (e.g., critical flicker frequency), or neuropsychological [66,67]. Of note, psychometrical testing needs to be oriented to the cognitive domains mostly altered by the initial phase of HE (e.g., cognitive speed, divided attention, sustained attention, inhibition, working memory) and be properly standardized for single patients, since cognitive performance depends on age and education and, most probably, geographical/national factors [66]. Thus, locally standardized Z-score based techniques, such as the psychometric HE score PHES [68], are preferable. Recently, a very rapid oral technique based on animal naming has been suggested [69], but needs confirmatory studies. Computerized tests based on chronometric techniques have been used [70–72] and may be more repeatable since they are based on the repetition of many trials, but only well-educated, cooperative individuals can be easily tested. Further, any kind of mild psychometrical/neurophysiological dysfunction in cirrhosis cannot be immediately attributed to minimal HE, since mild cognitive dysfunction can have many concurrent causes [73,74].

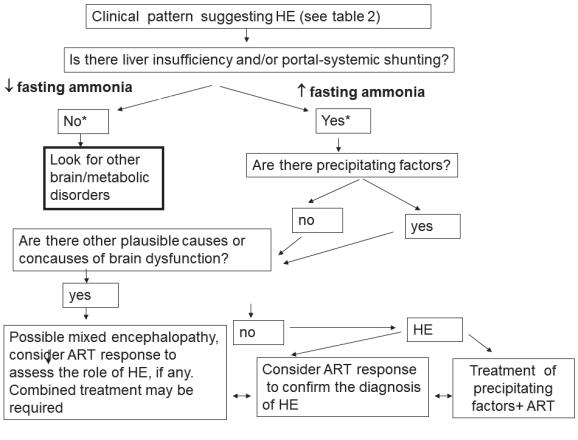


**Figure 2.** Flow chart for the diagnosis of hepatic encephalopathy (HE) (modified from [50]). ART: ammonia-reducing treatment (non-absorbable antibiotic ± disaccharides). * Low ammonia is considered to have high negative predictive value for HE [8], since it suggests: (1) relatively good liver function, (2) negligible portal-systemic shunting, and (3) negligible gut dysbiosis in cirrhosis.

## 4. Conclusions

The existence of liver failure, PSS, prior HE, frailty, and precipitating factors increase the risk of HE. However, the risk provided by these conditions (with varying degrees) cannot be exactly quantified, and neither can the risk provided by their interaction. At present, therefore, they may only confer a subjective degree of confidence relating to the a priori likelihood for the diagnosis of HE.

Another milestone for diagnosis depends on the recognition of the phenotype of HE [50], which is unspecific. Thus, alternative or concurrent conditions should be considered, particularly for patterns where a rapid alternative diagnosis may have high relevance with regard to the outcome (for example coma). Other conditions that require intensive work are the presentations characterized by prominent motor features or persistent fluctuating mental alterations. In most cases the diagnosis is simple, particularly when there is strong a priori probability that the patient only has liver disease and the phenotype of HE is one of delirium. In other conditions, the diagnosis can be more challenging. It is reasonable to assume that HE can be confirmed if a full-dose regime of non-absorbable disaccharides and non-absorbable oral antibiotics (i.e., a regimen significantly reducing plasma ammonia) improves or completely reverts symptoms in a few days. Finally, after having reached a correct diagnosis of the existence of HE, multidimensional qualification is required to characterize the type, severity, time course, and precipitant-favoring factors, in accordance with present practice guidelines.

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## Abbreviations

| | |
|---|---|
| HE | Hepatic encephalopathy |
| ALF | Acute liver failure |
| AASLD | American Association for the Study of Liver Disease |
| EASL | European Association for the Study of the Liver |
| PSS | Portal-systemic shunt |
| ACLF | Acute-on-chronic liver failure |
| AISF | Italian Association for the Study of the Liver |
| ICU | Intensive care unit |
| DDEI | Dehydration/diuretics/electrolyte imbalance |
| CI | Confidence interval |
| MOF | Multi-organ failure |

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# Gut Microbiota Modulation and Fecal Transplantation: An Overview on Innovative Strategies for Hepatic Encephalopathy Treatment

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**Simple Summary:** Treatment of advanced liver disease and its complications continue to be a challenge due to the complexity of this illness. In recent years, the gut microbiome has been recognized to play a beneficial role in our health. Studies have shown that overgrowth of harmful organisms in the gut can contribute to worsened outcomes in liver disease. Fecal microbiota transplant (FMT) is an approved and effective treatment in other gastrointestinal conditions. FMT involves the administration of a solution of a fecal suspension from a healthy donor into the intestinal tract of a recipient. This has led researchers to attempt this treatment in liver disease. There have now been small clinical trials showing that FMT is safe and could be effective in improving outcomes in advanced liver disease. There remain several questions to be answered before FMT is implanted in clinical practice, including the best route to administer this treatment, how many doses are needed to achieve a therapeutic response, and how long we need to wait between treatments. In this review paper, we explore the role of the gut microbiome in the human body with emphasis on the gastrointestinal system, how it changes in liver disease, and how we can improve it with fecal microbiota transplant.

**Abstract:** Hepatic encephalopathy (HE) is a major complication of cirrhosis, which is associated with gut microbial composition and functional alterations. Current treatments largely focus on gut microbiota using lactulose, rifaximin and other agents. However, despite these treatments, patients with HE have a high rate of readmission, morbidity and cognitive impairment. Fecal microbiota transplant (FMT) involves introduction of a donor microbiota into a recipient and is currently mainly used for recurrent *C. difficile* infection (rCDI). The role of FMT in cirrhosis and HE is evolving. There have been two randomized clinical trials (RCT) and several case reports/series in cirrhosis. Both RCTs were safety-focused phase 1 trials. One involved pre-FMT antibiotics and FMT enema versus standard of care, while the other involved 15 FMT capsules versus placebo without pre-FMT antibiotics. There was evidence of safety in both trials and the FMT group demonstrated reduction in hospitalizations compared to the non-FMT group. Changes in microbial function centered around short-chain fatty acids, bile acids and brain function showed improvement in the FMT groups. Long-term follow-up demonstrated continued safety and reduction in the antibiotic-resistance gene carriage. However, larger trials of FMT in HE are needed that can refine the dose, duration and route of FMT administration.

**Keywords:** cirrhosis; bile acids; antibiotic resistance; enema; capsules; hospitalizations; cognitive function

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## 1. Introduction

Liver cirrhosis has a multitude of debilitating complications including ascites, bleeding and hepatic encephalopathy (HE). HE is a major neurologic complication of cirrhosis and

is estimated to affect between 30 to 70% of patients with cirrhosis [1,2]. It often requires hospitalization, which has a significant economic impact. Studies have shown that the yearly total cost for patients with a primary diagnosis of HE is estimated at USD 620 million in the United States [3]. Cognitive impairment experienced by patients with HE can range from covert (minimal) to overt [2]. Covert HE can be subtle in nature and requires the use of specialized tests for detection. In contrast, overt HE is characterized by behavioral changes, confusion and lethargy [2]. Regardless of type, HE portends a poor prognosis and is associated with increased morbidity [1]. It not only negatively impacts the patient's quality of life but also places a heavy burden on family and caregivers. Moreover, individuals who experience HE have a high chance of recurrence [1,2].

The exact pathogenesis for the development of HE is yet to be determined. Certain mechanisms have been implicated and have been recognized for years including increased production of neurotoxins, impairment of neurotransmission, systemic inflammation, alteration of the blood–brain barrier and alterations in energy metabolism [4,5]. It is clear that no single entity is solely responsible for HE, and it is a synergistic effect of multiple mechanisms that lead to this illness. In recent years, it has been increasingly recognized that the gut microbiota plays a large role in the development of HE [6–8]. This is most evident by the beneficial role of antibiotics in the treatment in HE, which typically decrease the intestinal population of urease-producing microbes [6–8]. This suggests that HE is a disease of the gut–liver–brain axis whereby dysregulation in gut microbiota can lead to many downstream effects including bacterial translocation and toxin production subsequently causing systemic and neurological inflammation [6–8]. This has led to the evaluation of gut microbiota as a potential therapeutic target, particularly with fecal microbiota transplantation (FMT), and early studies have shown promising results [9].

## 2. Composition of Gut Microbiota

The number of microbes in the gut vastly outnumber the number of somatic cells in the human body [10–13]. There are more than a trillion unique microbial species found throughout the gastrointestinal tract [10–13]. At birth, the gut is relatively sterile, and microorganisms start colonizing after feeding [10–13]. The composition of the gut microbiota differs among individuals and is altered during times of illness and with dietary changes. The majority of organisms tend to be anaerobic bacteria with the greatest representation by species that belong to the phyla Bacteroidetes and Firmicutes [10–14]. Additionally, the population of microbes increases distally with the greatest amount and diversity of microbes seen in the distal small intestine and colon [10–14]. The stomach and proximal intestine contain a small number of organisms ($10^2$), which are made up of a combination of Gram-positive and Gram-negative bacteria as well as fungal species such as *Lactobacillus*, *Streptococcus*, *Helicobacter* and *Candida* [10–14]. The distal intestine and colon contain high levels ($10^{6-12}$) of predominantly anaerobic bacteria including Firmicutes and Bacteroidetes phyla as well as *Clostridium* species [10–14].

## 3. Gut Microbiota Function

The gut microbiota is involved in several normal physiological processes. It facilitates digestion by extracting and absorbing carbohydrates, amino acids, lipids, vitamins and bile acids. It also inhibits growth of invasive microorganisms by preferentially utilizing available resources, by producing anti-bacterial molecules and by contributing to the maintenance of the intestinal immune system. This interplay between the human body and the gut microbiota is a beneficial synergistic relationship that promotes the health of both parties and maintains homeostasis. This state of homeostasis is referred to as eubiosis.

As alluded to earlier, illness can significantly alter the composition of the gut microbiota. This alteration of its composition is known as dysbiosis, which is defined as a pathological condition that disturbs the state of homeostasis. The microbiota responsible for maintaining homeostasis is mainly the Firmicutes organisms, which have been shown to be severely decreased in liver disease [15,16]. Dysbiosis is heavily implicated in cirrhosis

and HE. The etiology for dysbiosis in cirrhosis is presumed to involve reduced levels of bile acids and short chain fatty acids (SCFA), small intestinal bacterial overgrowth (SIBO), and immune dysregulation [17–25]. Bile acids are thought to be protective against dysbiosis, as they are involved in the lysis of pathogens [19–22]. Bile acids have also been shown to regulate innate and adaptive immune inflammatory signaling in the gut by modulating the differentiation of Th17 and $T_{reg}$ cells [23]. Patients with cirrhosis produce lower levels of bile acids due to poor biosynthetic function and impaired intestinal secretion [19–22]. SIBO is commonly seen in cirrhosis; this increases the quantity of pathological organisms and their metabolites, which has several downstream effects including changes to intestinal permeability [24–26]. SCFA are by-products of gut bacterial carbohydrate metabolism, and they have been shown to be integral in maintaining luminal pH, intestinal motility and enterocyte structure [23]. SCFA also regulate immune response in gut lymphoid tissue by inhibiting macrophages, dendritic cells and inflammatory cytokines [23]. Furthermore, gut lymphoid tissue express pattern recognition receptors such as toll-like receptors, which recognize commensal bacterial antigens, and this leads to a cascade of signals that ultimately lead to the differentiation of naïve T cells [23]. The absence or reduction in these commensal bacterial antigens hinders the proliferation and differentiation of gut lymphoid population [23]. In summation, dysbiosis in cirrhosis promotes a pro-inflammatory and immunosuppressed state.

## 4. Changes to Gut Microbiota in Cirrhosis and Hepatic Encephalopathy

Many studies have shown that there is a significant difference between the stool microbiota composition in healthy individuals compared to individuals with cirrhosis. Beneficial organisms that are normal residents of the gut and contribute to the state of homeostasis are referred to as autochthonous organisms. Studies have shown that in cirrhosis there is a reduction in autochthonous organisms such as Bacteroidetes and an increase in harmful organisms such as *Enterobacteriaceae* [27,28]. The data from human studies on gut microbiota in cirrhosis are summarized in Table 1. On the one hand, autochthonous organisms have several beneficial roles including the production of bile acids and SCFAs (e.g., butyrate). On the other hand, pathogenic organisms such as *Enterobacteriaceae* produce endotoxins and lipopolysaccharides, which promote inflammation. The outgrowth of these pathogenic organisms results in the loss of beneficial autologous species, resulting in reduced levels of bile acids and SCFAs and serves to increase intestinal inflammation and permeability, which in turn allows bacterial translocation and systemic inflammation. Interestingly, several studies have shown that not only does the microbiota detected in stool change but also the microbiota detected in the oral cavity. These organisms such as *Streptococcaeae* and *Veillonellaceae* are seen more abundantly in the oral cavity in individuals with cirrhosis and are correlated with a worse disease prognosis. In addition, studies have shown that dysbiosis in cirrhosis is not only limited to stool and saliva but also in the colonic and duodenal mucosa as well as in the liver and ascitic fluid. This further supports the principle of cirrhosis and its complications being a systemic disease with multiple pathophysiologic mechanisms in play.

The changes in composition of the microbiota in cirrhosis are important because of their effect on metabolite and byproduct levels and their ability to affect the body. Many metabolites require microorganisms for their production including bile acids, dimethylamine/trimethylamines and hippurate [17–22,35,46]. As described earlier, endotoxins and lipopolysaccharides increase in cirrhosis when the population of deleterious organisms increase. These perturbations are further exacerbated by underlying portal hypertension in cirrhosis, which leads to bypassing of the reticuloendothelial system and delivery of these metabolites to the systemic circulation. Autochthonous organisms such as Clostridia are responsible for converting primary bile acids to secondary bile acids, which subsequently contributes to fecal bile acid concentrations [17–22,35]. Reductions in fecal bile acid was observed in patients with cirrhosis with a concomitant increase in serum bile acid. Methylamines are associated with atherosclerosis and cardiovascular disease and is now

recognized to play a role in liver disease [47–49]. Alteration of levels of bacterial-derived methylamines was observed in individuals with cirrhosis [47–49]. Hippurate is a product of bacterial metabolism of dietary polyphenols, and low levels of hippurate have been observed in individuals with liver failure and are linked to the degree of hepatocellular reserve [25,50]. Changes to relative abundance of *Bacteroidaceae* in cirrhosis is thought to perhaps contribute to reductions in hippurate levels [36].

**Table 1.** Microbial changes in patients with cirrhosis.

| Study | Population | Changes in Gut Microbiota | Additional Findings |
|---|---|---|---|
| Chen et al., 2011 [29] | 24 Controls<br>36 Cirrhosis | Increased: Proteobacteria<br>Decreased: Bacteroidetes | Child–Pugh score correlated positively with *Streptococcaceae* and negatively with *Lachnospiraceae* |
| Lu et al., 2011 [30] | 32 Controls<br>31 Cirrhosis | Increased: *Enterobacteriaceae*<br>Decreased: Firmicutes | Statistically significant decrease in *Bifidobacterium* to *Enterobacteriaceae* ratio in patients with decompensated hepatitis B cirrhosis. |
| Bajaj et al., 2012 [31] | 10 Controls<br>25 Cirrhosis (17 with HE) | Increased: *Enterobacteriaceae*, *Leuconostocaceae*, *Lactobacillaceae*, *Alcaligenaceae*, *Fusobacteriaceae*<br>Decreased: *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiales XIV* | Specific bacterial families (*Alcaligenaceae*, *Enterobacteriaceae*, *Porphyromonadaceae*) were seen more commonly in patients with HE and were associated with alterations in cognition and inflammation. |
| Mutlu et al., 2012 [32] | 18 Controls<br>28 Alcoholics without cirrhosis<br>19 Alcoholic with cirrhosis | Increased: Bacteroidetes<br>Decreased: Proteobacteria | Dysbiosis seen in both alcoholic groups regardless of presence of cirrhosis. |
| Bajaj et al., 2012 [33] | 17 Controls<br>60 Cirrhosis (24 with HE) | Increased: *Clostridium*, *Acidaminococcus*, *Enterococcus*, *Burkholderia*, *Ralstonia*, *Proteus*<br>Decreased: *Dorea*, *Subdoligranulum* | Significant differences in mucosal microbiota between HE and patients without HE, reduction in *Roseburia* and increases in *Enterococcus*, *Veillonella*, *Megasphaera* and *Burkholderia*. |
| Zhang et al., 2013 [34] | 26 with HE<br>25 Cirrhosis without HE | Increased: *Streptococcus salivarius* in HE | *Streptococcus salivarius* correlated negatively with cognitive function. |
| Kakiyama et al., 2013 [35] | 14 Controls<br>47 Cirrhosis | Increased: *Enterobacteriaceae*<br>Decreased: *Lachnospiraceae*, *Ruminococcaceae*, *Blautia* | Decreased fecal bile acids and reduced secondary bile acid conversion in patients with cirrhosis. |
| Bajaj et al., 2014 [36] | 15 Controls<br>15 Cirrhosis | Decreased: *Lachnospiraceae*, *Ruminococcaceae* | Increased *Streptococcaceae* after PPI therapy in all groups. |
| Bajaj et al., 2014 [15] | 25 Controls<br>219 Cirrhosis | Increased: *Enterococcaeae*, *Staphylococcaceae*, *Enterobacteriaceae*<br>Decreased: *Ruminococcaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Porphyromonadaceae* | Increase in *Enterobacteriaceae* after HE episode. |
| Qin et al., 2014 [16] | 83 Controls<br>98 Cirrhosis | Increased: *Veillonella*, *Streptococcus*, *Clostridium*<br>Decreased: Bacteroidetes | Significantly increased population of oral flora in cirrhosis. |
| Tuomisto et al., 2014 [37] | 14 Controls<br>13 Cirrhosis | Increased: *Bacteroides* spp., *Enterobactericaeae*, *Enterobacter* spp. | Patients with alcoholic cirrhosis had increased amounts of enterobacteria in feces. |
| Bajaj et al., 2015 [38] | 32 Controls<br>102 Cirrhosis | Increased: *Enterobacteriaceae*, *Enterococcaceae*<br>Decreased: *Lachnospiraceae*, *Ruminococcaceae*, *Clostridiaceae* | Patients with cirrhosis had dybiosis in saliva and stool. |
| Bajaj et al., 2015 [39] | 94 Controls<br>278 Cirrhosis | Increased: *Lactobacillaceae*, *Enterococcaceae*, *Enterobacteriaceae*, *Pasteurellaceae*<br>Decreased: *Bacteroidaceae*, *Porphyromonadaceae*, *Clostridiales XIV*, *Lachnospiraceae*, *Ruminococcaceae* | Patients with cirrhosis and diabetes were found to have increased *Bacteroidaceae* and reduced *Ruminococcaceae* in stool. |

**Table 1.** *Cont.*

| Study | Population | Changes in Gut Microbiota | Additional Findings |
|---|---|---|---|
| Chen et al., 2015 [40] | 50 Controls<br>79 Cirrhosis | Increased: *Pasteurellaceae*, *Streptococcaceae*, *Enterococcaceae*<br>Decreased: *Bacteroidaceae*, *Ruminococcaceae*, *Lachnospiraceae* | Individuals who developed HE had reduced population of *Lachnospiraceae*. |
| Ahluwalia et al., 2016 [41] | 40 Controls<br>147 Cirrhosis | Increased: *Lactobacillaceae*, *Enterococcaceae*, *Clostridiales XIV*, *Lachnospiraceae*, *Enterobacteriaceae* | Patients with cirrhosis and HE had increased *Staphylococcaceae*, *Enterococcaceae*, *Porphyromonadaceae*, *Lactobacillaceae* |
| Chen et al., 2016 [42] | 28 Controls<br>30 Cirrhosis | Increased: *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium*, *Prevotella* | PPI reduced *Cloacibacterium* and increased *Dialister* |
| Santiago et al., 2016 [43] | 17 Controls<br>60 Cirrhosis | Decreased: *Clostridiales*, *Roseburia faecis*, *Alistipes putredinis*, *Oscillospira*, *Mogibacteriaceae*, *Dehalobacterium* | Patients with ascites had elevation in markers of serum microbial translocation. Fecal microbiome composition was more altered in patients with ascites compared to those without. |
| Dubinkina et al., 2017 [44] | 72 Alcoholics<br>27 Alcoholic cirrhosis | Increased: *Streptococcus constellatus*, *Streptococcus salivarius*, *Veillonella atypica*, *Veillonella dispar*, and *Veillonella parvula*<br>Decreased: *Parabacteroides* | Increased abundance of oral microbes in patients with cirrhosis. |
| Sung et al., 2019 [45] | 13 Controls<br>97 Cirrhosis<br>(62 with HE) | Increased: *Veillonella parvula*, *Clostridium* XI, *Prevotella*, *Enterococcus*, *Schlegelella*, *Megasphaeae*, *Lactobacillus*<br>Decreased: *Phascolarctobacterium*, *Bacteroides*, *Alistipes* | Increased abundance of *Alistipes*, *Bacteroides* and *Phascolarctobacterium* associated with HE recurrence. |

All these changes to microbiota population and function have been invariably linked to complications of cirrhosis including HE. For instance, patients with HE have an increased population of *Veillonellaceae*, which was associated with increased levels of IL-6, TNF-a, IL-2 and IL-13 [51]. Elevations in pro-inflammatory cytokines are thought to have contributed to poorer cognition in patients with HE [6]. HE is associated with over-abundance of ammonia partly due to urease-producing organisms. Individuals with HE and poor cognition were found to have higher population of urease-producing Proteobacteria [6]. Another study showed that *Blautia*, *Fecalibacterium*, *Roseburia* and *Dorea* were associated with good cognition, while pathological organisms such as Enterococcus was linked to poor cognition [33]. Furthermore, an over-abundance of ammoniagenic oral organisms such as *Streptocoocus* species were found in cirrhotic patients with HE [34]. Endotoxin-producing organisms were also found in higher abundance in the oral microbiota in patients with HE, which is correlated with a pro-inflammatory state with increased levels of IL-1B, IL-6 and IgA [34]. Magnetic resonance imaging (MRI) has been utilized in some studies to evaluate the link between the composition of gut microbiota and neuronal function [41]. By using brain MRI, patients with higher levels of Enterobacteriaceae were found to be have astrocytic changes typically seen in hyperammonemic states [41]. Furthermore, patients with higher levels of *Porphyromonadaceae* were found to have non-hyperammonemic neuronal changes [41].

As described earlier, SIBO is commonly associated with cirrhosis, affecting up to 59% of these patients [52–63]. The gold standard diagnostic method is a demonstration of greater than $10^5$ colony forming units per millimeter in the proximal jejunum via aspiration during endoscopic examination. SIBO typically shows an over-abundance of gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae* [52–63]. These organisms are linked to the development of decompensated cirrhosis and the development of HE due to their ability to translocate [52–63]. One meta-analysis demonstrated that the prevalence of SIBO in cirrhosis is significantly greater than its prevalence in otherwise healthy individuals [58]. In this study, the presence of SIBO in cirrhotic patients was correlated with an increased probability of HE compared to those without SIBO [58]. SIBO

alone, therefore, does not predispose to liver disease, but it does predispose to worse outcomes when it coexists with systemic inflammation, immunodeficiency, increased intestinal permeability and decreased intestinal motility.

## 5. Modulation of Gut Microbiota in Hepatic Encephalopathy

The first-line therapy for HE is to target the precipitating episode, which can range from bleeding, dehydration, constipation to infection. Other therapies can be started once the precipitating episode is identified and treated. The most common therapies for HE include non-absorbable disaccharides such as lactulose and lactitol as well as antibiotics such as rifaximin. These therapies work by decreasing serum ammonia levels by accelerating intestinal transit and by modifying intestinal bacterial metabolism and abundance. As the influence of gut microbiota composition on cirrhosis and HE has been clearly demonstrated by many studies, the next natural step is to therapeutically target the gut–liver axis. This has been done with dietary changes, prebiotics, probiotics and more recently with fecal microbiota transplantation (FMT).

Diets rich in plant protein, fermented milk products, and vegetables have shown to be beneficial to the gut microbiota. Plant protein has been linked to greater abundance of autochthonous organisms and reduction in pro-inflammatory organisms [64]. We demonstrated that diet not only affects gut microbiota but also modulates hospitalization risk [65]. This was done by comparing decompensated and compensated American patients with cirrhosis to matched cohorts in Turkey. Turkish patients were found to have significantly higher microbial diversity with higher intake of vegetables, chocolate, coffee, tea and fermented milk intake predicting a higher microbial diversity. Furthermore, the Turkish cohort had a lower risk of 90-day hospitalizations.

Administration of probiotics and prebiotics is another technique to modulate the gut microbiota. The purported mechanism of probiotics is to directly increase the population of beneficial bacteria. The data on probiotic therapy are conflicting, especially for treating gastrointestinal pathologies other than liver disease; however, one study showed benefit in cirrhosis complicated by HE [66,67]. Saab et al. demonstrated that probiotics compared to placebo decreased hospitalization rates in patients with cirrhosis and HE and prevented progression to overt HE in patients with underlying covert HE [68]. Prebiotics are typically non-digestible fiber compounds that feed beneficial bacteria in the digestive system. Prebiotics have been shown to promote the growth of organisms that produce SCFAs and thus preventing intestinal permeability [67].

## 6. Fecal Microbiota Transplantation in Hepatic Encephalopathy

FMT involves the administration of a solution of a fecal suspension from a donor into the intestinal tract of a recipient. This can be administered in several formulations including enema, via colonoscopy, or in capsular form. This therapy serves to directly change the gut microbiota composition. Previous studies have shown that FMT is effective in conditions associated with dysbiosis particularly recurrent *Clostridoides difficile* and ulcerative colitis [69,70]. As repeatedly demonstrated, cirrhosis and, in particular, HE is associated with dysbiosis. Reversing dysbiosis and restoring eubiosis can potentially reduce systemic inflammation, preserve gut membrane integrity, prevent bacterial translocation and maintain production of bile acids.

A case report by Kao et al. was the first attempt at treating HE with FMT [71]. Cognition was assessed with the inhibitory control test and the Stroop test, and they showed that the patient's cognition improved with consecutive FMT until it stabilized by the fourth week after a total of three FMTs. The patient's cognition reverted to baseline after 14 weeks after withdrawal of FMTs. Interestingly, *Lachnospiraceae*, which is associated with better cognition, was found to be in reduced quantities in this patient.

The aforementioned results were promising and lead to the first randomized control trial studying the efficacy of FMT in HE, which was conducted by our group. In this study, a single stool specimen was used for the experimental group by identifying a donor

with the highest relative abundance of *Lachnospiraceae* and *Ruminococcaceae* [72]. A total of 20 cirrhotic patients with recurrent HE (defined as two or more episodes) were enrolled and randomly assigned to the standard of care and to the FMT group. Both groups were to continue their lactulose and rifaximin. Prior to receiving the FMT enema, the experimental group received a five-day treatment of antibiotics to increase the success of donor bacterial colonization. The FMT with antibiotic pretreatment was well tolerated, and the patients were followed for up to 150 days. There was a significant reduction in serious adverse events in the experimental group compared to the control group. Five patients in the control group had a recurrent episode of HE, whereas no patient in the FMT group developed further HE. The psychometric hepatic encephalopathy score (PHES) and EncephalApp Stroop (EAS) were used to assess cognitive ability. These cognitive assessments showed that there was a significant improvement in cognition in the FMT group at day 20 compared to baseline.

A follow-up study was performed to demonstrate the long-term safety of FMT. The study was carried out in a similar fashion to our previous study; however, all participants received proton pump inhibitors in addition to lactulose and rifaximin. The participants were followed for 12 months, and there were significantly fewer hospitalizations and HE episodes in the FMT group compared to the control group [73]. Furthermore, cognition measured by PHES and EAS was found to be significantly better in the experimental group compared to their control counterparts. Similar to the case report by Kao et al., the abundance of the autochthonous organisms *Lachnospiraceae* and *Ruminococcaeae* was not statistically different between the two groups despite the FMT donor's microbiome being rich in these organisms. We concluded that FMT is safe for long-term use; however, this study was limited by a small sample size. This was then followed by a phase 1, randomized, placebo-controlled trial to determine the safety of capsular FMT. The groups were followed for five months after administration of FMT capsules obtained from a single donor with the highest relative abundance of *Lachnospiraceae* and *Ruminococcaceae*. Only one serious adverse event was reported in the FMT group as opposed to 11 in the control group, suggesting that FMT capsules are well tolerated and safe [74]. Patients who received FMT capsules had improved cognition when assessed with EAS; however, PHES did not improve. The participants had their microbiomes analyzed, and the FMT group had increases in duodenal *Ruminococcaeae* and *Bifidobacteriaceae* and decreases in *Streptococcaceae* and *Veillonellaceae*. Moreover, there were reductions in sigmoid and stool populations of *Veillonellaceae*. As described earlier, *Streptococcaceae* is an ammionagenic organism and is linked with development of HE. There was a non-significant trend toward fewer hospitalizations in the FMT group. Although the sample size was small, this was one of the first studies to demonstrate that FMT capsules can decrease relative abundance of microorganisms associated with the progression of cirrhosis. A case series published by Mehta et al. performed FMT via colonoscopy in patients with cirrhosis and recurrent HE [75]. Although this retrospective study only had 10 participants, it demonstrated that there was a sustained clinical response in six patients 20 weeks after treatment. These patients showed reductions in arterial ammonia concentration, Child–Pugh score, and model for end-stage liver disease score.

We recently completed a phase 1, double-blind, randomized clinical trial to study the effects of FMT enema on alcohol-use-disorder-related cirrhosis [76]. Although not designed to study FMT on outcomes of HE, this study demonstrated reduced alcohol craving, reduced urinary ethyglucuronide, improved cognition and psychosocial quality of life at day 15 compared to placebo. Moreover, there was a reduction in serum IL-6 and LPS binding protein and increased butyrate/isobutyrate compared to baseline in the FMT group. These metabolic changes are presumed to be due to the increased abundance of *Ruminococcaceae* in the treatment group. In regard to safety, there was a lower rate of serious adverse events in the FMT group compared to placebo.

Antibiotic resistance is a frequent complication of cirrhosis that leads to poor outcomes. FMT offers a promising therapy that may reduce the population of multidrug resistance

organisms. We recently studied this by evaluating the expression of the antibiotic resistance gene (ARG) in patients with decompensated cirrhosis before and after healthy donor capsule and enema FMT [77]. There were 20 patients with cirrhosis in each trial (capsule and enema FMT), and all patients were on rifaximin, lactulose and proton pump inhibitors. ARGs were identified using metagenomics, and changes in ARG abundance were studied within and between groups. Expression of beta-lactamase was decreased post capsule FMT compared to baseline. Beta-lactamase, vancomycin and rifamycin ARGs were significantly lower at 4 weeks post-FMT compared to placebo. A reduction in rifamycin ARG in the interventional group was associated with cognitive improvement. In the enema FMT trial, beta-lactamase and vancomycin ARGs were decreased at day 7 post treatment compared to standard of care, and this reduction in ARGs persisted until day 15. These data suggest that ARG abundance is largely reduced after FMT in decompensated cirrhosis regardless of the route of administration.

## 7. Future Directions and Challenges

There are several ongoing and future trials studying several aspects of FMT including different routes of administration. The PROFIT trial is an ongoing study designed to assess the effect of FMT delivered directly into the small bowel of patients with cirrhosis [78]. In contrast to previous studies, the patients in this study are not to be pretreated with antibiotics. The idea is that direct instillation of FMT into the jejunum can directly target SIBO. This study is powered to study the feasibility and safety of this technique and not to assess clinical outcome. Preliminary data from this trial were recently presented at the 2020 digital international liver congress [79]. Twenty-one patients with confirmed cirrhosis were included, and 15 patients received FMT and six received placebo. Plasma ammonia was significantly reduced at day 30 compared to baseline. There was also a non-significant increase in plasma ammonia in the placebo group. Ammonia levels in stool were found to be increased in the placebo group but not in the FMT group.

A common criticism of the aforementioned studies is small sample sizes. This is certainly an issue, as small sample sizes can lead to inflated false discovery rate and inflated effect size estimation. Several large studies are ongoing and actively recruiting, one of which we are conducting [80]. This trial includes 100 participants with four groups randomized to oral and/or rectal FMT.

These early studies have shown promising results in treating HE with FMT. Several questions remain answered. One question is safety. Our studies have shown that FMT appears to be well tolerated and safe. A case report by DeFilipp et al. highlights the importance of donor screening to limit transmission of microorganisms [81]. They reported transmission of drug-resistant E. coli in two patients who had undergone FMT obtained from the same donor. Both patients developed bacteremia and one died. Other rare cases of bacteremia and death have been reported suggesting that FMT is not a benign procedure. Careful selection of donor and recipient must be done to minimize the risks. The FDA has updated their protocol on FMT in 2019 mandating that donors be screened for multi-drug-resistant organisms. However, since patients with cirrhosis have compromised intestinal membrane integrity and are immunocompromised, there is potential of transmission of other pathogenic organisms that are not routinely screened for.

The risk to benefit ratio of using FMT to treat chronic liver disease, cirrhosis and hepatic encephalopathy is yet to be established. The benefits are assuredly high, as there is no equivalent alternative therapy available to address the issue of dysbiosis in chronic liver disease. As mentioned previously, the risks include transmission of resistant organisms or other deleterious traits that are not yet measured. Furthermore, patients need to be accepting of this therapy, and it needs to be universally available. For this to occur, certain aspects regarding the implementation of FMT in clinical practice need to be established, particularly regarding the amount of donor material required, optimal route of administration, length and frequency of treatment, and interval between treatments. While FMT has been successful for the treatment of refractory *Clostridium difficile*, whereby gut microbiota

is typically sterilized by antibiotics prior to replacement with a single inoculation of small amount of a donor's sample, it may not be as simple in chronic liver disease. The dysbiosis in cirrhosis is complex and is not necessarily equivalent amongst patients, which makes its treatment selection challenging. Patients with cirrhosis are routinely treated with antibiotics for HE and spontaneous bacterial peritonitis, which further disturbs the microbiota. Dosing time is likely to be important, and preliminary data from the PROFIT trial, while small in number, have demonstrated this [82]. It is likely that multiple doses are required to achieve a meaningful therapeutic response. As mentioned previously, we have a large clinical trial with 100 participants to address the question of treatment mode of delivery and dose range (0–3, clinicaltrials.gov ID NCT03796598). Our study consists of four groups: dual oral and rectal FMT, oral FMT and rectal placebo, oral placebo and rectal FMT and oral and rectal placebo. Rectal-administered treatment is given once at day 2, and oral administered treatment is given at day 2 and day 30. Participants are then followed for 6 months, and the primary outcome of the study is to determine serious adverse events related to FMT. Secondary outcomes at 6 months include changes in microbial diversity in stool/blood/saliva, changes in intestinal permeability and change in HE status as determined by changes in EAS or PHES. To further assess treatment delivery, the PROFIT trial will be extending their study to recruit 300 more patients over two years to undergo multiple doses using a capsule delivery model in contrast to their previous endoscopically administered treatment. The possibility of long-lasting cure from FMT alone is highly unlikely given the multi-factorial nature of chronic liver disease. The complexity of chronic liver disease and cirrhosis require a multi-faceted treatment approach. It is clear that FMT is not intended to be used as a solo therapy and simply augments the existing armamentarium for treating liver disease.

The need for pre-procedure antibiotics for sterilization has not been studied extensively. Whether antibiotics need to be withheld post-FMT is not clear either, as this may not be feasible in many patients. Multiple routes of administration have been evaluated with many showing successful results; however, the superiority or non-inferiority of one route versus another is unknown. Utilizing FMT in clinical practice will only be possible once we find solutions to these questions.

## 8. Conclusions

Gut microbiota has been repeatedly demonstrated to play a major role in liver disease and its complications including HE. Many patients continue to have progressively worsening cirrhosis and more frequent and recurrent episodes of HE despite treatment. The gut microbiota represents an attractive therapeutic target. Early studies have shown encouraging findings with improvement in cognition and reduction in HE episodes following FMT, but larger studies are required and underway. There remains a need to standardize the treatment and explore the best route options along with integrating it with current therapies for HE.

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# Shunt-Induced Hepatic Encephalopathy in TIPS: Current Approaches and Clinical Challenges

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**Abstract:** Transjugular intrahepatic portosystemic shunt (TIPS) is an established treatment tool in decompensated liver cirrhosis that has been shown to prolong transplant-free survival. Hepatic encephalopathy (HE) is a frequent complication of decompensated cirrhosis, eventually induced and/or aggravated by TIPS, that remains a clinical challenge especially in these patients. Therefore, patient selection for TIPS requires careful assessment of risk factors for HE. TIPS procedural parameters regarding stent size and invasive portosystemic pressure gradient measurements thereby have an important role. Endovascular shunt modification, in combination with a conservative medical approach, often results in a significant reduction of symptoms. This review summarizes HE molecular mechanisms and pathophysiology as well as diagnostic and therapeutic approaches targeting shunt-induced HE.

**Keywords:** hepatic encephalopathy; transjugular portosystemic intrahepatic shunt; liver cirrhosis

## 1. Background

Transjugular intrahepatic portosystemic shunt (TIPS) placement is an established method in the management of complications of portal hypertension [1,2]. With technical progress and increasing evidence, TIPS has improved transplant-free survival and TIPS-associated complications were vastly reduced [1,3–5]. However, hepatic encephalopathy (HE) occurs frequently after TIPS procedures with an incidence of 20% to 50% [6,7]. The mechanism of HE is complex, including reduced hepatic filter function in liver dysfunction and splanchnic blood shunting into the systemic circulation, as well as an overproduction of enteric neurotoxins and increased cerebral inflammation and neurotoxins [8–12]. Yet, hyperammonemia remains the central underlying cause [8–12]. The clinical effects range from mild cognitive alteration to coma, and are commonly graded using the West Haven Criteria [13,14].

Historically, post-TIPS HE pharmacological approaches are directed at reducing enteric neurotoxin production and absorption, which is increasingly being questioned today [12,15–17]. However, up to 8% of TIPS patients develop refractory HE, which is often associated with further deterioration of liver disease [18,19]. In these cases, endovascular shunt modification is the only therapeutic option besides liver transplantation [18,20]. TIPS modification reduces the portosystemic shunt volume and can improve HE [21].

Despite these advances, there are still uncertainties regarding the appropriate workup for TIPS patients [22]. Moreover, prevention and management of post-TIPS HE are still in need of improvement [1,22]. Correct patient selection for TIPS requires careful assessment of risk factors for

HE to prevent complications which may weaken the improved hemodynamic results and worsen the patient's life quality or expectancy [22–24]. Furthermore, the history of HE with increased severity itself is one of the main risk factors for HE recurrence in cirrhosis, and also an important predictor of post-TIPS HE [25–27].

## 2. Pathogenesis and Molecular Mechanisms

Understanding the underlying pathophysiology and molecular mechanisms of HE is essential for targeted management. Several new pathogenetic mechanisms have recently been identified while neurotoxicity from hyperammonemia remains the central underlying cause of HE [10,11,28,29].

### *2.1. Ammonia Homeostasis in Normal Liver Function and Hepatic Failure*

Figure 1 illustrates the gut–liver–brain axis pathway considering porto-systemic hemodynamics. Under physiological conditions, ammonia enters the portal circulation and is cleared by the urea cycle in the liver which is then excreted by the kidneys and metabolized in skeletal muscle (Figure 1A) [10,30]. In chronic liver disease or hepatic failure, blood flow becomes hepatofugal and retrograde into the portal vein, resulting in splanchnic blood shunting into the systemic circulation (Figure 1B) [30]. Brain and muscle tissue use the enzyme glutamine synthetase to detoxify ammonia by synthesizing glutamine from glutamate [31]. The kidneys are able to release ammonia from the glutamine incurred by the brain and muscles into the urine using the enzyme glutaminase, but this mechanism is oversaturated in severe or chronic hyperammonemia [31].

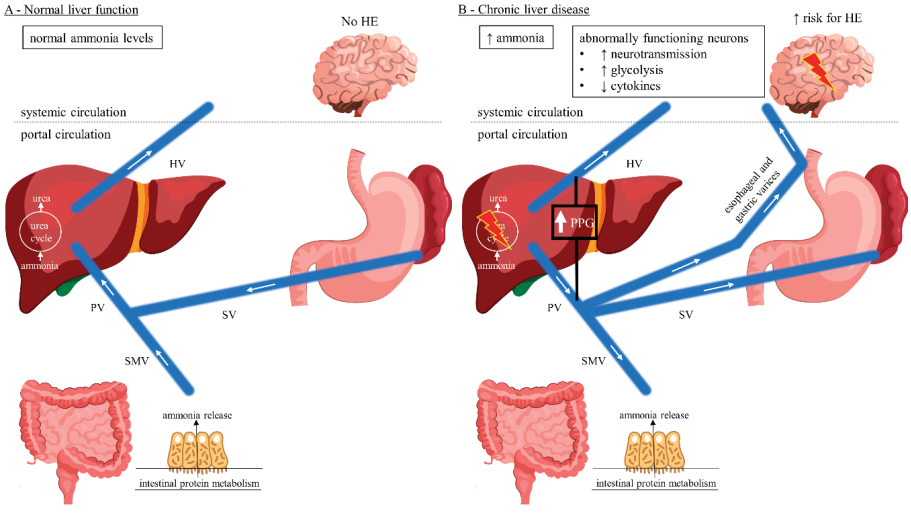


**Figure 1.** *Cont.*

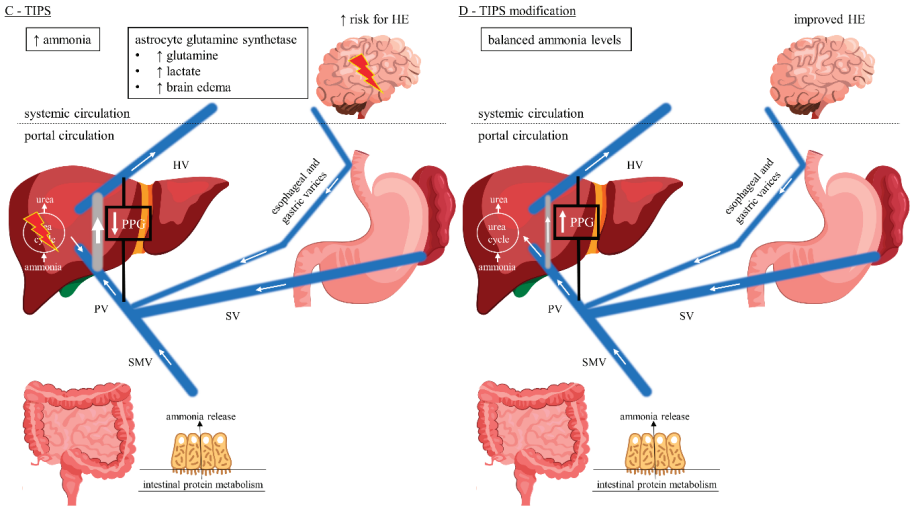


**Figure 1.** Gut–liver–brain pathway of HE considering porto-systemic hemodynamics. (**A**) Normal liver function with hepatopetal blood flow. Ammonia released from the gut enters the portal circulation and is detoxified in the liver via the urea cycle. Normal systemic ammonia levels, no HE being present. (**B**) Chronic liver disease with hepatofugal blood flow reversal resulting in development of porto-systemic collaterals and splanchnic blood shunting into the systemic circulation. Hepatic urea cycle is bypassed resulting in elevated systemic ammonia levels with increased risk for HE due to neuronal dysfunction. (**C**) TIPS with predominantly hepatopetal blood flow towards the low-pressure shunt rather than liver parenchyma, achieving decreased splanchnic blood shunting. Intrahepatic portal vein flow is hepatofugal and into the shunt. The hepatic urea cycle is again bypassed resulting in elevated systemic ammonia levels with increased risk for HE, in an acute setting, due to the increased metabolism of ammonia to glutamine by the astrocytes, with a subsequent tendency to edema. (**D**) Endovascular shunt modification decreases the shunt flow achieving hepatopetal flow reversal in the portal vein. Ammonia detoxification in the liver via the urea cycle is increased, thereby improving HE. This cover was created with resources from Freepik.com. Abbreviations: HE, hepatic encephalopathy; HV, hepatic vein; PPG, portosystemic pressure gradient; PV, portal vein; SMV, superior mesenteric vein; SV, splenic vein; TIPS, transjugular intrahepatic portosystemic shunt.

### 2.2. Cerebral Ammonia Metabolism

Astrocytes are of central importance for the maintenance of adequate neuronal function and play a central role in the pathophysiology of HE [10]. They are the only central nervous system (CNS) cells capable of detoxifying ammonia by synthesizing glutamine from the excitatory neurotransmitter glutamate [32,33]. Glutamine increases the permeability of the blood-CSF barrier [33]. Acute HE, e.g., post-TIPS, is caused by a rapid rise in ammonia levels and often associated with generalized swelling of the astroglia, which clinically may present as cerebral edema (Figure 1C) [9,33–35]. In contrast, a long-term increase in serum ammonia levels usually does not show clinical signs of cerebral edema [9,36]. CNS cells exhibit osmotic adaptive mechanisms which may explain the lower frequency of brain edema in chronic hepatic failure [36]. Here, hyperammonemia results in direct neuronal toxicity and altered neurotransmission leading to HE (Figure 1B) [9,37].

### 2.3. Ammonia Homeostasis among TIPS Patients

Among TIPS patients, there is predominantly hepatopetal blood flow towards the low-pressure shunt rather than liver parenchyma, whereas intrahepatic portal vein flow is hepatofugal and towards the shunt (Figure 1C) [30]. Moreover, following TIPS there is an upregulation of glutaminase activity

in the gut resulting in increased intestinal ammonia production [30,38]. On the other hand, the body composition can alter among TIPS patients resulting in reversal of sarcopenia and thereby improving ammonia metabolism in skeletal muscle [39,40]. However, acute post-TIPS HE is caused by a rapid short-term increase in ammonia levels and often associated with cerebral edema [9,33–35]. In contrast, chronic/late post-TIPS HE with a long-term rise in serum ammonia levels usually does not present as cerebral edema [9,36]. Endovascular shunt modification reduces the shunt flow and achieves hepatopetal flow reversal in the portal vein (Figure 1D) [30]. Consequently, the intestinally derived ammonia shunt is reduced and perfusion to the hepatocytes is increased, thereby improving HE [30].

### *2.4. Additional Mechanisms Underlying HE*

Besides the direct correlation between blood ammonia levels and the degree of HE, further mechanisms in HE have recently been discussed [41,42]. An increased inhibitory neurotransmission by γ-aminobutyric acid (GABA) is another possible factor [11]. GABA can be formed in the colon by bacterial decarboxylation of glutamate, and, in the event of liver failure due to reduced hepatic clearance via the blood-CSF barrier, can reach the CNS [43]. The GABA receptor on the postsynaptic membrane contains binding sites for benzodiazepines [44]. By binding them, the affinity of the receptor for GABA itself is significantly increased [11,44]. This explains why benzodiazepines can trigger or worsen HE in cirrhosis of the liver [45]. It has also been shown that cirrhosis of the liver can lead to an increase in the concentration of so-called endogenous benzodiazepines [46]. A systemic inflammatory reaction with inflammatory cytokines and oxidative stress is assigned an important role [47]. Moreover, magnetic resonance imaging shows an increased deposition of manganese in the basal ganglia of patients with HE, which is neurotoxic and is usually excreted hepatobiliary [10]. In contrast to the aromatic amino acids, the branched-chain amino acids in serum are reduced in cirrhosis of the liver, since the former are degraded less in the liver and the latter are catabolized more in extrahepatic tissues [48,49]. The increased concentrations of aromatic amino acids are said to inhibit the intracerebral synthesis of dopamine and norepinephrine, while inactive false neurotransmitters are increasingly being produced [48,50]. In the colon there are toxic short- and medium-chain fatty acids as well as free, unconjugated phenols, toxic para-hydroxyphenolic acids and mercaptans (metabolites of the amino acids tyrosine, phenylalanine and methionine), which enter the CNS as a result of liver insufficiency and via portosystemic shunts, where they also have neurotoxic effects [50,51].

## 3. Epidemiology and Clinical Presentation

With a prevalence of 20–80%, HE is one of the most important comorbidities in patients with advanced cirrhosis [25,52,53]. The 1-, 5- and 10-year cumulative incidence of HE is between 0% to 21%, 5% to 25%, and 7% to 42% [25], respectively. The creation of a portosystemic shunt can significantly worsen a HE or even cause it, while the overall prevalence of HE in patients with TIPS ranges between 10% and 50% [25,53]. Within 2 years after TIPS insertion, incidence of HE has been reported between 20% and 55% [25,53].

Considering its complexity, HE can be characterized using four parameters (Table 1): (1) underlying disease, (2) severity of clinical manifestation, (3) time course, (4) existence of precipitating factors [25,54,55].

(1) According to the underlying cause, HE is subdivided into type A (due to reduced detoxification performance of the liver in acute liver failure), type B (if the hepatic detoxification function is bypassed by portosystemic bypass or shunt) and type C (by combining the mechanisms mentioned above in cirrhosis) [25,54,55].
(2) Clinical effects range from mild confusion to coma and are commonly graded by the West Haven Criteria [14,54]:

- Grade 0 (minimal)—normal state of consciousness, objectifiable only by neuropsychiatric tests;

- Grade 1—slight mental slowdown, disturbed fine motor skills;
- Grade 2—increased fatigue, apathy, flapping tremor/asterixis, ataxia, slurred speech;
- Grade 3—somnolence, marked disorientation, rigor, stupor;
- Grade 4—coma.

**Table 1.** Clinical characterization of HE (adapted from AASLD/EASL guidelines [54]).

| Type | Grade | | Time Course | Spontaneous/Precipitated |
|---|---|---|---|---|
| A | Minimal | Covert | Episodic | Spontaneous |
| | I | | | |
| B | II | | Recurrent | |
| | III | Overt | | Precipitated factors |
| C | IV | | Persistent | |

However, this classification is increasingly being questioned because of its subjective nature and impaired suitability for follow-up, and has been extended by the subdivision into covert and overt HE according to the International Society for Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) guidelines [56,57].

(3) Based on its time course, HE is classified into an episodic (HE bouts more than 6 months apart), a recurrent (HE bouts within a time frame of 6 months or less) and a persistent (patterns of behavioral alterations that are always present interspersed with relapses of overt HE) form [54,55].

(4) In the event of overt HE occurrence, triggering factors such as constipation, infections, gastrointestinal bleeding, electrolyte imbalance, diuretic over dosage or taking benzodiazepines, analgesics or hypnotics must always be cared for [25,54,55]. In the absence of precipitating factors, HE is considered to be spontaneous [54,55].

## 4. Risk Factors of HE in Cirrhosis and Following Tips

Despite the increased risk of developing HE or worsening it on the basis of chronic liver disease, TIPS has been well established in the therapy of refractory complications of portal hypertension such as variceal bleeding, ascites, hepatic hydrothorax and hepatorenal syndrome [1,2]. To ensure prevention and improve management of post-TIPS HE, careful patient selection for TIPS considering specific risk factors of HE is required [22–24]. Within this context, it is important to differentiate specific predictive risk factors of HE in cirrhosis and following TIPS placement.

### *4.1. Risk Factors in Cirrhosis*

Most episodes of overt HE occur secondary to precipitating factors, especially infections and gastrointestinal bleeding [25,54]. Moreover, several studies have identified multiple risk factors of HE in cirrhosis (Table 2) [58–62]. Among patients with liver cirrhosis, minimal HE, history of overt HE, sarcopenia, epilepsy, diabetes, higher creatinine and bilirubin levels, lower albumin levels, and use of proton pump inhibitors and non-selective beta blockers are the main risk factors for developing overt HE [25,58–62]. Minimal HE is not only one of the main risk factors for overt HE, but is also associated with the severity and progression of chronic liver disease [25,63]. In a retrospective study of 216 cirrhotic patients, the occurrence of minimal HE was associated with a 2-fold increase in the risk of developing overt HE [59]. Those results were in line with the results of a later prospective study of 310 cirrhotic patients while the risk of an overt HE was 1.79 (95% CI: 1.21–2.65) compared to those without minimal HE [62]. The same studies have identified overt HE as another important risk factor for HE recurrence (2.01–2.45-fold increase) [59,62]. Moreover, the number of HE episodes also revealed a direct association with the occurrence of further HE episodes [25,64]. Changes in serum

levels of albumin, bilirubin, and creatinine have also been identified as independent risk factors of HE among cirrhotic patients [25,58–62]. Hyperbilirubinemia and higher levels of creatinine significantly increased the risk of HE while an increase in albumin levels of 1 mg/dl reduced the risk of overt HE by up to 53% [58–62]. Ultimately, various comorbidities such as diabetes and epilepsy, sarcopenia and hyponatremia were assigned an increased risk of developing HE [25,58]. In addition, taking proton pump inhibitors and non-selective beta blockers also increased the risk of HE in cirrhosis (34–83%), whereas taking statins revealed a protective effect (risk reduction by 20%) [61,62].

**Table 2.** Recent relevant publications identifying specific risk factors of overt HE in cirrhosis (adapted from Elsaid et al. [25]).

| Reference | Study Design | Number of Patients | Risk Factor | Adjusted Hazard Ratio (95% CI) |
|---|---|---|---|---|
| Jepsen et al., 2015 [58] | Secondary analysis * | 862 | Diabetes | 1.86 (1.20–2.87) |
| | | | Child–Pugh class B | 2.57 (0.61–10.8) |
| | | | Child–Pugh class C | 4.32 (0.96–19.3) |
| | | | Bilirubin, per 10 µmol/l increase | 1.06 (1.03–1.08) |
| | | | Albumin, per 5 g/L increase | 0.68 (0.56–0.83) |
| | | | Sodium, per 5 mmol/L increase | 0.63 (0.53–0.74) |
| | | | Creatinine, per 10 µmol/L increase | 1.09 (1.05–1.13) |
| Riggio et al., 2015 [59] | Retrospective cohort | 216 | Previous overt HE | 2.01 (1.24–3.26) |
| | | | Minimal HE | 2.02 (1.23–3.33) |
| | | | Albumin level < 3.5 g/dL | 2.32 (1.37–3.93) |
| Ruiz-Margáin et al., 2016 [60] | Prospective cohort | 220 | Cachexia | 1.81 (1.08–3.03) |
| | | | Creatinine, per 1 mg/dL increase | 4.12 (1.57–10.77) |
| Tapper et al., 2018 [61] | Retrospective cohort | 1979 | Bilirubin, per 1 mg/dL increase | 1.07 (1.05–1.09) |
| | | | Albumin, per 1 mg/dL increase | 0.54 (0.49–0.60) |
| | | | Non-selective beta-blocker use | 1.34 (1.09–1.64) |
| | | | Statin use | 0.80 (0.65–0.98) |
| Nardelli et al., 2019 [62] | Prospective cohort | 310 | Albumin, per 1 g/L increase | 0.47 (0.33–0.69) |
| | | | Previous overt HE | 2.45 (1.66–3.58) |
| | | | Minimal HE | 1.79 (1.21–2.65) |
| | | | Proton pump inhibitors use | 1.83 (1.22–2.74) |

* data from 3 randomized trials; Abbreviations: CI, confidence interval; HE, hepatic encephalopathy.

### 4.2. Risk Factors Following TIPS

Among TIPS patients, older age, higher Child–Pugh and model of end-stage liver disease (MELD) score, history of HE pre-TIPS, low portosystemic pressure gradient (PPG), sarcopenia, and use of proton pump inhibitors were identified as potential risks for developing overt HE post-TIPS (Table 3) [25,26,65–68]. In a prospective study of 82 TIPS patients, older age was associated with an adjusted hazard ratio of 1.05 [66]. Those results were in line with the results of later retrospective studies of 284 and 264 TIPS patients [67,68]. Several studies have identified higher Child–Pugh/Child–Turcotte–Pugh and MELD score as independent risk factors of post-TIPS HE [25,26,65–68]. In a retrospective cohort of 279 TIPS patients, a unit-increase in the Child–Pugh score was associated with 1.2 higher odds of post-TIPS HE [25,65]. A prospective study of 82 TIPS patients reported similar results with an increased risk of post-TIPS HE by 29% [25,26]. Two retrospective ($n = 279$, $n = 284$) and one prospective study ($n = 46$) revealed that a one unit-increase in the MELD score was associated with 1.69 higher odds and 1.16-fold increase in the risk of post-TIPS HE, and 1.06-fold increase in the rate of new or worsening HE [25,26,65,67]. History of HE is not only one of the main risk factors for HE recurrence in cirrhosis, but also an important predictor of post-TIPS HE [25,27,66,67]. In a prospective study of 82 TIPS patients, the occurrence of HE was associated with a 3.16-fold increase in the risk of developing a post-TIPS HE [66]. A retrospective study of 284 patients reported that patients with pre-TIPS HE had 1.06-fold increase in the rate of post-TIPS HE [67]. Those results (older age, prior HE and higher Child–Pugh class/score) were similar to the results of an earlier meta-analysis

of 30 studies [27]. Later studies have identified PPG as another important risk factor of post-TIPS HE [25,65]. Odds for post-TIPS HE was increased 1.2-fold for each 1 mmHg decrease in the post-TIPS PPG [65]. Comparable to the risk factors of developing HE in cirrhosis, various comorbidities such as diabetes, sarcopenia and hyponatremia were associated with an increased risk of developing post-TIPS HE [26,68]. Moreover, using proton pump inhibitors were assigned a 3.19-fold increase in the risk of post-TIPS HE [67]

**Table 3.** Recent relevant publications identifying specific risk factors of post-TIPS HE.

| Reference | Study Design | Number of Patients | Risk Factor | Adjusted Hazard Ratio (95% CI) |
|---|---|---|---|---|
| Yao et al., 2015 [65] | Retrospective cohort | 279 | Pre-TIPS MELD<br>PPG post-TIPS | 1.69 (1.39–2.06) +<br>1.20 (1.07–1.34) + |
| Nardelli et al., 2016 [66] | Prospective cohort | 82 | Age<br>Child–Pugh score<br>Covert HE before TIPS | 1.05 (1.02–1.08)<br>1.29 (1.06–1.56)<br>3.16 (1.43–6.99) |
| Nardelli et al., 2017 [26] | Prospective cohort | 46 | Sarcopenia<br>Pre-TIPS MELD | 31.3 (4.5–218.07)<br>1.16 (1.01–1.34) |
| Lewis et al., 2019 [67] | Retrospective cohort | 284 | Age<br>Pre-TIPS MELD<br>HE before TIPS<br>Proton pump inhibitors use | 1.05 (1.03–1.07) *<br>1.06 (1.01–1.11) *<br>1.51 (1.04–2.20) *<br>3.19 (2.19–4.66) * |
| Yin et al., 2020 [68] | Retrospective cohort | 264 | Age<br>Diabetes<br>Child-Turcotte-Pugh class C<br>Sodium<br>Creatinine | 1.03 (1.00–3.21)<br>1.84 (1.06–3.21)<br>6.68 (1.68–8.89)<br>0.94 (0.88–0.99)<br>1.01 (1.00–1.03) |

+ = odds ratio; * IRR = incidence rate ratio; Abbreviations: CI, confidence interval; HE, hepatic encephalopathy; MELD, model of end-stage liver disease; PPG, portosystemic pressure gradient, TIPS, transjugular intrahepatic portosystemic shunt.

## 5. Shunt Diameter and HE

The first generation of TIPS stents has been widely used, and initial underdilatation was intended to balance portal hypertension reduction and adverse events due to excessive shunting, especially HE [69]. However, several studies have shown that initial underdilatated TIPS stents tend to passively expand over time (depending on the stiffness of the liver), thereby potentially increasing HE rate [69–72]. Since 2017, so-called controlled expansion stent grafts have been established [69]. They proved to prevent self-expansion and to keep a stable shunt diameter, thereby reducing TIPS-associated complications [69,73,74]. Besides therapeutic shunting, it is well known that spontaneous portosystemic shunts have also a significant influence on HE development [75]. Spontaneous portosystemic shunts are common in cirrhotic patients and larger single shunt diameters were associated with the development of HE [76]. In a recent retrospective multicentric study of 908 cirrhotic patients, the total cross-sectional spontaneous shunt volume/area (rather than single shunt diameter) has been identified as an independent predictor of HE and survival, and should be considered for risk stratification in the work-up of cirrhotic patients [77]. Regarding shunt diameter, is has been shown that 8 mm sized covered stents do not compromise shunt function but reduce hepatic encephalopathy compared to 10 mm sized stents [78].

## 6. Management and Outcome of Post-Tips HE

Historically, patients with post-TIPS HE were conservatively treated with a low-protein diet as well as nonabsorbable antibiotics and disaccharides to reduce intestinal neurotoxin production and absorption, which is increasingly being questioned today [12,15–17]. Considering the limitations of current standard-of-care medications, non-pharmacological treatment strategies targeting gut dysbiosis

and including probiotics and fecal microbiota transplants are increasingly used as alternative or supportive therapies [12]. Recent randomized controlled trials and meta-analyses indicated probiotics to be efficacious in treating HE compared with placebo, although there was no increased efficacy compared with lactulose [12,79–81]. Fecal microbiota transplantation (FMT) is increasingly established in the management of clostridium difficile infection [82]. Moreover, promising findings suggest that FMT may play an important role also in the management of other diseases associated with the disbalance of gut microbiota, including HE [82,83]. In the first randomized controlled trial, Bajaj et al. reported that FMT decreased HE recurrence, hospitalization, and improved cognitive functions compared to current standard-of-care medications (rifaximin/lactulose) [12,83]. Moreover, nutritional management and branched chain amino acids can be considered as adjunct therapies preventing degradation of skeletal muscles that detoxify ammonia [12,48,84].

However, up to 8% of patients develop refractory HE after TIPS which is often associated with further deterioration of liver function and poor prognosis [18,19,85]. In these cases, TIPS modification is increasingly integrated in multimodal treatment settings to avoid or delay liver transplantation [18,20,86,87]. Endovascular shunt modification can be performed either as partial occlusion with the insertion of a reduction stent or complete occlusion, each reducing the portosystemic shunt volume and thereby improving HE [18,87]. Within these concepts it is important to be aware of patient safety in the course of TIPS modification, particularly regarding the recurrence of the primary TIPS indication, especially variceal bleeding and ascites [19,20,86,87]. Recent main studies analyzing the effect of shunt modification in patients with refractory post-TIPS HE are summarized in Table 4 [4,19,66,87–89]. While shunt reduction at the time of the pivotal study by Kochar et al. was still a technical challenge, today, ready-to-use reduction stents offer the opportunity to easily downsize the shunts in a standardized manner [86,87,89]. From the data available, shunt reduction to 5 mm does not lead to relapse of variceal bleeding or refractory ascites in the majority of patients, demonstrating that reduction is mostly safe and should preferably be performed compared to complete shunt occlusion. However, endovascular shunt modification is not always successful in managing HE while possibly the presence of large collaterals and the deterioration of the liver function is more important than the changes in portal hemodynamics [38,87]. In a recent study, it has been shown that a higher HE grade after TIPS as only positive predictor for response to shunt modification, independent of liver function and PPG [89]. Non-responders reveal poor prognosis between 27–67% survival rate of 6-month follow-up following shunt modification, while liver transplantation remains the ultimate treatment [86,87,89].

**Table 4.** Recent relevant publications analyzing the effect of shunt modification in patients with refractory post-TIPS HE (adapted from Nardelli et al. [87]).

| Reference | No. with Refractory HE/Treated with TIPS | Child–Pugh Class | No. of Patients Improved | Recurrence of Primary TIPS Indication after Shunt Modification | PPG Pre (mmHg) | PPG Post (mmHg) |
|---|---|---|---|---|---|---|
| Nardelli et al., 2016 [66] | 3/82 | B: 1<br>C: 2 | 3 | - | 5.6 ± 3.2 | 12.1 ± 2.7 |
| De Santis et al., 2018 [88] | 2/38 | B: 1<br>C: 1 | 2 | Ascites 1<br>Bleeding 1 | 6.5 ± 2.6 | 12.7 ± 3.8 |
| Bureau et al., 2017 [4] | 1/29 | C: 1 | 1 | - | - | - |
| Rowley et al., 2018 [19] | 10/174 | - | 8 | - | 8.6 ± 4.1 | 13.0 ± 4.0 |
| Schindler et al., 2020 [89] | 20/344 | A: 7<br>B: 9<br>C: 4 | 11 | Ascites 2<br>Bleeding 1 | 7.7 ± 3.9 | 12.1 ± 4.4 |

- = not calculated or specified; Abbreviations: HE, hepatic encephalopathy; PPG, portosystemic pressure gradient, TIPS, transjugular intrahepatic portosystemic shunt.

## 7. Summary

Post-TIPS HE remains a clinical challenge. There are multiple factors impacting risk and prognosis of HE which have to be considered for the appropriate TIPS workup and targeted HE management. Moreover, besides the central thesis of hyperammonemia being the underlying cause of HE among TIPS patients, further molecular mechanisms have been identified and may also play an important role in the pathophysiology of HE. Current standard-of-care medications have their own limitations and non-pharmacological treatment strategies targeting gut dysbiosis may be the future of supportive therapy. In cases of refractory post-TIPS HE, endovascular shunt modification is increasingly established in multimodal treatment approaches to obviate liver transplantation. Here, TIPS reduction can be considered to be a safe treatment option that is frequently not associated with a relapse of the initial TIPS indication, such as variceal bleeding or ascites.

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# The Use of Administrative Data to Investigate the Population Burden of Hepatic Encephalopathy

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**Abstract:** Hepatic encephalopathy (HE) is a devastating complication of cirrhosis with an increasing footprint in global public health. Although the condition is defined using a careful history and examination, we cannot accurately measure the true impact of HE relying on data collected exclusively from clinical studies. For this reason, administrative data sources are necessary to study the population burden of HE. Administrative data is generated with each health care encounter to account for health care resource utilization and is extracted into a dataset for the secondary purpose of research. In order to utilize such data for valid analysis, several pitfalls must be avoided—specifically, selecting the particular database capable of meeting the needs of the study's aims, paying careful attention to the limits of each given database, and ensuring validity of case definition for HE specific to the dataset. In this review, we summarize the types of data available for and the results of administrative data studies of HE.

**Keywords:** cirrhosis; liver disease; epidemiology

## 1. Introduction

Cirrhosis is an increasingly common [1], morbid, and deadly condition [2]. The increased health care utilization [3], symptom burden [4], and mortality associated with cirrhosis is particularly driven by the development of hepatic encephalopathy (HE) [5]. HE is a syndrome of brain dysfunction caused by liver insufficiency and/or portal-systemic shunting that manifests as a spectrum of neuropsychiatric perturbations ranging from deficits in executive functioning to coma [6]. As such, HE is a clinical diagnosis best made in conjunction with a careful clinical examination and exclusion of other causes of altered mentation. Research on the burden and impact of HE at the population level is therefore challenging.

One solution is the use of administrative data. Such data is generated with each health care encounter to account for health care resource utilization and can be extracted into a dataset for the secondary purpose of research. The richness of the included variables, and therefore the questions for which a dataset is amenable, varies with the purpose of primary data collection. At a minimum, administrative databases include demographics and diagnosis or procedure codes (e.g., ICD-10) which are input by clinicians or staff for billing or resource monitoring. The contents of administrative data are only as valid as the methods used to record the clinical details. Administrative data cannot provide the accuracy and granularity of detail found in well-executed prospective clinical research. However, administrative data offers several advantages.

Administrative data allows an understanding of the impact of HE on the population. The careful, prospective, multicenter data needed to define the incidence, health care utilization, and clinical outcomes associated with HE has prohibitive costs. As such, the ability to extract insights from data recorded for other purposes is essential to extend our knowledge of HE epidemiology. To ensure

validity, this requires its own deliberate methodology. Herein, we review the tools required to analyze and what is known about HE from administrative sources.

## 2. Identifying Cirrhosis with Administrative Data

Cohort studies using administrative data to identify patients pose unique challenges to investigators wishing to communicate their results. Whereas prospective studies define cirrhosis using clinical criteria with prima facie validity such as histology or clinical criteria supported by imaging and laboratory evidence with an acceptable, largely unquestioned degree of uncertainty, administrative data lacks the assumption of validity. When clinicians or administrative staff process visit charges, they assign billing diagnoses utilizing a system known as the International Classification of Diseases (ICD). The ICD systems and codes utilized vary across time and locality. Whereas much of Europe has used the 10th iteration (ICD-10) for decades, the United States switched from ICD-9 to ICD-10 in October of 2015. These codes may be chosen incorrectly (reducing specificity) or the chosen codes may incompletely catalogue the patient's active problems (reducing sensitivity). Further, the temporality of codes can only be inferred. The first appearance of a code is felt to establish the index data for a diagnosis but this may lag. Similarly, the prior use of a code does not establish whether it is persistent, resolved, or entered in error.

The use of administrative data to identify patients is therefore dependent on the validation of the codes utilized. Algorithms for the identification of cirrhosis have been established by a number of investigators for a variety of datasets by using chart review to confirm the positive and negative predictive values of diagnostic coding schema [7–12]. In general, most approaches involve requiring a specific set of codes and multiple (>1) entries of the codes in outpatient records (or one entry in inpatient records). The performance of diagnostic codes is also etiology dependent. Performance is best for viral hepatitis, moderate for ALD, and worst for NAFLD [8,13,14].

## 3. Identifying Hepatic Encephalopathy with Administrative Data

Numerous studies have used administrative data to identify patients with HE, but only a few have validated the use of such data (Table 1). Kanwal and colleagues validated the use of the ICD-9 code for hepatic encephalopathy (572.2) in a Veterans Affairs (VA) cohort [15]. They found that the presence of at least one 572.2 code had high positive predictive value (0.86) and high negative predictive value (0.87) for a diagnosis of HE on detailed chart review with the denominator of persons with multiple cirrhosis codes. An algorithm based on the ICD-9 code for HE (572.2) and prescription fills for lactulose or rifaximin had moderate agreement with a chart review diagnosis of HE in a separate VA cohort [16]. Most published studies using administrative data to identify HE have used ICD-9 codes. However, to use United States data after 2015 when ICD-9 was abandoned, algorithms using ICD-10 are needed.

**Table 1.** Methods to Identify Hepatic Encephalopathy Using Administrative Data.

| Tool | Description | Study | Database | Relevant Result | Validated Method for Identifying HE or Cirrhosis | Limitations | Benefits |
|---|---|---|---|---|---|---|---|
| International Classification of Diseases, 9th Revision (ICD-9) | • International standard for defining and reporting diseases<br>• 9th revision was used in the United States from 1979 to 2015<br>• ICD-9 code for HE is 572.2 | V. Lo Re et al. (2011) | • Veterans Affairs | Nine of 295 patients with an ICD-9 code or laboratory value indicating liver dysfunction had an ICD-9 code for HE; the PPV of this code was 0.11 and estimated NPV of 0.99 | HE | ICD-9 is not being coded in the United States after 2015, so available data ranges are limited; Variable accuracy in coding | International; Currently best validated; Specific code for HE |
| | | Goldberg et al. (2012) | • Local registry (two tertiary care centers) | Presence of one inpatient or outpatient ICD-9 code for cirrhosis, chronic liver disease, and a hepatic decompensation (of which HE was one), the PPV of 0.85 for confirmed cirrhosis | Cirrhosis | | |
| | | Kanwal et al. (2012) | • Veterans Affairs | After identifying cirrhosis patients with ICD-9 codes and laboratory data, at least one ICD-9 code for HE had PPV of 0.86 and NPV of 0.87 for confirmed HE | HE | | |
| | | Nehra et al. (2013) | • Local registry (single hospital system) | ICD-9 code for HE had PPV 0.92 and NPV 0.36 for identifying confirmed cirrhosis; did not report if it identified HE | Cirrhosis | | |
| | | Lapointe-Shaw et al. (2018) | • Two Canadian hospitals | Having a single hospital diagnostic code for cirrhosis, including 572.2, was specific for cirrhosis (0.91–0.96 depending on subcohort), but not as sensitive (0.57–0.77); however, the authors did not specify in how many cases 572.2 was used vs. other codes | Cirrhosis | | |

**Table 1.** *Cont.*

| Tool | Description | Study | Database | Relevant Result | Validated Method for Identifying HE or Cirrhosis | Limitations | Benefits |
|---|---|---|---|---|---|---|---|
| International Classification of Diseases, 10th Revision (ICD-10) | • United States began using ICD-10 in 2015<br>• Many countries began using this system earlier<br>• No specific code for HE, instead many use K72.90 | Thygesen et al. (2011) | • Danish National Registry of Patients | The PPV of one inpatient or outpatient ICD-10 code for moderate/severe liver disease, which included K72.90, correctly identifying cirrhosis was 1.00; however, the authors did not specify in how many cases K72.90 was used vs. other codes | Cirrhosis | Only available in the United States 2015 and thereafter | International; Required to use data after 2015 in the United States; Readily available in most databases |
| | | Mapakshi et al. (2018) | • Veterans Affairs | Unable to validate the use of ICD-10 codes for HE because there were no HE events during the study period | Neither | | |
| | | Tapper et al. (2020) | • Development cohort: single academic center<br>• Validation cohort: Veterans Affairs | In a validation cohort of veterans with HCV, ICD-10 code K72.90 identified development of HE with PPV 0.90 and NPV 0.93 | HE | | |
| | | Lapointe-Shaw et al. (2018) | • Two Canadian hospitals | Having a single hospital diagnostic code for cirrhosis, including K72.90, was specific for cirrhosis (0.91–0.96 depending on subcohort), but not as sensitive (0.57–0.77); however, the authors did not specify in how many cases K72.90 was used vs. other codes | Cirrhosis | | |
| Prescription Data | • Record of a medication prescription | Tapper et al. (2020) | • Development cohort: single center<br>• Validation cohort: Veterans Affairs | In a validation cohort of veterans with HCV, lactulose prescription had PPV of 0.73 and NPV of 0.99 for HE diagnosis, while lactulose or rifaximin prescription had a PPV of 0.71 and NPV of 0.99 | HE | Not available in every database | Lactulose therapy for overt HE is nearly uniform |
| Combination | • ICD-9 + prescription data | Kaplan et al. (2015) | • Veterans Affairs | An algorithm based on the ICD-9 code for HE and prescription fills for lactulose or rifaximin had weighted kappa agreement of 0.51 with the CTP-subscore for HE | HE | Not available in every database | Using multiple modalities in one algorithm can enhance predictive value |

ICD, International Classification of Diseases; PPV, positive predictive value; NPV, negative predictive value; CTP, Child-Turcotte-Pugh.

Unfortunately, the ICD-10 system lacks a code for HE. In this vacuum, coders will use a handful of different options. As we have found, across the US this most frequently this involves the code K72.90, which is technically "hepatic failure, not otherwise specified." The code K72.90 had excellent positive and negative predictive value for the development of HE in a prospective cohort of Child A and B cirrhosis [17]. The same code also successfully identified HE in a VA cohort meeting a validated definition of cirrhosis [17]. Several groups have used this ICD-10 code as one of many to identify cirrhosis; however, the specific performance of K72.90 in those algorithms is unknown [12,18,19].

Prescription data is accessible in many administrative databases. The treatment of HE is nearly uniform with one or two medications: lactulose and rifaximin. Consistency in HE treatment across different geographies and patient subgroups enhances the utility of prescription data in identifying the diagnosis. We found that a prescription for lactulose or rifaximin had high negative predictive value (0.99) and substantial positive predictive value (0.71) for HE [17].

Multiple gaps persist. Data are lacking regarding whether a given coding algorithm can identify patients with early stages of HE or whether diagnostic coding schema generalize between countries. Further, non-ICD-9 coding algorithms have only been validated in cohorts with known cirrhosis. These algorithms are not yet validated for use in larger, less-defined samples.

## 4. Administrative Databases: Which to Use

In Table 2, we detail the data elements and outcomes available in each dataset.

**Table 2.** Potential Administrative Data Sources for Hepatic Encephalopathy Research.

| Data Sources | Population | Data Elements | Outcomes | Validated Definition of Cirrhosis | Validated Definition of HE | Limitations |
|---|---|---|---|---|---|---|
| Veterans Affairs (VA) | National health care for US veterans | ICD-9/10CPT<br>Physical exam<br>Pharmacy<br>Laboratory<br>Imaging | • Hospitalization<br>• Mortality<br>• Transplant<br>• Cost | Kanwal et al. (2012)<br>V. Lo Re et al. (2011) | Kanwal et al. (2012)<br>Kaplan et al. (2015)<br>Tapper et al. (2020) | Male<br>Missing outside data<br>VA population and access to care may differ |
| Medicare | United States ≥65 years old | ICD-9/10CPT<br>Pharmacy | • Death<br>• Health care utilization<br>• Linked cohorts such as the Health and Retirement Study or Cardiovascular Health Study can provide additional outcomes relating to functional disability and cognitive function | Rakoski et al. (2012) | None | No laboratory data<br>Relies on diagnosis and procedure codes |
| National Inpatient Sample (NIS) / National Readmissions Database (NRD) | United States<br>Nationally representative sample<br>All payers | ICD-9CPT | • Length of stay<br>• Discharge disposition<br>• Inpatient mortality | None | None | No laboratory data available<br>Relies on diagnosis and procedure codes alone and is subject to misclassification<br>Inability to link hospitalizations to individual patients limits longitudinal follow-up post-discharge |
| Private Insurance Claims Data | United States<br>Private insurance represents ~50% total market, often through employer | ICD-9CPT<br>Pharmacy<br>Laboratory | • Hospitalization<br>• Direct health care costs<br>• Limited death data | None | None | Relies on diagnosis and procedure codes<br>Enrolled only while covered<br>Often missing death data |

**Table 2.** *Cont.*

| Data Sources | Population | Data Elements | Outcomes | Validated Definition of Cirrhosis | Validated Definition of HE | Limitations |
|---|---|---|---|---|---|---|
| National Patient Registries | Denmark, Sweden, Ontario | Includes detailed information on clinical characteristics, laboratory data, imaging, procedures and outcomes | • Hospitalization<br>• Death<br>• Additional data depending on registry | Thygesen et al. (2011) Lapointe-Shaw et al. (2018) | None | Country and health care system specific |
| Organ Procurement and Transplant Network (OPTN) | United States Listed for liver transplantation | Manually entered detailed pre-, intra-, and post-transplant clinical information | • Data on liver transplantation, and post-liver transplant outcomes<br>• Linked by UNOS to social security death index | None (manually input by transplant program) | None (manually input by transplant program) | Considerable selection bias given limited to transplant centers and listed patients<br>Potential for misclassification due to inaccurate completion of questionnaire<br>ELTR: No information on patient ethnicity or socioeconomic information |
| European Liver Transplant Registry (ELTR) | Europe (155 centers from 28 countries) | Detailed information on liver transplant indications, transplant types and complications | • Death<br>• Transplant outcomes | None (manually input by transplant program) | None | |

Some elements of this table were adapted from Moon et al. (2019) [20].

### *4.1. US Data*

Many databases have been used to study the population burden and impact of HE. In the US, the lack of nationalized health care creates the central limitation of administrative data. Data for each patient is often dispersed across multiple payers and therefore databases. The Veterans Affairs (VA) data is rich and incudes diagnostic/procedure codes, laboratory data, and pharmacy records. However, even veterans receive care, both out- and inpatient at outside facilities with variable reconciliation of events and prescriptions. HE code and prescription-based algorithms have been validated using both ICD-9 and 10 [16,17].

The Organ Procurement and Transplant Network (OPTN) offers a database that includes all persons waitlisted for liver transplantation with granular data that is regulated by OPTN rules and manually entered by each transplant center. Among administrative data sources, OPTN data is unique given the richness of physiological variables and the intrinsic validity of the clinical diagnoses. A history of HE is recorded and HE is graded using the West-Haven scale.

The National Inpatient Sample (NIS) is an all-payer database of admission-level inpatient encounters strengthened by complete billing and in-hospital outcome data but lacking in laboratory and prescription information or data following discharge. The National Readmissions Database (NRD) is a sample of NIS data accounting for most states and hospitals contained within the NIS. In the NRD patients can be linked between hospitalizations by a unique identifier allowing for studies of readmissions albeit without accounting for the competing risk of post-discharge mortality. Although ICD-9 based algorithms for HE have been applied to these databases presuming similar performance compared to the VA, none have been validated [21].

Patients aged ≥65 years as well as those who are disabled or requiring hemodialysis are eligible for government insurance with Medicare. At a minimum, Medicare data includes longitudinal, patient level data linked to vital status records as well as comprehensive diagnosis/procedure codes and medications that are provided by a health care facility. The kind of research that can be performed

using Medicare varies according to the data-elements at the investigator's disposal. Algorithms using ICD-9 derived from the VA have been validated in Medicare data [22].

Finally, many investigators have used commercial claims data to study cirrhosis and HE-related outcomes [23,24]. Commercial claims vendors use highly varied data sources ranging from one sole insurer (Optum/United Health) or a pooled dataset from many employer-based insurance plans [25]. The richness of the claims data varies, some offer linkage to the originating provider-type while others do not, some offer laboratory claims but not the results of those laboratory tests. As such, careful inspection of the database's data elements is necessary to understand the ability of each to capture the incidence, prevalence, burden, and outcomes of HE as well as the determinants thereof.

### *4.2. International Data*

Canada has a universal health system, administered semi-independently by each of its 13 provinces and territories. The Institute for Clinical Evaluative Sciences holds administrative data for all Ontario residents utilizing publicly available insurance. Databases containing billing claims, hospitalization records, and death data are linked. Methods for identifying cirrhosis in claims data, validated in other cohorts, have been applied to this database [26]. Lapointe-Shaw and colleagues have validated the use of combinations of ICD-9 and 10 codes to identify cirrhosis and decompensated cirrhosis, but not HE specifically [12]. Outpatient physician claims for cirrhosis were sensitive but not specific, likely due to financial incentives provided for including a visit diagnosis of cirrhosis.

The National Patient Register contains diagnosis and hospital contact data on the entire population of Denmark since 1977. Diagnoses after 1994 were made using the ICD-10 coding system, and are notably entered by a physician, not other administrative personnel. Two studies have validated the use of ICD codes for cirrhosis in this registry [19,27], and numerous investigations into cirrhosis have been performed with it [5,28–30]. Jepsen and colleagues have reported on the incidence of HE in a cohort of alcohol-related cirrhosis from this registry, but the HE was identified by chart review and not administrative codes [5]. To date, no studies have validated the use of ICD-10 codes for HE in the Danish public registry.

Several studies of cirrhosis epidemiology have used a southern Swedish cohort, developed from a comprehensive population registry [31–34]. These studies initially identified 4611 patients with ICD-10 codes for cirrhosis, but 2950 were excluded by chart review as not meeting criteria for cirrhosis [31]. The authors identified the incidence of HE, defined as a prescription for lactulose. Another group recently used the Swedish National Patient Register, which collects ICD-10 codes for all specialty care in Sweden, and validated codes to identify cirrhosis [35]. No administrative codes have yet been used to identify HE within these cohort.

The European Liver Transplant Registry (ELTR), similar to UNOS in the United States, collects manually entered data regarding liver transplant indications and complications from 28 countries in Europe. While this registry includes pre-transplant data from patients with cirrhosis, there are no published studies of HE in this cohort.

## 5. Identifying Risk Factors for Hepatic Encephalopathy

Cohort studies aimed at identifying the incidence of new or interval HE will require patient samples with risk factors for HE development. Most studies have done this by identifying cirrhosis or a common cause of chronic liver disease, such as hepatitis C virus infection. As described above, algorithms for identifying cirrhosis have been validated in multiple datasets by multiple authors [7–9]. Coding algorithms have also been used to successfully identify cohorts with alcohol liver disease [8], non-alcoholic fatty liver disease [14,36], hepatitis C virus infection [8,37–39], and—with slightly less success—chronic hepatitis B virus infection [8,38,39]. Using the US Medicare database, we identified a cohort of patients with cirrhosis whose risk of incident diagnoses of HE were influenced by etiology (particularly alcohol-related liver disease), the presence of portal hypertension, comorbidities, and polypharmacy (particularly benzodiazepines, opioids, and proton pump inhibitors) [40]. The risk of

HE was 11.6 per 100 person-years. Using the US VA database, we found that persons with cirrhosis and portal hypertension or an AST-to-Platelet Ratio Index >2.0 had a cumulative incidence in excess of 40% at 5 years. The specific risk factors identified included disease severity (albumin, total bilirubin), nonselective beta-blocker use, and statin therapy (inversely associated) [41].

## 6. Outcomes of HE

Several studies have used administrative data to describe the outcomes of persons with HE (Table 3). Scaglione demonstrated that HE was independently associated with mortality after hospitalization while Wong showed that grade of HE at the time of transplant evaluation was associated with increased mortality on the waitlist [24,42]. We showed using Medicare data that the median survival after HE was approximately 1 year for persons ≥65 years old as well as those with ascites prior to HE. In a claims database of privately insured persons, we found that the overall cumulative incidence of death at 1 year was 19% [25]. Stepanova and Hirode both examined the NIS and found that the in-hospital mortality and costs associated with hospitalizations for HE from 2005 to 2014 were approximately 17% and $17,000 [43,44]. Roggeri examined the global annual health care costs for Italian patients with HE and estimated approximately $15,000 USD [45].

**Table 3.** Administrative Studies Detailing the Outcomes Associated with Hepatic Encephalopathy (HE).

| | Study | Population | Definition of HE | Outcome(s) |
|---|---|---|---|---|
| Incidence/ Prevalence | Tapper | US Veterans with APRI>2.0 2005–2015 | ICD-9 572.2 or the use of lactulose and/or rifaximin | The cumulative probabilities of overt HE at 1, 3, and 5 years was 22.6%, 36.9%, and 43.6% |
| | Tapper | US Medicare 2008–2015 | | Incidence rate: 11.6 per 100 person-years |
| | Nilsson | Sweden, 43% with ascites | Lactulose use | Cumulative incidence at 1 and 10 years, 6.4% and 26% |
| Mortality | Wong | Transplant waitlisted Americans 2003–2012 | Manually entered grading | HE is associated with mortality: Grade 1–2 1.1.3 (1.02–1.26) Grade 3–4: 1.65 (1.44–1.89) |
| | Scaglione | Privately insured Americans with cirrhosis and a readmission 2010–2014 | 572.2 | Adjusted mortality associated with HE 1.14 (1.04–1.24) |
| | Tapper | US Medicare 2008–2015 Optum commercial claims 2008–2015 | ICD-9 572.2 or the use of lactulose and/or rifaximin | Median survival 0.95 and 2.5 years for those ≥65 or <65 years old; 1.1 and 3.9 years for those with or without ascites |
| Post-transplant mortality | Wong | Transplant waitlisted Americans 2003–2013 | Manually entered grading | HE is associated with mortality: Grade 3–4: 1.27 (1.17–1.39) |
| Inpatient outcomes | Hirode | Hospitalized Americans 2010–2014 | ICD-9 572.2 | In-hospital mortality 12.3% from 13.4% Cost per admission 16,168 to 16,919 |
| | Stepanova | Hospitalized Americans 2005–2009 | ICD-9 572.2 | In-hospital mortality 15.6% to 14.3% Cost per admissions 16,512 to 17,812 |
| | Tapper | US Medicare 2008–2015 Optum commercial claims 2008–2015 | ICD-9 572.2 or the use of lactulose and/or rifaximin | 11.8 (IQR 2.9–38.0) hospital days per person-year Combination lactulose and rifaxmin use associated with lower hospital days and 30 day readmission |
| Costs | Roggeri | Hospitalized Italians 2011 | ICD-9 572.2 | Annual HE costs: 15,295 USD |

## 7. Pitfalls of Administrative Data

There are three central limitations inherent to administrative data research: validity, completeness, and descriptive fidelity. First, we review, in Table 1, the database definitions of HE which have been validated. There are likely additional methods to identify patients with HE beyond this table. Codes such as 'hepatitis C with coma (ICD-10 B19.21)' or 'encephalopathy (G93.41)' may rarely be used to describe HE but we do not know their accuracy. Furthermore, the current method for identifying HE using ICD-10 codes requires pre-specifying a population with known liver disease. This method enhances data validity at the cost of inclusiveness. Using this method also makes the accuracy of identifying HE dependent on the techniques used to identify the liver disease population. Validated methods to identify HE without yet established cirrhosis coding are needed. Second, as reviewed in Table 2 and expanded upon above, each database varies with respect to its data elements or cross-sectional versus longitudinal design. Accuracy of diagnostic codes vary by population and database, possibly secondary to differences in reimbursement. Furthermore, in the context of disparate sources of health care funding, such as in the US, it can be unclear which portion of a given patient's health care experience is captured within the dataset. Third, even valid and complete data may not be appropriate for specific aspects of HE care. No study, for example, has discerned the impact of covert from overt HE.

## 8. Future Directions

Future study should target two core areas: first, identify strategies to use multiple administrative data tools in tandem to identify patients who develop HE amongst those at risk; and second, linkage of administrative data to clinical care.

HE can be accurately identified by claims or prescription data, when done so within a cohort of known risk (i.e., HCV, cirrhosis). The next step is being able to expand these searches into larger population cohorts, by utilizing tools to first identify those at risk of HE. Natural language processing (NLP) holds potential future promise as an addition tool, beyond those discussed above, to identify patients with cirrhosis and risk of developing HE. NLP allow for automated extraction of text from medical charts, and could supplement administrative codes by also identifying "splenomegaly" or "varices" in radiology and endoscopic reports. An algorithm combining administrative codes and NLP of radiology report impressions had high (>90%) positive and negative predictive value for identifying cirrhosis [46]. A strategy that successfully uses multiple tools simultaneously including medications, laboratory values, codes, and NLP may optimally identify those at risk for HE from large databases.

Additional work must be done to leverage administrative data for clinical care. If hospital systems could efficiently and accurately identify patients at risk for the development of HE through administrative data, then those patients could be seamlessly incorporated into population health cohorts and targeted with additional resources. Given the availability of risk scores for HE using administrative data, these could be calculated and displayed at the point of care to influence decision making. If patients at hospital discharge could be automatically and accurately identified at high risk for recurrent HE, then linking those patients to close outpatient follow up and resources could optimize management. Finally, automated identification of patients at risk for HE with administrative data could facilitate clinical trial enrollment for studies aimed to treat this condition, and accelerate the pace of scientific discovery.

## 9. Conclusions

We cannot understand the societal burden of HE without administrative data. Rigorously collected data from prospective cohorts are essential tools for HE research. A research agenda that excludes the use of administrative data, however, does so at the peril of crucial insights. While each data stream is affected by its own pitfalls, those of administrative data are not intrinsically greater than

conventional cohort studies. As reviewed, the tools required to avoid the pitfalls of administrative data are straightforward and readily available.

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