Hepatic Encephalopathy

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ABSTRACT

Hepatic encephalopathy (HE) is a prognostically relevant neuropsychiatric syndrome, which occurs in the course of acute or chronic liver disease. Besides ascites and variceal bleeding it is the most serious complication of decompensated liver cirrhosis. Ammonia and inflammation are major triggers for the appearance of HE, which in patients with liver cirrhosis involves pathophysiologically a low grade cerebral edema with oxidative/nitrosative stress, inflammation and disturbances of oscillatory networks in the brain. Severity classification and diagnostic approaches regarding mild forms of HE are still a matter of debate. Current medical treatment predominantly involves lactulose and rifaximin following rigorous treatment of so-called known HE precipitating factors. New treatments based on improved pathophysiological understanding are emerging.

[H1] INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome, which can occur in the course of acute or chronic liver disease. Most cases of HE are associated with liver cirrhosis. As a complication of liver cirrhosis, HE is frequent, indicative of a poor prognosis and associated with a reduction of health-related quality of life.

Symptoms of HE largely comprise cognitive and fine-motor disturbances of varying severity, which can be ascribed to a slowing of cerebral oscillatory networks. The previously held view that episodes of HE will completely resolve after appropriate treatment of HE, underlying liver disease must be called into question, because several studies showed a persistence of mild cognitive disturbances, irrespective of demographic factors or the etiology of liver cirrhosis (for review see[1]) and possibly involving premature astrocyte senescence and neuronal death. HE in liver cirrhosis is accompanied by a low grade cerebral edema, whereas overt brain edema is an extremely serious and often fatal complication of acute liver failure or congenital hyperammonemic disorders.

Undoubtedly, ammonia and inflammation are major triggers in the pathogenesis of HE and recent studies provide novel mechanistic insight into ammonia toxicity and the pathobiochemistry/pathophysiology of HE. This article largely focuses on HE in the patient with cirrhosis, because HE is much more common in these patients than in those with other liver diseases and in whom low grade cerebral edema in combination with oxidative/nitrosative stress play a prominent pathogenetic role. Although many studies on HE and ammonia toxicity were conducted in animal models, key findings from these studies have also been confirmed

in human brain. HE symptoms are of varying severity and there is an ongoing debate regarding severity assessment and nomenclature of HE. This, and the dynamics of HE, which can resolve after correction of precipitating factors make clinical studies on the efficacy of medical treatment options more difficult.

[H1] EPIDEMIOLOGY

The epidemiology of HE is not well defined because the HE diagnostic criteria are not unanimous, the liver disease case mix is highly variable, HE has no specific WHO International Classification of Diseases code, and because there are few population-based studies of relevant format. Furthermore, as HE is variable over time, prevalence estimates may be inaccurate. Nonetheless, the available reports taken together show that HE is now the most frequent, devastating, resource-demanding complication of cirrhosis and closely associated with a poor prognosis [2].

[H2] Prevalence and Incidence

HE is categorized as type A, B, or C, and graded between minimal and grades I-IV [3]. HE in acute liver failure is HE type A. It is the most important clinical event defining acute liver failure, Therefore. the incidence rate of HE type A is largely the same as for acute liver failure[4]. This is a rare condition with a reported incidence of about 0.5 per 100.000 per year [4].

HE primarily or exclusively caused by porto-systemic shunts is type B HE. Such shunts arise spontaneously as a result of portal vein hypertension and allow blood from portal vein-drained viscera to bypass the liver and to directly enter the systemic circulation [3,5], HE type B is less rare than HE type A, but is still uncommon. Incidence estimates are based on the conditions that most often give rise to the shunting, such as portal vein thrombosis.. Of these patients, 6-13% at some time experience HE [6]. HE after placement of a Transjugular Intrahepatic Porto-Systemic Stent Shunt (TIPSS) to ameliorate severe portal hypertension particularly in cirrhosis, is thus usually a mix of HE types B and C (cf. below). The reported one year post-TIPSS HE incidence ranges from 10% to 50% [7]. HE thus remains the limiting factor for the utility of TIPSS, because it is a risk factor for HE,

HE caused primarily or exclusively by loss of functional liver mass due to cirrhosis is type C HE [3]. This is by far the most common and clinically prevailing variant of HE. The global burden of cirrhosis is at least 125 million patients [8]. How many of these develop HE is not exactly known, but assuming that they have approximately the same risk as that reported in most studies, the percentages given below can be multiplied by this prevalence to give an idea of

the global prevalence of HE. The clinically undiscernible form is minimal HE that requires psychometric methods for detection, but despite its innocuous presentation it is closely associated with severe loss of quality of life [9]. It has a very high prevalence being observed in 40-60% of cirrhosis patients [10,11] and within one year in about 33% progresses into clinically manifest HE. The mildest clinically detectable but still non-disorientational cerebral involvement is grade I HE, which is less frequent with an estimated prevalence of 15-25% of cirrhosis patients[10]. However, an even higher proportion of these, 50% within one year, progresses into clinically manifest HE [10]. Clinically manifest HE, i.e. HE grade II or above is most often marked by disorientation and is present in 10-15% of cirrhosis patients at diagnosis. Grade II is distinguished by disorientation, the higher grade III in addition by somnolence, and grade IV by a coma-like state. In 40% of patients with HE grade II or above HE will recur within one year [12,13]. Persistent HE is the rarest form. No good prevalence data are available but patients with this type of HE are intensively pharmacologically treated and in some cases need liver transplantation. Most of such patients have advanced cirrhosis with extensive porto-systemic shunting. The MELD (Model for End-stage Liver Disease) score, which is widely used for prognostication of patients with cirrhosis does not correlate with HE severity[14].

HE presenting in so-called acute-on-chronic liver failure (ACLF) is considered within the type C domain. However, over the past 10-years, it has become clear that both clinically, pathophysiologically and prognostically, ACLF is distinct from HE that occurs in patients with no ACLF. Typically, HE in ACLF is characterised by more frequent cerebral edema, marked disturbances in cerebral oxygenation, systemic and neuroinflammation, higher ammonia levels and risk of short-term mortality[15–18]. This type of liver failure is defined by the occurrence of other organ failures, such as renal, immunological or circulatory [15]. HE occurs in about 60% of such patients and here HE is considered a sign of “brain failure”, which is part of the organ failure count that defines the prognosis of acute-on-chronic liver failure patients.

[H2] Burden of disease

HE disrupts personality, self-reliance and capability for every-day living. The experience of having HE is highly distressing and perceived by the indivual as multiple losses of eg. independency and social interaction, and a sustained fear of recurrence[19]. This together with the weakening of cognitive coherence gives rise to the serious loss of quality of life, dealt with in detail later. The personality disruption also poses widespread distress, uncertainty, and anxiety to the caregivers, particularly those in the family[20,21]. There is a need for continuous information both to the reversibility of HE and the risk for recurrence. As regards direct institutional health care cost it is huge because of the large number of patients at risk, frequent hospital admissions and the need for close monitoring, intensive care support and prolonged

periods of hospitalization. There are only estimates from the US and they rose by 30% from 2010 to 2014, to 12 billion dollars per year for that population [22]. This cost burden can be extrapolated and expanded to most Western societies. It will continue to grow with the increasing incidence of cirrhosis of non-alcoholic and alcoholic etiology. The hospital cost of admissions with HE by 50% exceeds that of cardiac failure and chronic pulmonary disease [23]. There are no reliable estimates of the societal burdens of HE but they can be expected to be significant. Most HE patients cannot have a permanent job, many do not contribute towards society welfare and weigh on other sources for life sustenance, including public or private pensions where such are available[24]. Only limited data are available on the social/monetary impact of HE in low/middle income countries. Presence of minimal HE at the time of presentation also demonstrated higher burden on their caregivers[21].

[H2] Risk factors for HE (see Table 1)

Risk factors can be classified according to the organs involved in HE development including liver, portal hypertension, kidney, gut-liver axis, genetic background, drugs and accompanying diseases. These can be stratified as predisposing or precipitating risk factors..

Predisposing risk factors.

Liver dysfunction: commonly levels of albumin (< 3.5 g/dl) and bilirubin (≥ 2.1 mg/dl) and MELD score have been shown to predict events of HE. [25,26] According to a recent scoring system, bilirubin and albumin could independently predict HE[27]. A patient without previous episodes of HE or any genetic risk would have a 43.8% risk of an HE episode within 3-years with albumin ≥40 g/dL but bilirubin>8 mg/dL. Likewise, the same risk can be anticipated if levels of bilirubin are ≤1.8 mg/dL and albumin<20 g/dL[27]. Nevertheless, MELD score does not correlate with HE severity[14]. **Portosystemic shunts**: the cross-sectional area of the severity of porto-systemic shuntings (SPSS) was related to HE episodes and survival [28]. Total area of SPSS is calculated as the sum of the cross-sectional area (area=pr^2) of all shunts detected. A value >83 mm^2 increased risk of overt HE and shorter survival. In patients with preserved liver function (MELD<11) SPSS could be responsible for HE and its occlusion the first therapeutic choice. In patients with liver impairment SPSS increased morbidity and mortality[28].

Although TIPSS increased the incidence of HE by 10-50% at one year[29], covered stents [30] and early TIPSS seem to reduce it [31,32]. Genetic background: it is relevant because patients bearing variants in the promoter gene of kidney-type glutaminase (GLS-1) were predisposed to increased incidence of overt HE, linking genes with increased risk in patients with previous bouts of overt HE [18,30].

Precipitating risk factors.

Kidney dysfunction: both acute kidney injury (AKI) and hepatorenal syndrome (HRS) together with diuretic overdose promote hyponatremia and HE. Patients with serum sodium <130 mEq/l had increased risk of developing HE within one year[26]. Additionally, serum creatinine values greater than 1.2 mg/dl increased the risk of overt HE more than 3-fold. **Systemic inflammation** is mainly associated with bacterial translocation and was considered to be a prerequisite for cognitive impairment in cirrhotic patients with hyperammonemia [33], being the precipitating factor of the HE bouts in up to 50% of the cases [34,35]. **Disruption of gut-liver axis** with changes on the microbiome has been linked to HE. Patients with cirrhosis had enriched pathways related to ethanol production, GABA metabolism, and endotoxin biosynthesis[36]. Indeed, the relative abundances of *Alistipes*, *Bacteroides*, *Phascolarctobacterium* were associated with HE recurrence [37]. Moreover constipation and small intestinal bacterial overgrowth (SIBO) were associated with increased risk of HE [38]. **Drugs**: alcohol consumption, proton-pump inhibitors (PPI)[39,40] and drugs targeting the central nervous system (CNS) (mainly benzodiazepines, GABA-ergic drugs and opioids) by several unrelated mechanisms were also associated with increased risk of HE [12]. **Gastrointestinal bleeding** has been associated with increased risk of HE but some studies reported negative and controversial data suggesting that bleeding is more related to the risk of infection and liver dysfunction rather than HE [15]. **Diabetes mellitus** was identified as a risk factor for the occurrence of first episode of HE in patients with ascites[41,42]. The association between type 2 diabetes (T2DM) and HE remains controversial. T2DM is associated with chronic inflammation and gut dysbiosis which could promote HE but many patients receive anti-diabetic drugs such as metformin which could counterbalance the risk precluding independent associations[43,44].

Also **epilepsy** increases the risk of overt HE in cirrhotic patients[45]. Data supporting association between epilepsy and HE are merely descriptive and a link between epilepsy, anti-epilepsy drugs and HE remains unknown.

Malnutrition and sarcopenia also increase the risk for HE [46]. Furthermore, **higher age** was identified as an independent predictor of HE[47].

Risk factors of HE have been summarized recently (for review see [12] .

[H1] MECHANISMS/PATHOPHYSIOLOGY

[H2] General aspects.

Hepatic encephalopathy is a frequent complication of acute and chronic liver failure for both of which a variety of animal models have been established [48,49]. Hallmarks of HE in acute and chronic liver failure are astrocyte swelling, cerebral oxidative stress, microglia activation and altered neurotransmission. However, cerebral edema in HE is frequent in acute liver failure, whereas it is low grade only in chronic liver failure[50–52]. Moreover, glutamatergic neurotransmission is enhanced in HE in acute liver failure, resulting in neuroexcitation, whereas it is impaired in chronic liver failure and inhibitory neurotransmission (neurodepression) tends to be enhanced [53].

It is generally accepted that ammonia, which is insufficiently eliminated in liver cirrhosis, as well as inflammation play the major role in the pathogenesis of HE. However, also hyponatremia, sedatives of the benzodiazepine-type, neurosteroids, manganese, mercaptans, bilirubin, zinc, phenols, short-chain fatty acids, bile acids and amino acid imbalances (low branched chain/aromatic amino acid ratio) have been implicated as further neurotoxins or pathogenetic factors (for reviews see [2,54–62]). Ammonia as the most important neurotoxin in HE can readily cross the blood-brain barrier in its protonated and deprotonated form[63].

[H2] Low-grade cerebral edema and oxidative/nitrosative stress.

Overwhelming *in vitro* and *in vivo* evidence points to a central role of astrocytes in the pathogenesis of ammonia toxicity and HE, which are the major cell type for removal of ammonia via glutamine synthetase-mediated condensation of ammonia and glutamate to glutamine[64]. One consequence of hyperammonemia in liver cirrhosis is osmotic stress, in part due to intra-astrocytic glutamine accumulation with development of low grade cerebral edema, which is compensated by the release of organic osmolytes such as myo-inositol [50,65]. Depletion of this osmolyte pool in astrocytes, however, restricts the cell volume-regulatory capacity and renders the astrocyte vulnerable to other cell volume challenging agents with exacerbation of the low-grade cerebral edema and induction of oxidative/nitrosative stress in astrocytes. Evidence for a low-grade cerebral edema in patients with liver cirrhosis and HE came from studies employing 1H-MR-spectroscopy [50–52,65] and quantitative water imaging of the brain [66,67], whereas the presence of oxidative/nitrosative stress in brains from HE patients was shown in studies on *post mortem* human brain samples [68](for review see [69]).

According to current knowledge, HE can be seen as the clinical manifestation of a pathogenetic interplay between osmotic and oxidative/ nitrosative stress in the astrocytes [50,69,70]. This interplay is triggered not only by ammonia, but also by proinflammatory cytokines, hyponatremia (low serum sodium levels) and benzodiazepines [71–75]. Accordingly, astrocyte swelling and the formation of reactive oxygen and nitrogen species (RONS) represent a final

common path of action of heterogeneous and clinically well-known HE precipitating conditions [50,71,72,76]. These conditions include excessive protein intake, bleeding, trauma, infections, sedatives, metabolic acidosis, diuretic overdose, renal insufficiency and hyponatremia. Swelling and RONS formation mutually amplify each other in the astrocytes and HE-precipitating factors also act synergistically in the induction of astrocyte swelling and RONS formation [76,77]. Also RONS originating from other cell types in the brain such as neurons, microglia, and endothelial cells [78–80] and even from outside of the brain[81] may contribute to the swelling of the astrocyte. As depicted in the pathogenetic model in **Fig. 1**, the interplay between osmotic and oxidative/nitrosative stress triggers post-translational protein modifications, RNA oxidation, senescence, altered signaling and broad effects on gene expression. These alterations, which were also found in brains from patients with liver cirrhosis and HE, but not in those without HE, are thought to affect astrocytic/neuronal functions and synaptic plasticity and to trigger disturbances of oscillatory networks in the brain, which finally account for cognitive and motor HE symptoms[70,82,83].

[H2] Mechanisms underlying oxidative/nitrosative stress.

The induction of oxidative/nitrosative stress by HE-precipitating factors in rat astrocytes *in vitro* is triggered by an N-methyl-D-aspartate receptor (NMDAR)-dependent elevation of the intracellular calcium concentration $[Ca^{2+}]_i$ (**Fig. 2**)[73–75,84–86]. This NMDAR-activation may originate from an unlocking of the Mg^{2+} blockade of the NMDAR by membrane depolarization[87] and/or mechanical tension of the membrane [88] triggered by astrocyte swelling. Cytosolic phospholipase A_2 ($cPLA_2$)-dependent arachidonic acid release further amplifies $[Ca^{2+}]_i$ through prostanoid synthesis-dependent exocytosis of L-glutamate-containing vesicles (**Fig. 2**)[89].

The elevation of $[Ca^{2+}]_i$ by ammonia or hypoosmotic astrocyte swelling triggers the rapid formation of superoxide anion radicals (O_2^{-*}) by NADPH oxidase 2 (NOX2) [90] and the synthesis of nitric oxide (NO) through activation of the neuronal nitric oxide synthase (nNOS) [73,75,86,91]. Another source of NO in ammonia-exposed astrocytes is the inducible nitric oxide synthase (iNOS) which becomes upregulated in a NFκB-dependent way[86,92,93]. However, a pathogenetic relevance of iNOS for cerebral dysfunction in HE remains currently unclear, since iNOS is not consistently upregulated in animal models of HE [94–96] and was not elevated in *post mortem* brain samples from patients with liver cirrhosis and HE [68,80,97].

Also mitochondria contribute to O_2^{-*} formation in ammonia-exposed astrocytes *in vitro* and in animal models of HE [98,99]. This was proposed as a consequence of a glutaminase (GLS)-mediated mitochondrial hydrolysis of glutamine[79]. Importantly, recent studies confirmed the expression of the GLS isozymes 1 and 2 by astrocytes *in vitro* and in rat and human brain *in*

situ[100]. However, the mechanisms underlying the GLS-induced mitochondrial ROS formation still remain to be established.

Apart from being a source of ROS, mitochondria may further contribute to ROS formation in astrocytes in HE through the synthesis of neurosteroids due to an ammonia-induced upregulation of the peripheral-type benzodiazepine receptor (PBR)[101–103]. In line with this, elevated levels of $GABA_A$ receptor-modulating neurosteroids, such as pregnenolone, allopregnanolone or tetrahydrodeoxycorticosterone (THDOC) were reported in brains from animal models of HE and in human *post mortem* brain samples from patients with liver cirrhosis.[104,105] Importantly, neurosteroids were shown to trigger the formation of ROS in astrocytes in vitro through activation of the G protein-coupled bile acid receptor TGR5 which is expressed by astrocytes and neurons[106]. TGR5 is downregulated by ammonia in astrocytes *in vitro* and in brain of patients with liver cirrhosis and HE, which may reflect an adaption in order to ameliorate ROS formation (Fig. 2) [106]. However, TGR5 downregulation in the brain of patients with liver cirrhosis and HE may also compromise the well-known anti-inflammatory actions of TGR5 with so far unknown consequences.

The release of neurosteroids may be facilitated by the multidrug resistance protein 4 (MRP4) which is upregulated by ammonia in astrocytes through RONS-mediated activation of the peroxisome proliferator activating receptor-γ (PPARγ) [101]. Importantly, MRP4 mRNA and protein levels were also found to be elevated in *post mortem* brain tissue from patients with liver cirrhosis and HE, but not those without HE [101].

Ammonia also upregulates NOX isozyme 4 (NOX4) and heme oxygenase 1 (HO1) through *O*-GlcNAcylation-dependent transcription inhibition of HO1/NOX4-targeting microRNAs[107,108]. The resulting elevation of the intracellular levels of free ferrous iron and H_2O_2 was suggested to trigger the formation of hydroxyl radicals (OH^*) presumably *via* induction of the Fenton reaction[108]. This hydroxyl radical formation results in the oxidation of RNA and the induction of astrocyte senescence through the activation of the cell cycle master regulator p53 as described in detail in the following sections (Fig. 3). This pathway critically requires glutamine-dependent O-GlcNacylation, which again underlines the importance of glutamine formation in the pathogenesis of ammonia toxicity.

[H2] Functional consequences of oxidative/ nitrosative stress.

[H3] Covalent protein modifications. One important consequence of RONS formation in response to HE-relevant factors, are post-translational protein modifications (Fig. 2)[73–

[75,86,99,109]. Ammonia-induced RONS formation triggers protein tyrosine nitration (PTN) of a variety of proteins, such as glutamine synthetase (GS), the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), the extracellular signal-regulated protein kinase 1 (Erk1) and the PBR in astrocytes [73–75,86,109]. Importantly, an enhanced PTN and GS nitration were also found in brain of animal models of HE and in *post mortem* brain tissue from patients with liver cirrhosis and HE, but not in patients with cirrhosis without HE [68,86].. While the catalytic activity of GS was inhibited, the transport activity of NKCC1 was enhanced by tyrosine nitration. [86,109,110] Thus, in addition to intracellular glutamine accumulation, RONS-triggered-NKCC1 activation will contribute to astrocyte swelling in response to ammonia and the interplay between osmotic and oxidative/nitrosative stress. A prominent PTN was also observed in animal models of HE in astrocytes constituting the blood brain barrier (BBB) with yet unknown consequences for the integrity of the BBB [86,111]. The ammonia-induced ROS formation also triggers the carbonylation of proteins in astrocytes *in vitro* and elevated levels of carbonylated proteins were also found in brains of animal models of HE [99,109,112]. Similar to nitration, also carbonylation of the NKCC1 in ammonia-exposed astrocytes enhanced its transport activity[109].

[H3] Protein homeostasis. The ammonia-induced ROS formation in the astrocytes also affects mechanisms governing protein homeostasis (proteostasis) such as proteosomal degradation and autophagy. While the former is enhanced [99], the autophagic flux becomes impaired in ammonia-exposed astrocytes *in vitro* and also in brains from animal models of HE (Fig. 2). [113,114] Importantly, surrogate markers for autophagy were also upregulated in *post mortem* brain tissue from patients with liver cirrhosis and HE and a prominent nuclear accumulation of the autophagy adapter protein p62, which is known to be degraded during autophagy was specifically noted in Alzheimer type II astrocytes in HE. [115,116] These findings strongly suggest an impairment of autophagy in astrocytes in HE.

[H3] Mitochondria. Mild oxidative stress can trigger mitophagy (mitochondrial degradation by autophagy) in a mitochondrial fission-dependent manner [117]. In line with this, ammonia triggers mitochondrial swelling[118], reversible fragmentation of mitochondria and inhibits energy metabolism in astrocytes [113,119] (for reviews see [120,121]).

[H3] RNA oxidation. RNA also becomes modified by ROS in astrocytes exposed to HE-relevant factors, i.e. ammonia, inflammatory cytokines, benzodiazepine and hyponatremia *in vitro*. This was evidenced by the formation of 8-oxo-guanosine, a likely consequence of the ammonia-induced Fenton reaction[108,122]. Since RNA oxidation can impair the translation of proteins, oxidation of the glutamate aspartate cotransporter (GLAST, which is responsible for glutamate uptake from the extracellular space) mRNA may underlie the downregulation of GLAST in astrocytes in vitro and in brain in animal models of HE [122–124]. However, the relevance

of GLAST downregulation for synaptic glutamate clearance in HE remains to be investigated. Also the 18S ribosomal RNA subunit becomes oxidized in ammonia-exposed astrocytes, which may impair translation efficacy [122]. Elevated levels of oxidized RNA were also observed in brain in animal models of HE and also in *post mortem* brain samples from patients with liver cirrhosis and HE, but not from patients with liver cirrhosis without HE[48,68,94,122]. Interestingly, recent studies showed an upregulation of surrogate markers for endoplasmatic reticulum (ER) stress in ammonia-exposed astrocytes *in vitro* and in *post mortem* brain samples from patients with liver cirrhosis and HE[68,108,122]. One may speculate, that here ER stress is triggered by an improper translation of oxidized RNA. Oxidized RNA was also found at neuronal synapses and in RNA granules of neurons from ammonium acetate-treated rats, where it may disturb local postsynaptic protein synthesis and consequently impair related functions such as synaptic plasticity and memory formation [122].

[H3] Senescence. Ammonia also triggers astrocyte senescence through ROS-dependent p53-activation and transcription of p21 and growth arrest and DNA damage inducible protein 45α (GADD45α, Fig. 2). [108,118] Importantly, biomarkers for senescence were also elevated in *post mortem* brain tissue from patients with liver cirrhosis and HE, but not in those without HE.[118] As astrocyte senescence decouples neuronal networks and/ or inhibits neurogenesis, it may contribute to cognitive impairment in cirrhotic patients [118,125], which was shown to persist after resolution of an acute episode of overt HE. [126,127]

[H3] Gene expression. The RONS formation in astrocytes exposed to HE-relevant factors also triggers gene expression changes by releasing zinc ions from proteins in a nitric oxide synthesis-dependent way [91,102], thereby activating the metal responsive transcription factor 1 (MTF1)- and specificy protein 1 (SP1)-dependent gene transcription [91,102]. The resulting upregulation of zinc-chelating metallothioneins (MTs) may protect from cytotoxic effects of free Zn^{2+} ions. Activation of SP1 may enhance the transcription of the peripheral benzodiazepine receptor (PBR)**,** respectively (Fig. 2) [91,102]. Importantly, comprehensive HE-specific gene expression changes such as upregulation of several metallothionein isoforms were also detected in the cerebral cortex of patients with liver cirrhosis [97,128]. These transcriptome analyses identified about 600 genes whose expression was altered in patients with liver cirrhosis with HE but not in those without HE when compared to controls. This not only confirmed the relevance of findings derived from cell culture and animal models of HE, but also identified previously unknown biological processes with a possible involvement in the pathogenesis of HE. Such biological processes included genes involved in oxidative stress, altered zinc homeostasis, microglia activation and counteraction of proinflammatory signaling and inflammatory cytokine expression [97]. Chronic hyperammonemia also alters cerebral protein expression as shown by proteomics analysis of brains from liver-specific glutamine synthetase knockout mice[129],

[H2] Inflammation in HE

Appearance of MHE is associated with a shift in the immune system, with the expansion and activation of Th17, Th22 and Tfh CD4+ lymphocytes and increased serum levels of pro-inflammatory cytokines such as IL-6, IL-21, IL-17, IL-18, TNFα, IL-1β, IL-15, IL-22- as well as of CCL20, CXCL13 and CX3CL1. These alterations are transmitted to the brain and lead to an activation of the brain's immune system (neuroinflammation), which may alter neurotransmission and lead to cognitive and motor impairment [130] . Studies in patients and in animal models support that the sequence of events involved in triggering the early stages of MHE is that summarized in Figure 3. At advanced stages of HE all these processes occur simultaneously. Understanding the sequence of events involved in triggering MHE is essential to design and develop treatments to reverse MHE and HE.

Hyperammonemia and peripheral inflammation act synergistically to induce hepatic encephalopathy [33,131,132]. TNFα treatment of wild-type mice sensitizes the animals to the toxic effects of ammonia, whereas TNFα- or TNFα-receptor-1- deficient mice were protected in this respect[133]. The protection of TNFα-deficient mice against an ammonia load was paralleled by a decreased cerebral expression of NKCC1, which is expected to counteract ammonia-induced glia swelling. In line with this, TNFα induces synergistically with ammonia astrocyte swelling and protein tyrosine nitration of cultured astrocytes [71,76,77].

Hyperammonemia per se is enough to induce systemic inflammation, neuroinflammation and neurological impairment [134,135]. Altered gut-liver-brain axis also contributes to HE. Liver disease is associated with changes in intestinal microbiota. Colonic mucosal microbiota is altered in cirrhotic patients, especially in those with HE and this is linked with inflammation and impaired cognition[115]. Changes in microbiota can contribute and reinforce higher systemic inflammation and cognitive impairment in minimal HE (MHE) [136–139]. Systemic inflammation was suggested to play a main role in triggering MHE [140,141]. In animal models of MHE (porta-caval shunted rats) or chronic hyperammonemia prevention of systemic inflammation by intravenous injection of infliximab, an anti-TNFα antibody that does not cross the blood-brain barrier, also prevents the appearance of neurological impairment [134,142]. Furthermore, MHE appearance is associated with a shift in peripheral inflammation and immunophenotype, with increased differentiation and activation of Th22, Tfh and Th17 $CD4^+$ T lymphocytes and changes in specific cytokines [143]. Changes in extracellular vesicles have been proposed to contribute to development of liver disease and to be useful as biomarkers for diagnosis of different stages of liver diseases [144,145]. Damaged hepatocytes, non-parenchymal cells and infiltrated

inflammatory cells in the liver release large amounts of extracellular vesicles with altered cargo which contribute to the pathogenesis of liver disease and MHE. Also extracellular vesicles originated outside the liver contribute to progression of liver disease. The changes in the cargo of the vesicles is different at different stages of liver disease[146].

Cargo alterations in liver cirrhosis and hyperammonemia regard mainly proteins involved in biological processes of the immune system, increasing the content of TNFα and of its receptor TNFR1 and of proteins such as Hsp70, TIMP-3 or glutamine synthetase[147]. These changes contribute to MHE induction. In line with this, injection of extracellular vesicles from plasma of hyperammonemic rats induces cognitive impairment in normal rats [147]. The source of the extracellular vesicles inducing cognitive impairment remains unclear and it is not known if they are generated in the liver. Increased bile acids may also contribute to HE through sphingosine-1-phosphate receptor 2 (S1PR2) and TGR5 activation and increased C-C Motif Chemokine Ligand 2 (CCL2), which activates microglia and contributes to cognitive impairment [148].

Thus, there are several mechanisms by which peripheral alterations may be transmitted to brain to induce MHE. These include (a) Infiltration of peripheral lymphocytes and monocytes into the brain [149,150], (b) Infiltration of extracellular vesicles from plasma [147] and (c) activation by peripheral cytokines of their receptors in endothelial cells and transmission of signals to brain.

All these mechanisms finally lead to neuroinflammation with activation of microglia and astrocytes and increased synthesis of pro-inflammatory factors, which alter neurotransmission and trigger neurological impairment [130]. Microglia activation has been reported in post mortem brains from patients with HE [80,151,152]. However, microglia reactivity (pro- or anti-inflammatory) has not been shown consistently. Such discrepant results from different studies may be explained because different stages of HE progression and also different brain areas at similar stages of HE were investigated [80,97,135,151,152]. It should be noted that neither enhanced mRNA levels of proinflammatory cytokines, IL-1ß and TNFα nor increased protein levels for IL-1ß, TNFα, interferon-γ, interleukin-4 and interleukin-10 were detected in *post mortem* cerebral cortex from patients with HE[80,97,151].

[H2] Neurotransmission in HE

Previous attempts to ascribe HE to alterations of a single neurotransmitter and/or its receptors have failed; in fact multiple neurotransmitter/receptor systems were found to be deranged and many of these changes were brain region-specific and possibly stage-specific (for reviews see [55,153]).

Neuroinflammation interferes with different steps of neurotransmission, resulting in altered glutamatergic and GABA-ergic neurotransmission [130]. Also other neurotransmitter systems such as glycinergic[154], serotoninergic[155], cholinergic[156] and dopaminergic[157] systems are altered in HE, but the mechanisms involved are not well known. In the past, a generally increased GABA-ergic tone was considered to characterize HE. This concept was challenged recently when it became clear that hyperammonemia increases the GABA-ergic tone in the rat cerebellum but decreases it in the rat cortex [153]. The cerebellum in a rat model with hyperammonemia and MHE shows neuroinflammation, increased TNFα and increased membrane expression of its receptor TNFR1, as assessed in whole brain homogenates. This increases nuclear NF-kB, which activates transcription of pro-inflammatory IL-1β and TNFα, high mobility group protein B1 (HMGB1) and glutaminase, which increases glutamine breakdown and extracellular glutamate concentrations. This leads to increased glutamate and sodium uptake by activated astrocytes through the glutamate transporters GLT1 and GLAST. Increased sodium uptake and activation of astrocytes leads to reversal of the function of the GABA transporter GAT3, which releases GABA, increasing extracellular GABA, which leads to motor incoordination[130] . Enhanced GABA-ergic neurotransmission in cerebellum has been also reported in patients with HE [158]. A decreased cortical GABA-ergic tone was also found in patients with manifest HE employing a paired-pulse transcranial magnetic stimulation paradigm to investigate short-interval intracortical inhibition as a marker for GABA-ergic neurotransmission [159]. However, a similar study on patients with minimal HE found an increased GABA-ergic tone[160], suggesting that motor cortical GABA-ergic tone decreases with increasing HE severity and that alterations in neurotransmission may be different in different brain areas. In a pilot study golexanolone, a $GABA_A$ receptor-modulating steroid antagonist improved the cognitive performance of patients with covert HE [161].

The hippocampus in a rat model with hyperammonemia and MHE (as assessed by behavioral tests) shows neuroinflammation, with increased levels of TNFα and IL-1β. These cytokines activate their receptors in neurons and pathways which alter phosphorylation and membrane expression of AMPA and NMDA receptors, leading to impaired learning and memory. Normalizing TNFα levels or blocking IL-1 receptors restore different aspects of hippocampal neurotransmission and cognitive function, indicating that different pro-inflammatory factors induce different cognitive alterations by different mechanisms [130,162].

In animal models treatments targeting inflammation in HE can restore cognitive and motor function. This may be achieved by reducing peripheral inflammation with (a) anti-TNFα antibodies or ibuprofen; (b) microglia activation and neuroinflammation with sulforaphane, (c) inhibitors of p38 or S1PR2 antagonists; (d) reducing $GABA_A$ receptor activation with bicuculline, pregnanolone sulphate or GR3027, which antagonizes $GABA_A$ receptor-potentiating neurosteroids or (e) increasing extracellular cyclic guanosine monophosphate

(cGMP) per se or total cGMP with sildenafil [130,148]. Although not yet tested in humans, such treatments could be beneficial in patients with HE.

[H2] Cerebral oscillatory networks in HE

Oscillatory neuronal activity serves as a key mechanism of large-scale functional communication and integration across different brain regions and forms the basis of cerebral network interactions [163]. Recent work using whole head magnetoencephalography and electroencephalography in patients with HE has revealed a close association between clinical HE symptomatology and alterations of oscillatory brain activity across different frequency bands and functional subsystems of the brain[164]. Motor and attentional deficits are key symptoms of HE. Mini-asterixis, a postural tremor like-phenomenon with a frequency of 6-12 Hz in the upper limbs, has been shown to arise from an abnormally slow thalamocortical and corticomuscular oscillatory drive[165,166]. The frequency decrease in corticomuscular drive is paralleled by a decrease in the critical flicker frequency (CFF) in HE[167] suggesting that slowing of oscillatory activity represents a common pathophysiological mechanism across modalities underlying diverse clinical HE symptoms.

In agreement with this concept, attentional deficits in HE have been shown to be directly related to changes in oscillatory brain activity. When required to shift attention between visual and auditory stimuli in a crossmodal attention experiment, HE patients exhibited a marked negative correlation between occipital gamma band oscillations to visual stimuli and HE disease severity as assessed by the CFF[168]. Moreover, HE patients lacked the physiological power modulation of visual oscillatory activity in the gamma band around 60 Hz associated with attentional shifts. Since gamma oscillations are instrumental in directing attention to stimuli [169] these findings indicate that slowing of occipital gamma band oscillations mediates an impairment of top-down attentional mechanisms of HE patients in this task. Thus, attentional deficits in HE are related to changes of the oscillatory gamma band activity, agreeing with the hypothesis that slowing of distinct oscillatory brain activities underlies the different clinical symptoms of HE. However, so far it remains unclear to what extent the reported gamma band changes are not only symptom-related but also specific to HE.

Disease-related slowing of oscillatory brain activity has also been shown to affect the somatosensory system. May et al. reported both, a slowed peak frequency of alpha oscillations and a slowing of stimulus-induced modulation of oscillatory activity in primary somatosensory cortex [170]. On the behavioral level it has recently been shown that HE patients are impaired in the temporal discrimination of tactile stimuli, which correlates negatively with the CFF[171]. It is likely but has not yet been directly investigated that this behavioral deficit is related to the

slowing of oscillations in primary somatosensory cortex since these oscillations define perceptual cycles underlying discrete processing of sensory input[172].

In resting state recordings HE patients also exhibit a more global slowing of oscillatory activity with especially prominent effects found for the occipital alpha band peak frequency [97,173,174]. A positive linear correlation was shown to exist between occipital alpha band peak frequency and the CFF suggesting a connection between spontaneous alpha band activity and visual temporal resolution. Similar to the link between slowed alpha oscillations in somatosensory cortex and impaired temporal tactile discrimination this connection between alpha band activity and visual temporal resolution can be explained by current models of perceptual cycles in the visual system[175]. As a possible neurochemical mechanism, occipital alpha band peak frequencies were found to be positively correlated to occipital GABA levels measured with MR spectroscopy. Together these findings revealed distinct disturbances of oscillatory brain activities in HE which in turn are related to neurochemical changes and result in clinically relevant behavioural disturbances. However, further research is needed to unravel molecular and cellular mechanisms of oscillatory dysfunction and to provide more direct evidence for a causal rather than correlational connection with clinical symptomatology.

Accumulating evidence, therefore suggests that alterations in oscillatory network activity provide a fundamental pathophysiological mechanism for linking neuronal dysfunction to the diversity of clinical deficits in HE (see Box **1**).

[H2] Cerebral vessel alterations in HE

Apart from astrocytes, microglia and neurons, also endothelial cell dysfunction was suggested to contribute to the pathogenesis of HE[78]. Further evidence from animal models of HE suggest an impaired cerebral blood flow, an enhanced permeability of the BBB and a reduced clearance of metabolites and toxins from the brain through the glymphatic system [176]. Considering the different brain cell types and associated vessels as an entity, it was suggested to describe HE as a global dysfunction of the so-called “neurogliovascular unit ” [177].

[HE2] Circadian rhythm alterations in HE

Patients with liver cirrhosis and HE frequently exhibit disturbances of the sleep-wake cycle, as reflected by insomnia and excessive daytime sleepiness and altered circadian melatonin and

cortisol blood levels[178]. Since astrocytes participate in the generation of circadian rhythms in the suprachiasmatic nucleus, altered circadian rhythmicity in HE was suggested to be a consequence of astrocyte dysfunction[178].

[H2] Sarcopenia in HE

Sarcopenia due to increased loss and decreased gain of muscle mass is a frequent complication in patients with liver cirrhosis (for review see [179]). Besides physical inactivity, also an ammonia-induced upregulation of myostatin was suggested to impair muscle growth and thereby reduce the muscle mass in patients with liver cirrhosis[180] . Since glutamine synthetase activity in the muscle may compensate for the impaired ammonia detoxification in the liver of cirrhotic patients, sarcopenia may contribute to the development of hyperammonemia and HE (for review see [179]).

[H1] DIAGNOSIS, SCREENING AND PREVENTION

[H2] Classification

Four items are used for classification of HE[3]. **(I)** The underlying condition: “type A” HE if this is acute liver failure, “type B” HE if it is portal-systemic shunting alone (in the absence of significant liver damage) and “type C” HE, which is associated with cirrhosis, with or without the contribution of portal-systemic shunting. **(II)** The severity of mental alterations (*vide infra*). **(III)** The time-course of mental alteration [episodic, recurrent (more than one bout of overt HE within 6 months), or persistent, if in between overt HE bouts the patient does not return to normal mental performance]. **(IV)** The precipitating events (infections, electrolyte disorders, gastro-intestinal bleeding, dehydration or diuretic overdose, sedatives, metabolic acidosis, constipation) and/or facilitating events (spontaneous/surgical shunts or TIPSS; see also above).

With regard to severity, HE has been traditionally divided into overt (neurological and/or psychiatric abnormalities which can be detected clinically) and minimal (abnormalities detectable only on neuropsychological, neurophysiological or psychophysical testings; Figure 4) [181]). The diagnosis of overt HE is primarily a clinical one and the stage-dependent symptomatology has been described in detail in [3,182](ref) and refers to alterations in the state of consciousness, intellectual function, personality-behavior and neuromuscular abnormalities.

This spectrum of disordered mental state and neuromuscular abnormalities in HE is summarized as West Haven Criteria[3].

As the clinical diagnosis of mild forms of overt HE [grade I according to the West Haven criteria[182,183]] is operator-dependent[184], it has been suggested [3,185] that HE is called overt when at least temporal disorientation or flapping tremor are present [≥ grade II according to the West Haven criteria [3,182,183]]; for a full grading algorithm see [3,182,186]. It should be noted that flapping tremor is actually no tremor, but a negative myoclonus. By contrast, grade I HE abnormalities, which are usually detected by caregivers or doctors who know the patient well, are grouped with abnormalities on testing (minimal HE) and referred to as covert HE by some. Whether the term 'covert HE' should be used remains a matter debate. The term, 'covert' implies 'hidden' but the entitity includes patients with grade 1 HE who exhibit signs and symptoms of HE. Furthermore, 'covert HE' is a heterogeneous syndrome [161], prognostically distinct from minimal HE [164] and there is no subgradation of severity. What is certainly of value in the covert/overt HE model is the fact that disorientation to time and/or asterixis (flapping tremor) identify grade II overt HE [3,157]. Fig. 4 summarizes the currently used severity classifications of HE.

The diagnosis of minimal HE is important because the condition is common (30-70% of patients with liver cirrhosis, depending on the tests and cut-offs utilised) and may be associated with an increased likelihood of subsequent overt HE episodes[187], and it is associated with poorer quality of life (vide infra)[188]. As a group, patients with minimal and grade I HE have also been shown to drive worse than their counterparts with cirrhosis and no neuropsychiatric impairment [189]. As HE affects multiple components of mental functioning, probably to a different degree at any given moment in time, the International Society for Hepatic Encephalopathy and Nitrogen Metabolism suggested that the diagnosis is based on more than one test, to be chosen depending on local experience [185]. However, limited information is available on how to combine different test strategies/results, and concordance between tests has been generally reported as low[190].

It has also been proposed to replace categorical classifications such as the West Haven criteria with continuous classification schemes, considering neuropsychiatric changes as a spectrum, which they effectively are. This continuum (low to high-grade HE) may capture neuropsychiatric changes from normality to unambiguous pathology[191,192], using objective and reproducible parameters. Within the low-grade HE, HE 0 to HE 2 are subsumed and continuously recorded and tracked in their course using objective, reproducible and change-sensitive parameters such as the Psychometric Hepatic Encephalopathy Score (PHES) or the Critical Flicker Frequency (CFF), *vide infra*[191,192]. Severe HE can be graded according to the Glasgow Coma scale, and a pragmatic criterion for separating between low- and high-grade forms is the need for hospitalisation due to neuropsychiatric symptoms[191,192].

[H2] Diagnostic approaches

Tests that have been used to diagnose minimal HE and/or quantify overt HE are neuropsychological, neurophysiological and psychophysical. Neuropsychological tests are closer to the phenotype one is trying to assess but they are prone to learning effects, and the existence of local reference values is crucial, as age and educational attainment are major confounders. Neurophysiological tests like the electroencephalogram (EEG) can be obtained in any degree of HE (also in uncooperative patients) but they are further away from the phenotype, and their recording/analysis requires equipment and expertise that may not be necessarily available to hepato-gastroenterology departments. A summary description of available tests of demonstrated usefulness in diagnosing HE is provided below.

[H3] Neuropsychological, paper&pencil or bed-side tests

The **psychometric hepatic encephalopathy score (PHES)** is a combination of five paper-pencil tests assessing cognitive/psychomotor processing, speed and visuo-motor coordination [193]. They are relatively easy to administer and have been translated into several languages and validated in many countries.

The **Animal Naming Test** (**ANT**; i.e. the number of animals listed in 60 seconds has been shown to compare favourably with more established and more complex minimal/covert HE measures, and to predict overt HE [194].

[H3] Neuropsychological, computerised tests

The **Continuous Reaction Time (CRT) test** relies on repeated registration of the stability of motor reaction time to auditory stimuli delivered via headphones. Age, sex and learning/tiring seem to have limited influence[195,196] .

The **Inhibitory Control Test (ICT)** is a response inhibition and working memory test with good validity but it requires highly functional patients[197,198].

The **Stroop test** assesses psychomotor speed and cognitive flexibility by the interference between recognition reaction to a coloured field and a written colour name; it is also available as an app[199].

The **SCAN test** measures the speed and accuracy of a working memory task (digit recognition) of increasing difficulty/cognitive load; it has been shown to have prognostic value[200].

[H3] Neurophysiological tests

The **EEG** can detect changes in cortical cerebral activity in patients with any degree of HE and ist reliability increases if evaluated by quantitative semi-automated spectral analysis rather than visually [201,202].

[H3] Psychophysical test

The **Critical Flicker Frequency (CFF)** is the frequency at which a flickering light (from 60 Hz downwards) appears to be flickering (as opposed to fixed) to the observer. Studies have documented its reduction with worsening HE and improvement after treatment [191,203,204]; it has been shown to be useful in predicting post-TIPSS HE [205,206].

The choice between tests depends on local experience, availability of pertinent norms (to allow adjustment for age and educational attainment, where needed) and the clinical in- and outpatients set-up. While further validation is needed, the ANT is likely to gain popularity. The tests listed above can be used to both diagnose minimal/covert HE and to quantify mild forms of overt HE, as they mostly require some degree of cooperation from the patient. The EEG can be used also in uncooperative patients, thus across the whole HE spectrum.

[H3] Serum biomarkers

3-nitrotyrosine is an oxidative stress marker in neurodegenerative diseases (for review see [207]). In a pilot study, 3-nitro-tyrosine in serum, a degradation product of tyrosine nitrated proteins was suggested as a peripheral biomarker of minimal hepatic encephalopathy[208]. Here, determination of 3-nitrotyrosine had a good sensitivity, specificity and positive and negative predictive values. However these findings need validation in a larger cohort. Also interleukin-6 (IL-6) was suggested as biomarker for minimal HE and a recent study reported IL-6 serum levels twice as high in patients with liver cirrhosis and mHE compared to those without mHE [209]. Furthermore, IL-6 in serum may identify patients with liver cirrhosis at high risk for overt HE [210].

[H3] Abdominal imaging

In patients with hepatic encephalopathy and less severe liver disease, abdominal imaging should be carried out in order to rule out spontaneous portosystemic shunts, which could be subject to embolization (see below)..

[H2] Brain Imaging in HE

Imaging techniques permit investigation of structural and functional neuropathology. Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain exclude the presence of other neurological diagnoses that may be confused with HE or may be coexisting with it[211,212]. However, most of the imaging techniques listed below are not suitable for routine examinations and accordingly are reserved for research purposes. Cerebral MRI with volumetric, diffusion-tensor (DTI), magnetization transfer (MTI) and functional imaging (fMRI) sequences facilitate assessment of brain water, atrophy, neuronal damage and functional connectivity in patients with chronic liver disease [213], while positron emission tomography (PET) gives insight into neurotransmitter imbalance and neuroinflammatory status[214].

MRI is the most popular imaging technique in HE research studies. Cerebral edema is often low grade and may be radiologically-undetectable [50]. However, MRI measurement of total brain volume can be useful[215] : a pilot study from Patel and colleagues was the first to utilize co-registered MRI techniques to determine serial changes in brain volume in HE [216]). The authors concluded that patients in their study treated with lactulose had reduced brain size, associated with improved cognitive performance.

MTI employs the differential magnetic properties of free and bound protons[217]. Intracellular proteins, phospholipids and nucleic acids bind protons tightly, but others are present unbound in the form of free-moving water molecules, which resonate more easily in a magnetic field and therefore are visualised differently to those protons firmly bound inside the cell[218]. MTI allows magnetic transfer ratios (MTR) of intracellular bound to free water content to be calculated, changes in which reflecting neuronal damage and increases in brain water content or membrane permeability[219–221].

Functional MRI (fMRI) measures paramagnetic changes in deoxyhaemoglobin occurring during the metabolic perturbation caused by neuronal activity [222]. The resultant blood oxygen level dependent (BOLD) signal highlights areas of neural function in the brain[223,224]. Unlike positron emission tomography (PET), which involves radioactive tracer injections, fMRI is an alternative functional brain imaging technique to PET, but without radiation hazard, thus allowing safe, longitudinal studies before and after treatment intervention [225]. Zhang and coworkers showed reduced BOLD signal in the default-mode network of the brain in HE

patients (more specifically, in the right middle frontal gyrus and in the left posterior cingulate cortex, areas of the brain involved in maintaining attention, which are normally highly active in alert individuals)[226],[227].

A read out on brain neurochemistry, including osmolyte status can be gleaned using MR spectroscopy (MRS) [228], allowing non-invasive insight into changes in intracellular osmolytes in various regions of the brain, reflecting brain swelling. Although first used in the 1980s, more recent technical improvements in MRI sequences and in MRI scanners themselves have improved resolution of metabolite signals[65,229,230]. The characteristic spectral appearance of HE using MRS are reductions in the myo-inositol (mI) and choline (Cho) resonances with increases in measurable glutamine (Gln), which may overlap with glutamate (Glu) in a composite resonance, often termed Glx [65,231–233]. This pattern of metabolite disturbance has been correlated to the severity of psychometric test dysfunction in HE patients[234]. Although much time has been invested in developing these techniques, an imaging protocol for clinical practice has not been agreed internationally and thus, these techniques remain confined to research studies or clinical trials.

Role of Ammonia measurements

Ammonia levels during health is extremely tightly regulated through intricate network of regulatory systems in many organs. Ammonia is produced mainly in the gut and kidneys and removed in the liver. In patients with cirrhosis, muscles play an important role in ammonia metabolism and sarcopenia is an important risk factor for the development of HE. As discussed in the previous sections, ammonia is central in the pathogenesis of HE. Therefore, the diagnosis of HE should not be sustainable in the absence of hyperammonemia. However, current guidelines do not recommend ammonia measurements as being essential in the diagnosis of HE. This is despite the existing data from several studies confirming that a diagnosis of HE is incompatible with normal ammonia levels. There is however, no direct relationship between grade of hyperammonemia and severity of HE[2], which may explain why ammonia levels are not suitable to guide therapy in clinical practice [235]. Elevated blood ammonia can predict HE-related hospitalizations and ammonia levels of >150 $\mu mol/L$ and >80 µmol/L define the risk of mortality in ALF and cirrhosis respectively[236–239]. Also, an increase in ammonia levels defines the risk of death whilst a reduction is associated with survival

[16,238,240]suggesting that sequential measurements of ammonia may be useful in clinical practice. Whether ammonia levels are of value in out-patients with stable cirrhosis to predict episodes of overt HE is currently unknown.

Given the overwhelming evidence for the potential role of ammonia measurement in the diagnosis and prognosis of HE, it is intriguing that ammonia measurements are not recommended for routine clinical use for patients with HE. The main issues with interpreting ammonia levels in clinical practice is the lack of standardisation of procedures for blood sampling, storage and its measurement, which can lead to erroneous results[241]. Although the measurement of ammonia is relatively straightforward, normal values in different hospitals vary considerably making it difficult to interpret absolute values across different hospitals. Arterial levels tend to be higher than venous levels, There seems to be limited advantage in obtaining arterial as opposed to venous samples for purposes of ammonia measurement[242,243]. Venous blood should be preferably drawn when the patients is fasting, Furthermore, for accurate measurements, the blood samples need to be collected in pre-cooled tubes, transported to the laboratory on ice and measurements made within 30 min. It is important to stress that reliable ammonia levels can only be obtained if standard operating procedures are in place. Capillary ammonia is best measured on blood obtained from the earlobe, as sweat artefact leads to overestimates in blood drawn from the fingertip[244,245] (Huizenga et al 1995;Bersagliere et al. 2013). If arterial or capillary ammonia are utilized, appropriate reference values should be used. Ammonia measurements are only problematic in terms of false positives and not of false negatives. Thus the exclusion of HE based on normal ammonia levels is unlikely to be affected by measurement issues[3],. However, the presence of hyperammonaemia does not necessarily imply the development of the HE phenotype. So, for the sake of simplicity, one could conclude that there is no HE without hyperammonemia, while hyperammonemia does not necessarily imply the presence of HE signs/symptoms.

[H2] Prognostic relevance

Minimal HE is a predictor of overt HE[187,246–248] , thus its detection should raise attention to the possibility of the occurrence of overt HE. While it has not been proven that treatment of minimal/covert HE prevents the occurrence of overt HE, this assumption seems reasonable. Overt HE is a predictor of death[15,249], and its appearance generally marks a significant worsening of both hepatic function and prognosis [13,250]. Therefore, after a first bout of overt HE the patient should probably be referred to a liver transplant centre. In the transplant setting an episode of overt HE increases mortality in patients with the same MELD[251]. As outlined above, even after resolution after an attack of overt HE, patients may show persistent cognitive deficits (for review see [1]).

[H2] Differential diagnosis

Although the clinical symptoms of HE in a patient with liver cirrhosis are fairly typical, other neuropsychiatric diseases need also to be considered. Main differential diagnosis comprises sleep apnoe, Wernicke encephalopathy, alcohol withdrawal syndrome, infections and septic encephalopathy, hypo- and hyperglycemia, Wilson´s disease, sedative overdose, dementia, electrolyte imbalances, uremia and hepatocerebral degeneration. Also hyperammonemic conditions as a result of inborn errors of metabolism, such as urea cycle enzyme defects must be considered (for reviews see [252,253]). Another major and important differential diagnosis are intracerebral bleedings following falls and trauma in patients with cirrhosis, which frequently have a compromized blood coagulation. Such bleedings may present with somnolence and are detected by cerebral imaging.

[H1] MANAGEMENT

[H2] General approach and nutrition

Guidelines for treatment of hepatic encephalopathy have been published in recent years by the European, Italian and American Associations for Study of the Liver, the Japanese Society of Hepatology, the International Society for Hepatic Encephalopathy and Nitrogen Metabolism and by the European Society for Parenteral and Enteral Nutrition[3,4,186,254–257].

The approaches to the management of patients with HE in patients with cirrhosis (Type C) can be considered under the following 3 domains. As the pathophysiology of Type A and B HE is different, approaches and goals of therapy are distinctive and will not be considered here further.

Primary Prophylaxis

An approach to the selection of patients is shown in Figure 6A. The aim of primary prophylaxis is to prevent the occurrence of a first attack of overt HE. All patients with liver cirrhosis should regularly undergo screening for complications of cirrhosis such hepatocellular cancer and HE. Whereas overt HE is easily diagnosed by the physician and family members, MHE require psychometric tests, which can be neuropsychological, neurophysiological or psychophysical (see above), The main purpose of identification of MHE is to select patients for primary prophylaxis. However, there is no general agreement which test should be preferred, but equipment availability, test experience of the physician, test duration and the education status, specificity and predictive ability of the test will be important determinants[258]. In a recent metanalysis comparing the existing tests used for the diagnosis of MHE, which would allow selection of patients for prophylaxis concluded that more studies were needed but the PHES test performed the best with a negative predictive value of 69-94%. Blood ammonia measurements can also be helpful, but their value as a screening parameter is not yet fully settled. Of interest would be home-monitoring devices of HE severity, which can be handled by the patient himself or by his relatives. The animal naming test[194] could be suitable for this approach and smartphone apps have been developed[259], however further studies are required to assess their role as home-monitoring approach. Both patients with MHE and those with grade 1 HE should be considered candidates for primary prophylaxis. The drug that has the best evidence for primary prophylaxis is lactulose, which can be administered orally or as enemas. Tolerability of lactulose may be compromized by its sweet taste, flatulence and diarrhoe, which can be avoided by proper dosage. Additionally, nutrition is an important adjunct. The adoption of small meals during the day and of a late evening snack is also recommended to reduce the periods of fasting which may cause protein catabolism[260]. In a randomized trial, patients with cirrhosis with MHE, when assigned to nutritional therapy (30–35 kcal/kg/day, 1.0–1.5 g vegetable protein/kg/day) vs no nutritional therapy (patients continued on their same diet) for 6 months, demonstrated an improvement in their cognitive performance (reversal of MHE in 71 vs 22%) [261]. Patients with cirrhosis frequently have zinc deficiency and oral zinc supplementation was reported to improve HE and health related quality of life [262]. Insomnia can be a symptom of hepatic encephalopathy. If it does improve in response to HE therapy, cognition behavioural therapy, hydroxyzine and chloralhydrate [263] might be considered. An algorithm describing screening and selection of patients for screening and institution of primary prophylaxis is shown in **Figure 6A.**

Treatment of an acute episode of HE

After identifying cognitive impairment or neurological symptoms in a patient with liver cirrhosis and the exclusion of other possible causes of altered mental status so that a diagnosis of HE is achieved, supportive measurements and treatment need to become operational. Supportive care will require different intensity according to the severity of HE. In a patient hospitalized with high-grade HE (grade III-IV) prevention of airway obstruction and aspiration pneumonia, care of possible harms caused by the patient's disorientation, care of iv lines, liquid balance, monitor of vital signs, urine output, renal function, pH, blood gases, electrolytes and glucose, are mandatory [264]. In any case, an intensive search for and rigorous treatment of factors known to precipitate HE episodes, such as infections, trauma, bleeding, high protein intake, constipation, diuretics overdose, sedatives, are mandatory and frequently correction of such precipitating factors will already improve the situation. Alterations in bowel function can represent a precipitant of HE and should also be considered and corrected. Even in a patient with moderate (grade II) HE, general care and monitoring should be adopted according with clinical conditions.

Consideration of nutritional status is appropriate in all patients with HE. Protein-calorie malnutrition and sarcopenia are associated with a lower capacity of ammonia detoxification [266]. At the same time chronic hyperammonemia can induce a decrease in protein synthesis through myostatin activation[267], triggering further muscle depletion. There is a general consensus in guidelines about the need to support patients with HE with adequate calorie and protein requirements[254]. Protein restriction, i.e. a reduction to 20-40 g/kg protein daily as suggested decades ago should not be practiced, because it may result in a catabolic state worsening the clinical situation[3,15,255].

An adequate protein intake (at least 1.2-1.5 g protein/kg/day) is also recommended in patients with overt hepatic encephalopathy to prevent muscle catabolism [254] . In those patients who cannot achieve this goal, oral branched-chain amino acid (BCAA) supplements may be of help [268,269]. A recent metanalysis showed that BCAAs improved the manifestation of overt HE but mortality was not impacted [269]. They act by increasing muscle protein synthesis. Further better controlled studies are needed before it can be recommneded for routine use. When oral diet is not feasible due to high-grade HE (grade III-IV), enteral nutrition can be utilized for a period of more intensive nutritional support and, when needed, parenteral nutrition is another option. In patients with recurrence or persistence of cognitive symptoms compliance to dietary prescriptions may be poor and should be reinforced through a multidisciplinary approach and repeated dietary counseling[255]. The involvement of the caregiver is crucial to increase patients' motivation and fulfilment of the nutritional regimen[270]. In the last years specific modifications of microbiome have been reported in patients with HE[138]. Whether a nutritional approach will modify patient's microbiota can be a setting of future research. An algorithm describing the treatment of patients with an acute episode of HE is shown in **Figure 6B.**

Secondary prophylaxis

There is a high risk of HE recurrence after recovery from an episode of overt HE, which provides a rationale for secondary prophylaxis. Lactulose and probiotics were shown to be effective in preventing recurrent episodes of HE [271–273]. Also rifaximin in combination with lactulose was effective and even superior to lactulose monotherapy[274,275]. Lactulose was also shown to be effective in primary prophylaxis of HE in patients with liver cirrhosis [276] as were probiotics[277].

[H2] Specific medical treatments

The main specific targets for the treatment of HE are ammonia and inflammation, which are the two most important mechanisms underlying its pathogenesis. Strategies to directly target the underlying neurological mechanisms have lagged behind generalised systemic approaches.

[H3] Ammonia as a target

Current therapeutic approaches focus on ammonia as the most important neurotoxin in HE. Ammonia is predominantly produced in the small bowel through the action of enterocytic glutaminase and also in the colon through the action of gut bacteria. This ammonia is removed mainly by the liver with its sophisticated structural and functional organization of ammonia-metabolizing pathways in the liver acinus (for reviews see [110,278]). In periportal hepatocytes ammonia is eliminated by urea synthesis, which depends on ammonia amplification by mitochondrial glutaminase, because of the low ammonia affinity of carbamoylphosphate synthetase. In liver cirrhosis this amplification process is strongly upregulated in order to maintain a life-compatible rate of urea synthesis, despite a severe decrease of the urea cycle capacity by about 80% (for details see [278]). The ammonia which escaped periportal urea synthesis is eliminated with high affinity by a small perivenous hepatocyte population (socalled perivenous scavenger cells) via glutamine synthesis. In liver cirrhosis their capacity to eliminate ammonia before the sinusoidal blood reaches the systemic circulation is strongly impaired and hyperammonemia ensues. In line with this, liver-specific deletion of glutamine synthetase in mice results in systemic hyperammonemia [48]. These scavenger cells not only exclusively express glutamine synthetase in the liver, but specifically also ornithine aminotransferase and uptake systems for aspartate, glutamate and related dicarboxylates [279],

which can provide the carbon skeleton for glutamine synthesis and a rationale for their supplementation. Also the muscles contain glutamine synthetase and can contribute to ammonia detoxication. The following therapies that have been trialled in patients with HE and target ammonia are described below.

Approved treatments

Lactulose: The mainstay of therapy of HE is the non-absorbable disaccharide, lactulose, which is considered the gold standard for primary prophylaxis and also the treatment of HE. Meta-analytical reviews of trials of lactulose versus no therapy demonstrated marked beneficial effects of lactulose on severity of HE, prevention of HE, serious liver-related adverse events (number needed to treat 4-6), and also a reduction in mortality (number needed to treat 20) [280]. The trial quality is variable but the results are the same in high-quality randomized controlled trials only. The major criticism of the evidence surrounding the trials of lactulose in HE is the lack of double blind, multicenter studies. Nevertheless lactulose is very cheap and thus cost-effective and readily available from many sources. The proposed mechanism of its action is through increasing the excretion of ammonia in the gut lumen by decreasing the fecal transit time and reducing its absorption by acidification of the stool. Its use can trigger gastrointestinal side effects such as nausea, vomiting, flatulence and diarrhoea, which are easily controlled by dose reduction.

Rifaximin: Rifaximin is an essentially gut restricted antibiotic with a wide spectrum of activity against a multitude of bacteria with less than 4% bioavailability after oral administration. The best evidence for its efficacy in regulated studies is as an add-on to lactulose to prevent recurrence of HE in patients with a previous episode of HE [274]. Its use in this indication is almost universal and recommended by the regulators. In single center studies, the beneficial effect of rifaximin has been shown to extend beyond HE to improvements in survival[281]. It is well-tolerated but its mechanism of action remains uncertain. It reduces ammonia concentrations modestly and surprisingly, its action to reduce bacterial translocation remains controversial [281–283]. Given the relatively low systemic absorption, development of bacterial resistance is likely to be small.

L-ornithine L-aspartate (LOLA): L-ornithine augments glutamine synthesis in perivenous scavenger cells by provision of glutamate. Also aspartate, which like 2-oxoglutarate is preferentially taken up by perivenous scavenger cells [279] can provide after transamination glutamate for glutamine synthesis. There are 8 randomised controlled clinical trial of LOLA for the treatment of patients with HE of variable quality[284,285]. Meta-analyses have suggested

beneficial effects of the drug on HE in the acute setting, but a lot of the existing data are from single center studies. Its use in the setting of prevention of occurrence or recurrence of HE remains a matter of debate. It seems to be safe but is available in only a few countries and can be considered a second line therapy in these situations.

Embolisation of portosystemic shunts: Some patients with cirrhosis develop large spontaneous portosystemic shunts for reasons that are not clear, which can result in severe HE. In these patients, radiological embolisation of the shunt reduces ammonia levels and is effective in reducing the severity of HE. Although there are no published randomised clinical trials, data from case series provide compelling evidence for its usefulness at least in the short term as most patients will ultimately need a liver transplant for long term survival [247,248]. The procedure is also considered safe and efficacious only in the patients with well compensated cirrhosis (MELD score<11), further limiting its application [247] .

Off-label or experimental approaches

Polyethylene glycol: This is thought to work in a manner similar to lactulose by increasing fecal transit but does so in a dramatic fashion producing profuse diarrhoea. A single dose was shown to improve the severity of HE compared with the control group but the ammonia levels were unchanged[286]. More confirmatory data are needed before widespread use.

Fecal transplantation and engineered bacteria: The hypothesis that the gut bacteria is important in modulating the severity of HE has been supported by recent data from early phase human clinical trials showing that fecal transplantation is safe and can lead to improvements in the severity of dysbiosis and markers of minimal HE [287,288]. However, the patients were being treated with lactulose and rifaximin making interpretation of the data difficult [287]. In an extension of the concept, specific bacteria were engineered to impact on ammonia metabolism and inoculated into the gut. Although the data in animal models were impressive, this benefit was not observed in humans leading to discontinuation of the clinical development programme [289] (https://investor.synlogictx.com/news-releases/news-release-details/synlogic-discontinues-development-synb1020-treat-hyperammonemia). In single centre, unregulated studies, probiotics have revealed evidence of efficacy in patients with minimal HE. The wide range of the available probiotics and the lack of standardization and regulated studies make it difficult to determine their generalised usefulness and require further studies before wide spread use can be considered[290].

Activated carbon microspheres. These are modified carbon microspheres (AST-120) or combination of micro-macrospheres (CARBALIVE), which act by adsorbing toxins including ammonia in the gut lumen. Human clinical trials of AST-120 failed to show clinical benefit in patients with minimal HE (ASTUTE study) and results of the early phase clinical trials of CARBALIVE are awaited [291].

Ornithine phenylacetate: This drug is being developed on the hypothesis of the synergistic action of L-ornithine as glutamate provider for glutamine synthesis, and phenylacetate, a drug widely used for the treatment of urea cycle disorders, which aims to remove glutamine by formation of phenylacetylglutamine [292]. This has been tested through early phase trials and recently reported the results of a Phase 2b study. The data suggested that the drug was safe but did not reach the primary end point of reduction in the time to resolution of HE [245]. The authors went on to perform an unplanned post-hoc analysis excluding patients with confirmed hyperammonemia at study entry that showed statistical significance in time to resolution of HE. Based on this post-hoc analysis, a pivotal phase 3 study is contemplated.

Glycerol phenylbutyrate: This drug is converted to phenylacetate and acts to trap glutamine. It has been repurposed from its primary use in patients with urea cycle enzyme deficiencies. It was shown in a large Phase 2 study in patients with HE to reduce ammonia and also to prevent HE recurrence in a trial similar to that of Rifaximin[293]. However, the follow up Phase 3 trial was not performed. The reasons behind this decision to not proceed is not clear.

VS-01: This novel approach involves the administration of specially engineered, biocompatible microspheres that have the capability of adsorbing ammonia into the abdomen. Preliminary results of a Phase 1b study were recently described providing data confirming safety and the proof of concept for the approach[294]. Further clinical trials are planned.

[H3] Inflammation as a target

Although there are a plethora of data providing incontrovertible evidence for the importance of inflammation in the pathogenesis of HE, very few approaches targeting inflammation have been trialled and are described below. Additionally, it is likely that reduction in ammonia itself reduces the severity of inflammation and other interventions such as antibiotic use may also reduce systemic inflammation.

Off-label or experimental approaches

Extracorporeal detoxification devices. Albumin is a multifunctional protein, which can modulate inflammatory responses[295]. The detoxification and the anti-inflammatory property of albumin

has also been harnessed in an extracorporeal device. In a regulated, multicenter, randomised, controlled trial, albumin dialysis using the Molecular Adsorbents Recirculating System (MARS) was significantly more effective in reducing time to HE resolution compared with the control group[296]. Although not widely available, it is used by some centers and should currently be considered a 'third-line' treatment. Preliminary results of a new device, DIALIVE, was recently described in patients with ACLF. This device aims to exchange albumin and remove damage and pathogen associated molecular patterns. Beneficial effects were seen in the severity of HE providing the rationale for future clinical trials[297].

Albumin: An early uncontrolled non-randomised study suggested possible benefits in patients with HE, but this was not confirmed in a randomised controlled clinical trial [298]. In another single center study, the combination of lactulose with albumin was, however, more effective than lactulose alone in the complete reversal of HE[299]. In a further randomised clinical trial [300], albumin was compared with placebo aiming at reduction in 90-day mortality of patients hospitalised with grade 2 HE. The study failed to meet this primary end point.

Golexanolone: This is a $GABA_A$ receptor modulating steroid antagonist that underwent an early phase clinical trial in patients with minimal HE against placebo[161]. The drug was safe and although the results of the effect of golexanolone on neuropsychlogical tests compared with placebo were not statistically significant, they showed trends towards improvement in the drug arm. Future trials are being planned.

[H4] Liver transplantation and Reversibility of hepatic encephalopathy

Liver transplantation remains the only rescue therapy for patients with HE and an assessement for transplantation should be considered in all patients presenting with the first episode of overt HE as these patients are at a greater risk of death[301,302]. Resolution of HE in the long term is the norm even in patients transplanted with severe ACLF and coma if the brain stem remains intact. Severity of HE should not be considered a contraindication for liver transplantation. However, the widespread adoption of current organ allocation systems involving the use of the Model for end stage liver disease (MELD)[303] disadvantages patient with HE as patients with severe and recurrent HE often have relatively low MELD scores[304], which is not considered in the formulae used for prioritisation of organs. In fact, adding HE to the MELD score improves its predictive ability[251].

Long term follow up studies in patients with HE who have undergone liver transplantation allows exploration of the question of reversibility of HE. Several studies even 30-years back questioned the lack of complete reversibility of minimal encephalopathy in patients undergoing

liver transplantation[305–307]. In better controlled, more recent studies, the lack of resolution of HE was confirmed to be more severe in those with previous episodes of HE[1,308]. In fact, subsequent studies correlating neurocognitive changes with neuroimaging [309], confirmed these findings and identified subgroups that continued to show evidence of reduction in brain neuronal mass. Further prospective studies are needed to better characterise these data considering the effects of calcineurin neurotoxicity and surgery (for review see [310]). Nevertheless, this idea of irreversibility of HE needs to explored further as emerging data start to point towards loss of neurons underlying the pathophysiology of HE.

In summary, most important therapeutic approaches for the treatment of HE are directed at eliminating and treating the precipitating factors and reducing ammonia. The only specific therapy that has been through extensive and rigorous testing is rifaximin. The mainstay of treatment for all patients with any grade of HE is lactulose despite paucity of multi-center, double-blind high quality clinical trial data. Rifaximin is reserved for the prevention of recurrence of HE and several second and third line off-label approaches can be used when other therapies have failed. Many other drugs and approaches such as polyethylene glycol-3350, nitazoxanide and fecal microbial transplant are in clinical trials, the results of which are awaited or to be confirmed [311–314](see also https://clinicaltrials.gov/ct2/show/NCT03796598). Liver transplantation remains the rescue treatment of choice but whether this results in complete resolution of neurocognitive functions remains a matter of debate.

[H1] QUALITY OF LIFE

“Health-related quality of life (HRQOL) is a broad and multidimensional concept, which includes all aspects of human well-being, physical and cognitive skills, social functioning, set of emotions, and psychological status” [315]. HRQOL is often impaired in patients with chronic liver diseases [19,316]. The HRQOL worsens with the progression of chronic liver diseases to advanced cirrhosis [317]. The development of hepatic encephalopathy in cirrhosis further impairs the HRQOL not only in patients but also among caregivers [318]. Among cirrhosis-specific decompensating events, HE is the sole event consistently associated with impaired HRQOL[319]. The commonly used tools for HRQOL assessment include Short-Form survey-36 (SF-36), Sickness Impact Profile (SIP) and the liver-specific Chronic Liver Disease Questionnaire (CLDQ) [317]. . SIP and CLDQ are more exhaustive as the SIP has 136-questions, split in psychological and physical dimensions and 12 other domains [320], while the CLDQ has 29

questions split in 5 domains (emotional function, systemic, activity, abdominal symptoms, fatigue, and worry)[321].

Subtle changes in cognitive and psychomotor deficits without overt signs of HE connotes the development of minimal hepatic encephalopathy (MHE) in cirrhosis [322]. MHE impairs the daily functioning, driving skills and HRQOL in patients with cirrhosis and is an important risk factor for the development of overt HE and mortality [320,322–324]. A recent study of patients with cirrhosis and their caregivers[21], demonstrated that caregiver burden scores increased significantly among patients with either previous OHE or MHE and correlated with liver disease severity scores and negatively with socioeconomic status. Patients with MHE often have a preserved basic day to day functioning but the complex activities requiring attention, information processing and psychomotor skills, such as planning a trip or driving a car are often affected. Evidence suggests that almost all scales of the SIP are impaired in patients with MHE [320,323,324].

Another problem in cirrhotics are the sleep disturbances that adversely affect HRQOL [325]. These disturbances have been reported in 26–70% cirrhosis patients and are more frequently noted in those with MHE [15,320,326–328]. Delayed initiation and frequent awakenings result in reduced sleep time and excessive daytime sleepiness that affects sleep satisfaction and result in poor HRQOL. Interestingly, the sleep disturbances at night-time are not related to HE but to the abnormalities in circadian rhythm among patients with cirrhosis[326]. Diet-induced (with oral amino acid) hyperammonemia has shown to induce sleepiness in parallel with rise in blood ammonia levels among both healthy volunteers and cirrhosis patients [329]. Ammonia levels have been correlated with excessive daytime sleepiness and increased risk of HE related hospitalizations and presence of portosystemic shunts [325]. Sleep disturbances and MHE significantly contribute to impairment in HRQOL among patients with liver cirrhosis [326,328].

Patients with liver cirrhosis and MHE more frequently have falls and fall-related injuries that affect HRQOL[330,331] . MHE contributes to falls in cirrhosis due to slowed reaction time, impaired attention and visuomotor coordination, and psychomotor speed. Intake of psychoactive drugs, poor muscle strength, and sleep problems (excessive daytime sleepiness and its adverse effect on attention and steadiness) may also aggravate increase the risk of falls in cirrhosis[270]. Thus, these risk-factors should be assessed in this population and therapeutic interventions must be designed for patients, such as, exercise to improve strength and balance, medication assessment to limit the use of benzodiazepines, antipsychotics, etc, and home modifications to reduce fall hazards.

Osteopenia and osteoporosis increases the risk of fractures in cirrhosis that may eventually be associated with surgeries and decompensations which could adversely affect the HRQOL in cirrhosis patients [332]. An emerging area of interest is the interaction between MHE and

predementia mild cognitive impairment (MCI), especially in older cirrhotic patients (>65 years), with evidence suggesting MHE to be independently associated with poor HRQOL irrespective of MCI[333].

MHE in cirrhosis has been associated with poor driving skills both on real road driving tests or on simulator tests [189,334–336]. MHE patients showed a greater impairment in categories like car handling, maneuvering, adaptation and cautiousness compared to non-MHE patients on real-time-road driving tests [336]. Cognitive decline in MHE patients has been associated with increased risk of accidents [337]. Epidemiologic studies also demonstrated that cognitive impairment is linked to traffic accidents and violations [338]. Patients with liver cirrhosis and MHE have less insight into their driving skills and tend to overestimate their driving skills [189,339]. A real on-road driving study with a multiple sensor and camera-equipped car showed that the presence of MHE or HE grade I did not necessarily predict inability to drive a car in the individual case [189]. Increasing HE severity however paralleled significant performance deficits in traffic safety parameters [189] . However, a couple of studies found no impairment in driving performance or increased accident rate in MHE [340,341].

Currently, no clear guidelines exist for restricting driving in patients with MHE with or without recent overt HE. However an ISHEN Consensus[342] suggests that a short objective and nonjudgmental driving history should be taken at each visit (such as *Do you drive? Have you had accidents or "near-misses"?*). Cognitive testing is not useful to determine who is a poor driver and is not recommended to restrict or resume driving. In those with recent (<3 months) episode(s) of overt HE, oral and written advice against driving should be given to patients and caregivers and be documented. In case the affected patients want to resume driving, they should schedule a formal driving reassessment with the local authorities based on local regulations[342].

Treatment-induced improvement in cognitive functions had shown to improve HRQOL in patients with cirrhosis [320]. Lactulose treatment was shown to improve both cognitive functions and HRQOL in cirrhosis patients with MHE[320]. Similarly, rifaximin treatment of patients with MHE improved both neuro-psychometric performance and SIP scores, confirming a strong relation between cognitive functions and HRQOL [324]. Lactulose treatment had recently been shown to improve gut microbiota and recovery from MHE in cirrhosis patients [343]. Treatment with rifaximin was also reported to improve simulator-based driving performance in patients with MHE [185]. Rifaximin treatment may also improve objective parameters of sleep architecture rather than subjective parameters of sleepiness and quality of sleep in cirrhosis patients with recurrent HE [344].

[H1] OUTLOOK

The problems regarding nomenclature and diagnostic approaches have been addressed above and require unification bearing in mind the need for an objective, clinically and scientifically sound approach. However, HE is a crude description of a neuropsychological syndrome and phenotype, which may encompass pathophysiologically different and heterogeneous entities. To unravel these factors requires the search for novel biomarkers and non-invasive methods for brain examination and will have important consequences for treatment in individual cases, in terms of personalized medicine. One example may be acute on chronic liver failure (ACLF) which should possibly be considered as a specific HE subgroup as its pathogenesis, clinical features and management may be different to the 'traditional 'overt HE'. Although considerable progress has been made in the pathophysiology/-biochemistry of HE, only a small amount of this knowledge has been translated up to now into clinical practice and treatment. Smartphone-based health monitoring may be applied to cirrhosis patients with HE and used for early detection of HE worsening[345]. Potential new treatment targets may focus on cerebral oxidative/nitrosative stress and the oscillatory networks in the brain. Investigation of treatment options may be facilitated by clinical trials, which require not only the definition of study populations but also of appropriate study endpoints and readouts for different forms of HE. This and the availability of novel biomarkers will also help to decide whether primary prophylaxis of HE should be considered in future as the mortality after the first HE episode is high[249]. Blood ammonia levels were shown to be a biomarker regarding prognosis of patients with liver cirrhosis[238] , although blood ammonia levels themselves do not necessarily correlate with HE severity, and should not be used in isolation to diagnose the presence of HE. Nevertheless, it is reasonable to assume that simple bedside tests may soon be developed that can be used in clinical practice. It is hoped that this review will stimulate further research on this important disorder.

Table 1: Risk factors for hepatic encephalopathy

	Risk factors	Association with HE	Average risk H.R. (95%CI)	Refs
Predisposing factors				
LIVER	Liver Dysfunction	Bilirubin level Albumin level	1.184 (1.04-1.36) 0.93 (0.89-0.97)	18
SPSS	Spontaneous Porto-systemic shunts	Total area > 83 mm^2	1.83 (1.14-2.93)	[28]

GENES				
	Genetic background	Glutaminase gene	2.1 (1.17-3.79)	[27,346]
	Previous episode of overt HE	Personal risk	4.22 (3.30-5.41)	[15]
Precipitating factors				
GUT-LIVER AXIS				
	Variceal bleeding	Not associated (?)	0.52 (0.37-0.73)	[15]
	Constipation and SIBO			[38]
	Infections	SBP, pneumonia, cellulitis, UTI	3.0 (2.4–3.8)	[34]
	Dysbiosis	Cirrhosis Dysbiosis Ratio		[37]
KIDNEYS				
	Renal Insufficiency	AKI - HRS	1.01 (1.00-1.02)	[29]
	Hyponatremia	0.08 by each mmol/L decreased	10.7 (4.4 – 26.0)	[26]
TIPSS	TIPSS	Early TIPSS & Covered stent Covered stent	1.08 (0.84-1.38) 1.26 (0.54-2.95)	[30,32]
DRUGS				
	CNS Drugs use	Benzodiazepines Gamma aminobutyric acid (GABA)ergics Opioids Proton pump inhibitors (PPIs)	1.24 (1.21, 1.27) 1.17 (1.14, 1.21) 1.24 (1.21, 1.27) 1.41 (1.38, 1.46)	[12,39,40]
	Alcohol consumption		1.44 (1.40-1.47)	
DIABETES MELLITUS	First time HE in pts. with cirrhosis and ascites		1.86 (1.20-2.87)	[41,42]
EPILEPSY	HE grade I-IV HE grade II-IV		2.12 (0.99-4.55) 3.83 (1.65-8.87)	[45]
SARCOPENIA				[46]
OLDER AGE				[47]

Figure 1: Model for the Pathogenesis of Hepatic Encephalopathy

HE-precipitating factors trigger astrocyte swelling and oxidative/nitrosative stress in astrocytes, which mutually enhance each other. This results in covalent modification of proteins and RNA, senescence and changes in cerebral gene expression, which lead to astrocytic/neuronal dysfunction, impaired synaptic plasticity and disturbance of oscillatory networks in the brain (#see also Table 1), which finally account for HE symptoms. § Genes with altered expression in *post mortem* brain samples from patients with liver cirrhosis with HE relate to oxidative stress, inflammatory pathways, microglia activation, receptor signalling, cell proliferation, apoptosis and others[97]. *see also Fig. 3. Redrawn from [70]. RONS, reactive oxygen and nitrogen species.

Figure 2: Molecular mechanisms and consequences of oxidative/nitrosative stress in astrocytes in HE

HE-precipitating factors such as ammonia and inflammatory cyctokines trigger an NMDA receptor-dependent elevation of the intracellular calcium concentration in astrocytes[73–75,86]. This leads to the formation of a variety of reactive oxygen and nitrogen species which modify proteins and RNA species, alter gene expression and signaling and induce senescence. NOX4 and HO1 play an important role in generating oxidative stress. Both enzymes are upregulated by ammonia in a glutamine synthesis-dependent manner involving O-GlcNAcylation-dependent downregulation of miR326-3p [69,108]. For details see Fig. 3 and text. Redrawn from [69].

Potential sites of inhibition: (1) NMDA receptor antagonist (*e.g.* MK801 [73–75,86,122]); (2) Cyclooxygenase inhibitors (*e.g.* acetylic salicylic acid[89,94]); (3) Nitric oxide synthase inhibitors (*e.g.* NG-monomethyl-L-arginine [73–75,86,91,93,102]); (4) Antioxidants/ RNOS scavengers (*e.g.* epigallocatechine gallate[91,122]; (5) Uric acid [86]; (6) NAPDH oxidase inhibitors (*e.g.*apocynine[90,107,108,118,121]); (7) Heme oxygenase 1 inhibitors (*e.g.* zinc protoporphyrin IX[107,108]) ; (8): Iron chelators (*e.g.* Bipyridine[108])

It should be noted that pharmacological interventions at these potential sites of inhibition have not yet been tested clinically and evaluated and are discussed here on a pure theoretical basis.

cPLA2, cytosolic phospholipase A2; GADD45α; growth arrest and DNA damage inducible 45 α; GLAST, glutamate/aspartate transporter; GLS, glutaminase: Glu, glutamate; HO1, heme oxygenase 1; iNOS, inducible nitric oxide synthase; MRP4, multi drug resistance protein 4; MT, metallothionein; MTF1, metal response element-binding transcription factor 1; NKCC1, Na^+-K^+-$2Cl^-$ cotransporter 1; NMDR, N-methyl-D-aspartic acid receptor; nNOS, neuronal-type nitric oxide synthase; NO, nitric oxide; NOX, NADPH oxidase; p21, cyclin-dependent kinase inhibitor 1; p53, tumor suppressor protein p53; PBR, peripheral-type benzodiazepine receptor; PPARα, peroxisome proliferator-induced receptor α; SOD, superoxide dismutase; Sp1, specificity protein 1; TGR5, G protein-coupled bile acid receptor 1

Figure 3: The ammonia-induced glutamine formation triggers oxidative stress in astrocytes through protein *O*-GlcNAcylation. In astrocytes, ammonia is utilized by glutamine synthetase (GS) to form glutamine, which is a rate-limiting substrate for the synthesis of activated N-acetyl-D-glucosamine (UDP-GlcNAc) within the hexosamine biosynthetic pathway. UDP-GlcNAc is a substrate of *O*-GlcNAc-transferase (OGT) which attaches GlcNAc moieties on serine or threonine residues in selected proteins. This inhibits

the transcription of the heme oxygenase 1 (HO1) and NADPH oxidase 4 (Nox4) mRNA-repressing micro-RNA 326-3p. The resulting upregulation of HO1 and Nox4 proteins elevates intracellular levels of free ferrous iron and H_2O_2, respectively, and thereby triggers the formation of hydroxyl radicals (OH*) in the Fenton reaction. Consequences of the enhanced OH* formation are RNA oxidation and astrocyte senescence. Adapted from[108].

Figure 4: **Steps involved in the process by which liver damage leads to cognitive and motor impairment in MHE and HE.**

As shown in Figure 1, hyperammonemia and inflammatory factors induce astrocytic and neuronal dysfunction which alters synaptic plasticity and oscillatory networks leading to the neurological symptoms in HE. Some additional details of the process by which liver damage leads to these neurological symptoms are the following: **(a)** Patients with liver cirrhosis show liver damage and inflammation, chronic hyperammonemia and altered microbiota. **(b)** Each of these factors per se (liver inflammation, chronic hyperammonemia and changes in the microbiome) is enough to induce peripheral inflammation, changes in the immunophenotype and in the cargo of extracellular vesicles. The features of these changes are different for each factor. **(c)** These peripheral changes are transmitted to the brain by different mechanisms. **(d)** This results in induction of neuroinflammation in different brain areas, which alters neurotransmission and neuronal connectivity. **(e)** Chronic hyperammonemia per se may also alter neurotransmission and induce neuroinflammation. **(f)** Altered neurotransmission leads to impairment of cognitive and motor function.
The characterization of the molecular mechanisms involved in each of the above steps would allow identifying therapeutic targets on which to act to reverse the cognitive and motor alterations in MHE and HE.

Figure 5 : Classification of HE severity.

According to the Westhaven criteria (WHC)[183] hepatic encephalopathy (HE) is classified into 4 stages (HE 1-4) in addition to minimal HE (MHE), which only shows abnormalities in psychometric testings. For a more detailed description of WHC see [3,182]. The covert/overt classification summarizes MHE and HE 1 as covert and HE 2-4 as overt HE, whereby the presence of asterixis (flapping tremor) defines overt HE. The low grade/high grade classification defines low grade HE as HE forms that do not require hospitalization and describes severity within the low grade HE range by means of critical flicker frequency (CFF)

or psychometric hepatic encephalopathy score (PHES) test results. High grade HE corresponds to patients requiring hospitalization and are further characterized by the Glasgow coma scale.

Figure 6: Algorithms for assessment of HE and treatment in (A) stable out-patients and (B) hospitalized patients with cirrhosis

Box 1: Pathophysiological changes in HE at the system level.

Primary motor cortex:

- Cortico-muscular coherence is slowed in HE in parallel with a slowed critical flicker frequeny (CFF)[167]
- slowed motor performance[83]
- reduced GABAergic tone in HE [159]

Primary somatosensory cortex:

- slowed stimulus related alpha band activity HE in parallel to the slowed CFF [170]
- impaired processing of temporal tactile stimuli [171]
- impaired thermal perception[347]

Occipital cortex:

- impaired temporal processing of visual stimuli reflected by a slowed CFF [203]
- slowed spontaneous M/EEG activity in the alpha-band and oscillatory activity [174,348]
- slowed attention related-oscillatory activity in the gamma band [168]
- decreased levels of GABA[349]
- increased ammonia levels [350]

Cerebellum:

- less cerebellar inhibition in HE [158]

- increased ammonia levels[350]

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