Review Article
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Hepatic Encephalopathy: An Overview of Basic Concepts and Mechanisms

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ABSTRACT

The liver failure induced encephalopathy is commonly referred as hepatic encephalopathy (HE). For many decades, scientists have tried to describe the symptoms of this disorder revealing a spectrum with different types of HE. Studies on the mechanisms underlying the pathogenesis of HE have implicated several factors, mainly neurotoxins, including ammonia, manganese, in addition to changes in various physiological factors, especially an imbalance between true and false neurotransmitters, as well as the involvement of various pro-inflammatory mediators. Such changes impact on the brain, which promote disorders in glia and neurons. This review focuses on the most relevant basic pathophysiologic mechanisms associated with HE.

Keywords: *Hepatic Encephalopathy, Pathophysiology, Liver Failure, Ammonia*

Introduction

Hepatic encephalopathy (HE) refers to central nervous system (CNS) disturbances arising from both acute and chronic hepatic failure as well as from extrahepatic origins (e.g., Porta-caval anastomosis) (Funakoshi and Blanc, 2013). The prevalence of HE in a population of cirrhotic patients is 60 to 80% in a subclinical form (MHE: Minimal Hepatic Encephalopathy) and 30 to 45% in a clinical form (CHE: Clinical Hepatic Encephalopathy). Different types of liver failure have been described and, accordingly, different forms of HE evolve depending on the neurological alterations involved. An important is its complexity, and scientists have attempted to establish a common and consensual definition of the term "hepatic encephalopathy", as well as an appropriate classification of its pathological basis. At the 11th World Congress of Gastroenterology in Vienna (1998), a consensus on the exact definition and etiological

classification of HE was determined (Butterworth *et al*., 2009). The definition of HE was henceforth "a spectrum of neuropsychiatric abnormalities in patients with hepatic insufficiency following the exclusion of other disorders of the central nervous system"(Ferenci *et al*., 2002).

The pathophysiology of HE involves several endogenous, as well as exogenous factors. Therefore, it has been challenging to decipher mechanisms involved in this disorder. Since the time of Nencki and Pavlov's works (Hahn *et al*., 1893), the implication of ammonia pathogenesis has revealed motor disorders associated with HE. A wide range of HE patients exhibit profound motor disorders having clinical features of Parkinson's disease, including, tremor muscle rigidity and akinesia (Butz *et al*., 2010). These patients showed an increase in T1-weighted Imaging, and an accumulation of Manganese (Mn) in the basal ganglia, particularly the globus pallidus (Hermann *et al*., 2018). Such data led scientist to establish a solid link with this heavy metal in the pathophysiology of HE (Pujol *et al*., 1993).

The implication of neurotoxins, especially ammonia and Mn in HE, emphasizes their influence on glial and neuronal cells. Several cellular and molecular studies have reported the involvement of ammonia and Mn in "astrocytes swelling" known to occur ubiquitously in the brain of chronic forms of HE. Such gliopathy is often referred to as Alzheimer's type II astrocytosis (Hazell *et al*., 2006). The consequences of such an effect are directly linked to glutamatergic uptake and neurotransmission. Rather than the effect of neurotoxins, the impact of liver failure, on brain implicates different pathways that interferes not only with glutamate neurotransmission, but also implicates dysfunctions in catecholaminergic neurotransmitters, as it decreases their efficiency through the production of false neurotransmitters (Palomero-Gallagher and Zilles, 2013).

This review focuses on basic mechanisms underlying the pathogenesis of HE; the implication of ammonia and Mn; as well as the impact of liver failure on true and false neurotransmitters. We also review the impact of HE on different neuronal and glial functions.

History, Symptomatology and Classification of HE

The history of HE goes back over two /millennia to the Age of Pericles (Classical Greece) when Hippocrates identified for the first time the neuropsychological characteristics of HE in a patient suffering from acute liver failure (Summerskill *et al*., 1956). Such association had to wait until the 18th century to be evoked again in 1761, when Morgagni described the mental dysfunction in cirrhotic patients (Morgan *et al*., 1989). As the practice of surgical training had advanced in the research fields, Eck (1877) described, in a pioneering work, the first surgically constructed portocaval shunt in dogs carrying blood from the portal vein to the inferior vena cava. Subsequently, feeding operated dogs with meat induced anorexia,

loss of coordination, and stupor, resulting in coma and death (Nencki *et al*., 1895); a reaction which has been named the "meat intoxication syndrome." Such neurotoxic related behavior was first associated to ammonia in Pavlov's laboratory (Hahn *et al*., 1893). Since then, seminal works were carried out to increase our understanding of the liver's fundamental role in the central and peripheral levels of ammonia.

To better understand the associated neuropsychiatric manifestations to HE, Sherlock and coworkers developed a comprehensive study in 1954 describing the clinical manifestations of 18 patients with liver injury and neurological signs (Sherlock *et al*., 1954). All patients, showed a disturbance of consciousness accompanied by a reduction in facial expression, motility disorder, discourse, visual perception, spatial disorientation and a predominance of visual hallucinations. All of these symptoms were associated with changes in personality, even at early stages of the disease (Ferenci *et al*., 2002). Subsequently, neurologists attempted to adopt a classification of HE types based on their etiologies and associated symptoms.

In the late 1990s, despite the earlier descriptions of HE syndrome, the nomenclature, diagnosis and quantification of the neuropsychiatric abnormalities in liver disease were on hold until the commission of Vienna in 1998. At this meeting, held as part of the 11th International Congress of Gastroenterology. From the time of their meeting, scientists have tried to adopt a consensual classification of HE based on their etiologies and associated symptoms. The last class of HE (class C), was subdivided into three subtypes (Fig. 1):

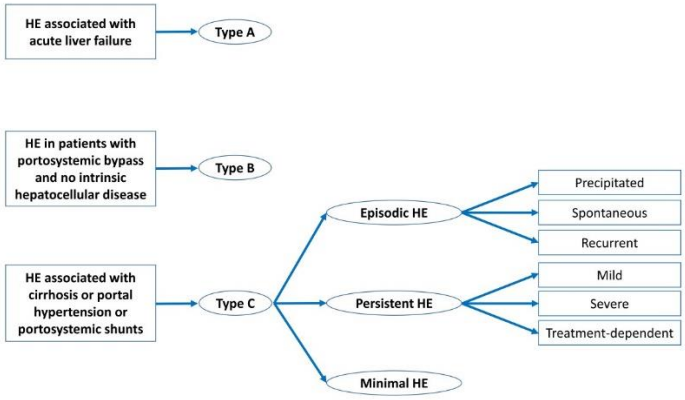

Figure 1: Classification of hepatic encephalopathy (HE) types as proposed by the "Working Party" at the 11th World Congress of Gastroenterology, 1998, Vienna, Austria (Prakash and Mullen, 2010).

a-Episodic HE: Occurring episodically and required hospitalization.

b-Precipitated HE: Induced by one of the following factors (Munoz, 2008).

Dehydration, Gastrointestinal haemorrhage; Certain spontaneous bacterial infections (peritoneal, urinary, dermal, or pulmonary); Constipation; Excessive protein intake, etc, Drugs acting on the central nervous system (neuroleptics), Hypokalemia renal insufficiency, urinary obstruction, hyponatremia, surgery, portosystemic trans-jugular shunt, acute hepatitis, drugs inducing hepatic insufficiency, hepatocellular carcinoma, terminal liver injury.

b-1-Spontaneous HE: when the precipitating factor is not determined.

b-2-Recurrent HE: when two episodes of HE occur within one year.

b-3-Persistent HE: cognitive deficit with a negative impact on social and professional abilities of the patient, along with a persistence of non-cognitive abnormalities (extrapyramidal syndrome and sleep disorders). It is further subdivided into:

- **Mild HE** (grade 1),
- **Severe HE** (grade 2 to 4), depending on the degree of impairment of the disorder.

c-Treatment-dependent HE: when symptoms develop rapidly after medication withdrawal.

d-Minimal: (Sub-clinical) showing no recognizable clinical symptoms of cerebral dysfunction (Ferenci and al., 2002).

Clinical Features of HE

The complexity of brain function partly explains the difficulty of its examination. Hence, several diagnostic systems have been proposed to fully cover the symptoms of HE. However, according to the Vienna Conference, it is better to use simple methods for HE diagnosis. Several simple neurological scales and neuropsychological tests have been developed; an example is presented below (Table 1).

Table 1: The clinical stages of EH.

Stages (grade of EH)	Characteristics
0	No anomaly detected
1	Shortening of attention span, alteration of calculation skills (addition, subtraction), mild euphoria and anxiety
2	Lethargy, apathy, temporal disorientation, personality change, inappropriate behavior
3	Drowsiness, semi-stupor, maintaining responses to stimuli, confusion on awakening, gross disorientation.
4	Coma, absent or reduced response to stimuli, non-testable mental state.

Pathogenesis of HE

For more than fifty years, scientists and clinicians around the world have studied the pathogenesis of HE. Despite the progress accomplished for the understanding of HE pathogenesis, a full comprehension of the underlying mechanism has not yet been established. However, several hypotheses have been proposed to dissect the mechanism of such a pathology, and each of them draws its proof from

innumerable experimental and clinical studies. We describe below the most critical hypotheses:

Ammonia Hypothesis

Ammonia (NH3) is a small water-soluble molecule generated in the digestive tract from the breakdown of food's nitrogen components by deamination of glutamine and degradation of urea by urease of gut bacteria (Clemmesen *et al*., 1999). From the portal system, this molecule reaches the liver where it transforms into other metabolites. The hepatic metabolism of ammonia shows a regio-specific heterogeneity; at the level of the periportal hepatocytes, NH3, is incorporated in glutamine, which is then converted into urea and subsequently eliminated in the kidneys along with urine. At the perivenous level, NH3 is associated with either glutamate by glutamine synthase to form glutamine, or with oxiacids (oxaloacetate or 2-oxoglutarate) to form aspartate and glutamate (Häussinger *et al*., 2000). However, in all cases of hepatic insufficiency, this function is impaired either by the inability of hepatocytes to metabolize ammonia, or by its passage into the circulation outside the liver as a result of collateral bypasses which deprive the liver from its purifying function. As a result, ammonia accumulates in the blood stream (Clemmesen *et al*., 1999; Bengtsson *et al*., 1991).

The first scientific evidence of such postulate, holding their drafts of previous works of Eck (1877), who described for the first time the effects of a meat-based diet in dogs undergoing porto-caval anastomosis. The main observed symptoms, were a decrease in motor coordination, stupor and coma, suggesting that the nitrogen metabolite, derived from meat were the main causal factor of this disorder called "meat intoxication syndrome."

Moreover, this neurotoxin could easily cross a deficient blood-brain barrier (BBB) into the CNS parenchyma (Larsen *et al*., 2001) where it will be highly concentrated. Indeed, an increase in brain ammonia levels, ranging from 0.05-0.1mM in normal animals, to 1-5mM had been reported in animal models of acute and chronic HE (Mousseau *et al*., 1997; Vogels *et al*., 1997). Other studies have shown that ammonia is involved in the pathogenesis of HE. Thus, in 1991, Lockwood *et al*., by means of radiolabeled nitrogen isotopes (13NH3) in Positron Emission Tomography (PET) imaging, demonstrated that the cerebral absorption of ammonia in patients with HE is greater than in normal cases, suggesting the likely implication of NH3 in the pathogenesis of HE (Lockwood *et al*., 1991). Ammonia impairs neuronal function by various direct and indirect mechanisms (Michalak *et al*., 2001) and has also been reported to inhibit excitatory postsynaptic neurotransmission in both the brain and spinal cord (Norenberg, 1996). One report cites a possible action on neuron-astrocyte complex, either by inhibiting glutamate reuptake or by affecting post-synaptic glutamate receptors (Mousseau *et al*., 1993).

In experimental models of acute liver failure, brain blood flow of ammonia exceeds 45-fold the normal level (Dejong *et al*., 1992). Astrocytes ensure a nutritional and a neuronal support functions, and detoxification of nervous tissue from these neurotoxins by the amination of glutamate into glutamine. Accordingly, glutamine accumulates in astrocytes, leading to an increase in intracellular osmotic pressure and astrocyte swelling (Häussinger *et al*., 2000).

Moreover, the increment of ammonia level crossing the BBB has been shown to modulate both excitatory and inhibitory neurotransmissions (Szerb and Butterworth, 1992). In chronic HE patients, cerebral glutamate is decreased as a result of loss of specific neuronal glutamate transporter (Glu-T) affinity to its ligand (glutamate), thereby reducing neuro-excitation (Norenberg, 1996). Moreover, astrocyte swelling is compensated by the release of osmolytes such as taurine and myoinositol in extracellular space (Ferenci *et al*., 2002). Such adaptive response may elicit a depletion of astrocytic myoinositol which has been linked to a sudden and profound development of HE symptoms (Shawcross *et al*., 2004) (Fig. 2).

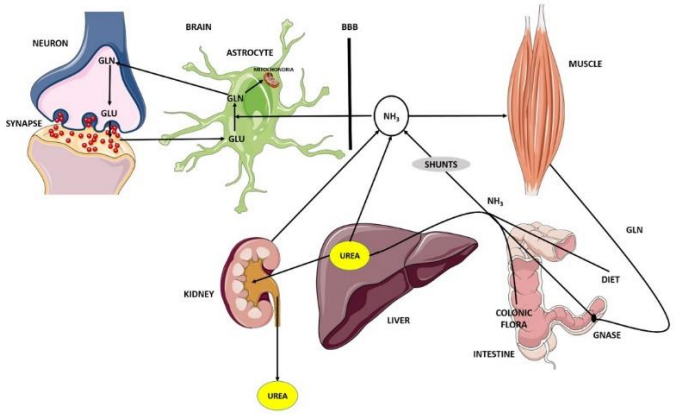

Figure 2: Inter-organ ammonia trafficking and metabolism. Ammonia is generated in the gut from nitrogenous compounds from the diet, deamination of glutamine by glutaminase; and the metabolism of nitrogenous substances by the gut bacteria. In normal circumstances, most of the ammonia is metabolized to urea in the liver. Portal-systemic shunts and liver failure cause a rise in blood ammonia level that may affect brain function by inducing disturbances in astrocytes which may impair mitochondria and the glutamate-glutamine trafficking between neurons and astrocytes. Skeletal muscle is capable of decreasing blood ammonia by metabolizing ammonia to glutamine. The kidney also has an important role in determining levels of blood ammonia by excreting urea in the urine and by generating ammonia. NH3, ammonia; GLU, glutamate; GLN, glutamine; GNASE, glutaminase; BBB, blood-brain barrier.

Ammonia is generated in the gut from dietary nitrogen components, deamination of glutamine by glutaminase, and the metabolism of nitrogenous substances by intestinal flora. Under normal conditions, most of the ammonia is metabolized to urea in the liver. Portosystemic shunting and hepatic insufficiency cause further elevation of circulating ammonia levels that could alter nerve functions directly or

indirectly via an impairment of the astrocytic function, thereby altering mitochondria and the trafficking of glutamate-glutamine between neurons and astrocytes. Skeletal muscles are able to reduce the level of circulating ammonia by metabolizing it to glutamine. Kidneys also determine circulating levels of ammonia either by excretion of urea or by the genesis of ammonia (Córdoba-Aguilar, 2008) (Fig. 2).

Otherwise, previous studies showed an inter-individual variation in serum ammonia levels which implies failures in the renal function, presence or absence of sarcopenia, and diet such factors lead to a high degree of variability (Ghabril *et al*., 2013; Vierling *et al*., 2016). Besides, circulating ammonia levels which are found to be higher in cirrhotic patients with a history of HE, are not well correlated with the severity of HE. Hence, even in the absence of neurological abnormalities, some patients exhibit an elevated ammonia levels (Ong *et al*., 2003). Ammonia as a neurotoxic agent implies its ability to cross the blood-brain barrier via the arterial vascularization, therefore, venous ammonia levels, generally the most commonly measured, may not be considered as the concentration of ammonia which exert its neurotoxic effects (Nicolao *et al*., 2003).

The Manganese Hypothesis

Manganese (Mn) is a neurotoxin that preferentially trends to deposit at the level of the basal ganglia, especially in the globus pallidus. MRI studies showed a hyperintensity signal on T1-weight images of the globus pallidus in more than 80% in cirrhotic patients (Fig. 3) (Mullen and Jones, 1996; Rose *et al*., 1999). Moreover, the signal intensity correlated well with the presence of extrapyramidal symptoms (Kulisevsky *et al*., 1992; Spahr *et al*., 1996). Noteworthy, this phenomenon has also been shown to diminish hepatic function (Aggarwal *et al*., 2006; Naegele *et al*., 2000). Mn is also suspected of causing changes in astrocytes of the basal ganglia, thus promoting the formation of Alzheimer's type II astrocytosis. The deposition of Mn in these areas may also explain the parkinsonian symptoms (resting tremor, muscle rigidity, mild akinesia, slow movements) observed in some patients with HE (Krieger *et al*., 1995). An angiographic study in cirrhotic patients with high concentrations of Mn in the pallidum showed the presence of large portosystemic collateral vessels originating from the mesenteric vein (Inoue *et al*., 1991). A study of autopsied samples from the globus pallidus of cirrhotic patients who died with hepatic coma showed an elevated levels of Mn (Krieger *et al*., 1995). Prolonged exposure to Mn induces extrapyramidal symptoms, while repetitive Mn administration in non-human primates (monkeys) results in hyperintensity of the T1-weighted signal of the globus pallidus (Newland *et al*., 1989). A recent study in cirrhotic patients has shown that exposure to Mn reduces glutamate uptake by cultured astrocytes (Hazell and Norenberg, 1997), and increases expression of the glycolytic enzyme glyceraldehyde-3- phosphate dehydrogenase (Hazell and Butterworth, 1999), suggesting that Mn affects both the glutamatergic system and cerebral energy metabolism in HE.

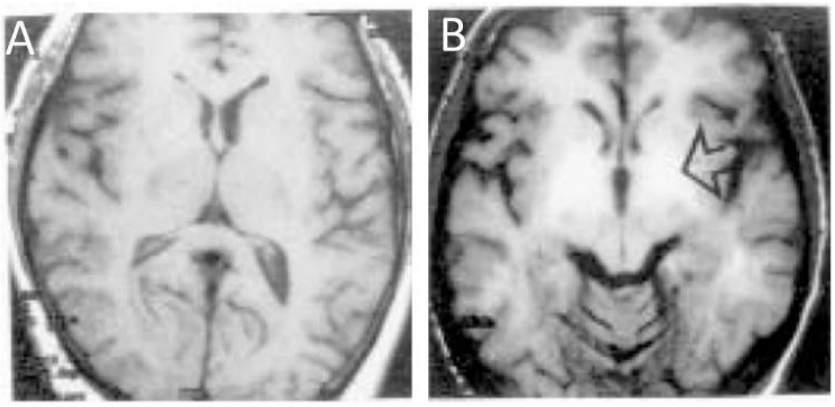

Figure 3: MRI of a healthy control (A) and an alcoholic cirrhotic patient of the same age (B). the arrow show abnormally bilateral hyperintensity signals in the globus pallidus. Such abnormality is due to Mn deposits (modified according to (Lockwood *et al*., 1997).

The ability of astrocytes to scavenge Mn suggests that its accumulation by these cells could be the cause of Alzheimer's type II astrocytosis. In non-human primates, ammonia poisoning induces the appearance of this kind of astrocytic abnormalities (Pentschew *et al*., 1963), suggesting that Mn, in addition to ammonia, contributes to the morphological and functional astrocytic changes characteristic of HE (Pentschew *et al*., 1963) (Fig. 4).

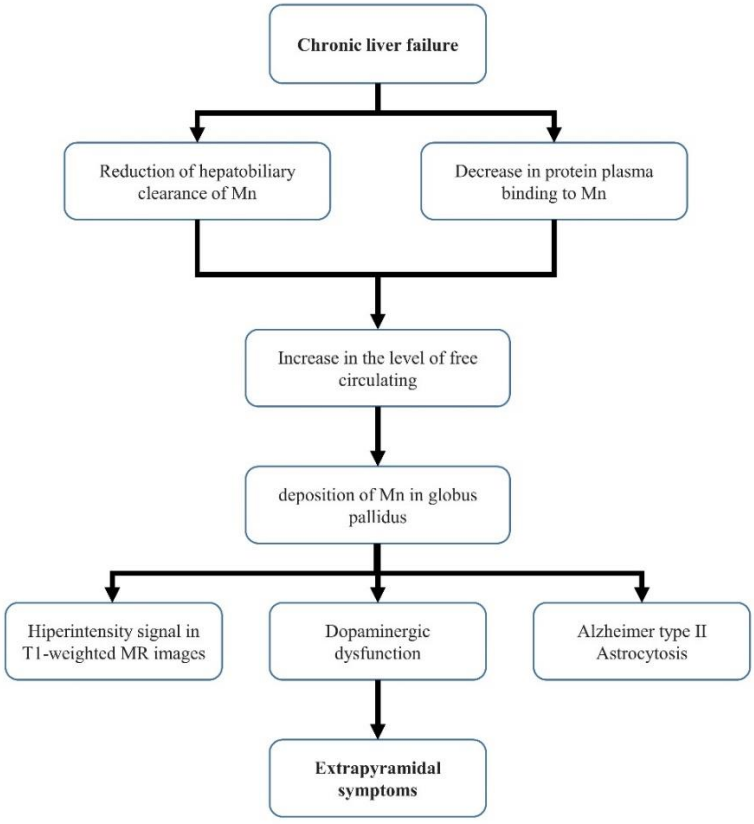

Figure 4: Mechanism of manganese neurotoxicity in chronic HE (modified according to (Prakash and Mullen, 2010).

Hypothesis of Branched-Chain Amino Acids and False Neurotransmitters

Fisher and Baldessarini proposed the hypothesis of false neurotransmitters 49 years ago. They postulated that the neurological disorders that occur in HE stages and in hepatic coma, especially a disorder in catecholaminergic neurotransmission (dopamine, norepinephrine), could be explained by a substitution of true neurotransmitters by false ones, such as octopamine and phenylethanolamine, in both the central and peripheral nervous systems (Fischer and Baldessarini, 1971). Even though these molecules are similar to true neurotransmitters, they have only 1/100 of their potential effect (Table 2), a fact which may importantly lead to a dysregulation on the modulation of neurotransmitters biosynthesis. Noteworthy, an important factor in the control of neurotransmitters biosynthesis is the central concentration of epinephrine and serotonin precursors. These precursors are aromatic amino acids, mainly tyrosine, phenylalanine and tryptophan, in which a positive correlation between their brain and plasmatic concentrations has been observed. During hepatic insufficiency, their levels are elevated as compared to other branched-chain amino acids such as valine, leucine and isoleucine (Orlowski *et al*., 1974). Accordingly, the cerebral flow of these aromatic amino acids is increased, and the synthesis of false neurotransmitters such as octopamine is favored (Buxton *et al*., 1974; Bloch *et al*., 1978). Subsequently, the circulating and central levels of dopamine and norepinephrine are reduced (Dodsworth *et al*., 1974; Rolando *et al*., 2000).

Table 2: Neurotransmitters, their precursors and the corresponding false neurotransmitters.

Neurotransmitter	Precursor	The False Neurotransmitters
Dopamine	Tyrosine	Tyramine
Norepinephrine		Octopamine
Serotonine (5-hydroxytryptamine)	Phenylalanine	Phenethyamine
	Tryptophan	Phenylethanolamine
		Tryptamine

Inflammation

Infection and inflammation are common traits of fulminant acute HE. Infection is documented in at least 80% of patients with acute hepatic failure (AHF) (Rolando *et al*., 2000). It is manifested by an increase in serum proinflammatory cytokines levels, including TNF-α, IL-6 and IL-1β (Keane *et al*., 1996; Muto *et al*., 1988). The production of pro-inflammatory cytokines could be the result of a stimulation of the immune system by the release of modulators by necrotic hepatocytes. Clinical studies have shown that the presence of Systemic Inflammatory Response (SIRS) and/or infection, correlates well with the severity of HE and the increase in intracerebral pressure along with a high mortality rate (Rolando *et al*., 2000; Vaquero *et al*., 2003). Such findings supports the hypothesis of the role of inflammation in the

development of cerebral edema and HE during acute hepatic failure (Jalan and Williams, 2001). Although systemic inflammation has been well established for over a decade, evidence for neuroinflammation in liver failure was not provided until after the publication of a report suggestive of increased production of pro-inflammatory cytokines in the brains of patients with acute hepatic failure (Wright and Jalan, 2007). A significant correlation was observed between arterial cytokine content and intracranial hypertension; cervical cytokine flow was noted, consistent with cervical cytokine production. Subsequently, evidence of the existence of neuroinflammation was reported by (Jiang *et al*., 2009; de França MER and Peixoto, 2020; Butterworth, 2011; Chastre *et al*., 2012; Butterworth, 2013; Jayakumar *et al*., 2015).

Neuropathology of HE

Role of Astroglia

The multitude of histopathological and molecular studies in patients and animal models of chronic and acute hepatic failure with HE, reveal the absence of direct neuronal damage. HE represents gliopathies with morpho-functional changes of glial cells especially astrocytes (Norenberg, 1987; El Hiba *et al*., 2016; El Khiat *et al*., 2019). These abnormalities are referred to as Alzheimer's Type II astrocytosis (Fig. 5), whose the phenotype is characterized by large, pale nuclei and prominent nucleoli along with chromatin marginalization and cytoplasmic enlargement associated with proliferation of cytoplasmic organelles (Norenberg, 1987; Butterworth *et al*., 1987). These abnormalities are found in both the brain's gray and white matter. The number of astrocytes with these abnormalities correlated well with the severity of encephalopathy (Norenberg, 1987; Butterworth *et al*., 1987).

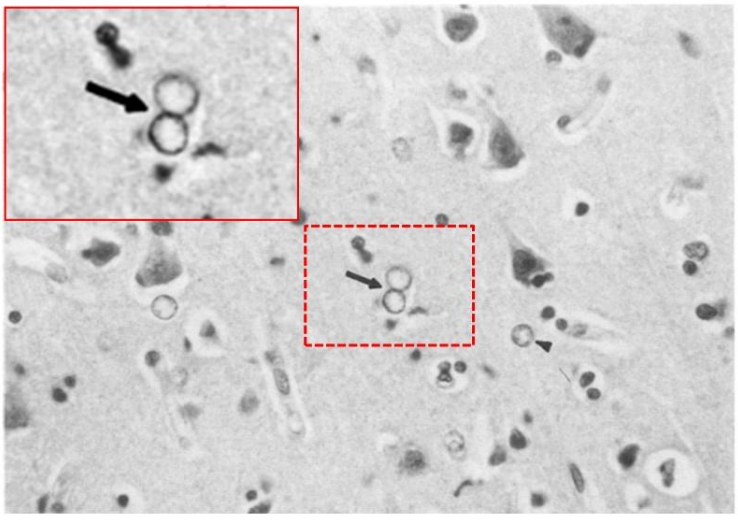

Figure 5: Light micrograph of post-mortem cerebral cortex from a patient dead with HE (Norenberg, 1987). Astrocytes show a prominent nucleus: enlarged, pale, and frequently found in pairs (arrow), a features of glial hyperplasia. Bar = 20 ~µm.

Several hypotheses have been proposed to explain such changes, namely the role of ammonia, which, once in the nervous tissue, stimulates astrocytes to reduce its concentration by incorporating it into glutamate to form glutamine. This induces an accumulation of the latter in astrocytes and an increased intracellular osmotic pressure, resulting in astrocyte swelling, a characteristic feature of Alzheimer's type II astrocytes (Häussinger *et al*., 2000).

Manganese is also considered as a possible cause of these anomalies. The histopathological study of brain samples from cirrhotic patients who died with hepatic coma, exhibiting a hyperintensity in the MRI of the globus pallidus, revealed the presence of Alzheimer's type II astrocytes (Kulisevsky *et al*., 1992). In addition, exposure to Mn reduces glutamate uptake by cultured astrocytes (Hazell and Norenberg, 1997). The large capacity of astrocytes to scavenge Mn (Aschner *et al*., 1992) suggests that its accumulation by these cells could be the cause of the development of Alzheimer's type II astrocytosis. Thus, in non-human primates, Mn intoxication induces the presence of Alzheimer's type II astrocytes (Pentschew *et al*., 1963).

HE And the Catecholaminergic System

HE and Dopamine

Clinical examinations and experimental studies in animal models and patients with HE have shown the presence of neuromuscular disorders such as tremor and muscle stiffness. Such symptoms belong to the extrapyramidal symptoms commonly observed in Parkinson's disease. Thus, it has been suggested that the extrapyramidal signs observed during episodes of HE could be the consequences of impaired dopaminergic neurotransmission (Vaquero *et al*., 2003; Blei and Cordoba, 2002). Such impairment is related to dopaminergic metabolism alteration rather than the levels of the neurotransmitter. Strikingly. the activity of monoamine oxidase (MAO) increases in the frontal cortex and the caudate nucleus in HE/cirrhotic patients (Rao *et al*., 1993). Even in animal models, alteration of aromatic amino acid metabolism cause changes in dopamine, DOPAC, and HVA levels (Murakami *et al*., 1992). Tyrosine hydroxylase level was also drastically decreased in the substantia nigra and the striatal outputs (El Hiba *et al*., 2012).

HE and Norepinephrine

In addition to dopamine, another catecholamine also appear to be involved in the pathophysiology of HE. Norepinephrine (NA) is a catecholamine that is a part of the DA biosynthetic pathway. Indeed, hepatectomy (Hadesman *et al*., 1995), liver devascularization (Murakami *et al*., 1992), as well as thioacetamide-induced hepatic impairment (Yurdaydin *et al*., 1990) in rats, all induce a

decrease in NE levels. In addition, hepatic coma is associated with increased extracellular NE and decreased density of NE binding sites: α1 and β1 of the frontal cortex and thalamus (Michalak *et al*., 1998). In addition, α1 and α2 NEergic receptors are overexpressed in the cerebral cortex of the porto-caval shunted rat (Song *et al*., 2002). These data support the possible involvement of NE in the pathophysiology of several neuropsychiatric disorders encountered in HE.

HE and Glutamate

Glutamate is an amino acid and an excitatory neurotransmitter in the central nervous system involved in the pathophysiology of HE. In patients with HE, glutamine levels are increased (Butterworth *et al*., 1987) and appear to imply a role of ammonia. The exposure of astrocytes to millimolar concentrations of ammonia, results in the reduced expression of glutamine synthetase (Girard *et al*., 1993), along with a decrease in the efficiency of the GLT-1 glutamate astrocyte transporter (Knecht *et al*., 1997), an essential factor in the inactivation of glutamate in the synaptic cleft (Schmidt *et al*., 1990). Several in vivo and in vitro studies have shown an alteration of cerebral glutamate transport in chronic and acute liver failure (Butterworth, 1993). In addition, the release of glutamate from the cerebral cortex is increased in port-caval shunted animals (Hori *et al*., 1997). Based on these studies, it has been suggested that HE is the possible outcome of a disruption of neuro-astrocytic glutamate trafficking (Butterworth, 1993). Noteworthy, decreased ability of astrocytes to recapture glutamate from nerve endings when exposed to pathological concentrations of ammonia has been identified (Norenberg, 1996). Molecular biology has also revealed a depletion of the expression level of the astrocytic GLT1 glutamate transporter proteins and genes in animal models of fulminant HE (Knecht *et al*., 1997).

HE and Serotonin

Several neuropsychiatric symptoms related to HE such as sleep disorders have been attributed to possible impairment of serotonergic neurotransmission. It has been shown that the concentration of the 5-HT precursor, L-tryptophan, is increased in cerebrospinal fluid (CSF) of cirrhotic patients with hepatic coma (Bergeron *et al*., 1990). Likewise, levels of the 5-HT metabolite; 5-hydroxyindolacetic acid (5-HIAA), have been shown to be increased either in the CSF or in brain tissue of patients (Bergeron *et al*., 1990) as well as animal models with severe encephalopathy secondary to chronic liver failure (Bengtsson *et al*., 1991). While the catabolic enzyme MAO-A, the activity is elevated in the brain of patients who died with in hepatic coma (Rao *et al*., 1993). However, in rats with bile duct ligation (HE type C), 5-HT levels seems to be reduced at the cirrhotic stage (El Hiba *et al*., 2012). Altogether, these data appear to indicate a synaptic 5-HT deficiency due to chronic liver failure. However, microdialysis studies in rats with either acute (Michalak *et al*., 1998) or chronic (Bergqvist *et al*., 1997) hepatic failures showed no change in

extracellular levels of 5-HT, more investigations regarding this aspect are warranted.

HE and GABA

Gamma Amino-butyric Acid (GABA) is the most common inhibitory neurotransmitters in the central nervous system. It is found in about 30% of synapses and has inhibitory properties on neuronal activity. The concept of GABA involvement in the pathophysiology of HE is relatively recent. It was introduced in the 1980s, suggesting that an increase in inhibitory neurotransmission of GABA is likely the cause of the impaired motor function and reduced levels of consciousness, which are characteristics of HE (Basile *et al*., 1991).

It has been postulated that peripheral GABA levels resulting from the bacterial activity of the intestinal flora, is not being eliminated by the defective liver, crosses the deficient blood-brain barrier and thereby participates in neuronal inhibition (Schafer and Jones, 1982). This hypothesis is well supported in animal models of hepatotoxicity, especially by galactosamine (Schafer *et al*., 1983). However, in humans, unlike animals, no alteration in levels of GABA nor its related enzymes were found during HE episodes (Butterworth *et al*., 1987).

Alternatively, recent studies have shown that hyperammonemia modulates the increase in GABAergic tone during hepatic failure (Schafer and Jones, 1982). Ammonia inhibits the astrocytic reuptake of GABA, increases Cl- currents by a direct action on GABA-A receptors and potentiates the binding of endogenous benzodiazepine agonists to the GABA-A receptors. This finding led investigators to assess the possible meliorative potential of benzodiazepine receptor antagonists such as Flumazenil in patients with chronic HE. Noteworthy, there was a reduction in GABAergic tone in some patients with stage IV HE (Pomierlayrargue *et al*., 1992).

Flumazenil likely acts by displacing endogenous agonists from their benzodiazepine receptor-binding site. However, other clinical studies have shown no beneficial effect on improvement of HE symptoms and patient survival (Goulenok *et al*., 2002). This suggests that the effect of this drug is not related to the inhibition of endogenous benzodiazepine receptor agonists and that other factors (ammonia and Mn) are likely involved.

Conclusion

While recent studies have led to the improvement of our understanding of HE pathophysiology, multiple aspects of the pathology are still far from being fully established. Despite the ubiquitous involvement of ammonia, in the neuropathology of HE implicating the neuronal as well as the glial

compartments, further elements such as inflammation, GABA, Mn and false neurotransmitters, need to be taken into account. Assembling and an interacting approach of key elements involved in such neuro- and gliopathies of HE is necessary for a better understanding of the mechanisms involved in hepatic encephalopathy.

Conflict of Interest Statement: The authors declare no conflicts of interest.

Author Contributions: AEK: study concept, writing the paper and participated entire work. MA & AD and ARJ: revised the paper. OEH & MN and HG: supervised the study and revised the manuscript.

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