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REVIEW

Dietary approach and gut microbiota modulation for chronic hepatic encephalopathy in cirrhosis

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Abstract

Hepatic encephalopathy (HE) is a common and serious neuropsychiatric complication of cirrhosis, acute liver failure, and porto-systemic shunting. HE largely contributes to the morbidity of patients with liver disease, severely affecting the quality of life of both patients and their relatives and being associated with poor prognosis. Its presentation is largely variable, manifesting with a broad spectrum of cognitive abnormalities ranging from subtle cognitive impairment to coma. The pathogenesis of HE is complex and has historically been linked with hyperammonemia. However, in the last years, it has become evident that the interplay of multiple actors, such as intestinal dysbiosis, gut hyperpermeability, and neuroinflammation, is of crucial importance in its genesis. Therefore, HE can be considered a result of a dysregulated gut-liver-brain axis function, where cognitive impairment can be reversed or prevented by the beneficial effects induced by "gut-centric" therapies, such as non-absorbable disaccharides, non-absorbable antibiotics, probiotics, prebiotics, and fecal microbiota transplantation. In this context dietary modifications, by modulating the intestinal milieu, can also provide significant benefit to cirrhotic patients with HE. This review will provide a comprehensive insight into the mechanisms responsible for gut-liver-brain axis dysregulation leading to HE in cirrhosis. Furthermore, it will explore the currently available therapies and the most promising future treatments for the management of patients with HE, with a special focus on the dietary approach.

Key words: Cirrhosis; Hepatic encephalopathy; Diet therapy; Gut microbiota; Leaky gut; Hyperammonemia; Prebiotics; Probiotics; Gluten-casein free diet; Gut microbiota transplantation

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Core tip: Hepatic encephalopathy (HE) is a serious complication of cirrhosis resulting from a multifactorial impairment of gut-liver-brain axis functioning. Multiple interrelated factors (*e.g.*, intestinal hyperpermeability, dysbiosis, hyperammonemia, inflammation) cooperate in its development. "Gut-centric" therapies, including non-absorbable disaccharides, antibiotics, prebiotics, probiotics, and fecal microbiota transplantation have been successfully employed to manage HE: pertinent current knowledge will be reviewed. Furthermore, the utility of dietary modifications in this context is increasingly recognized, thus opening a new promising research path. This review sheds light on dietary therapeutic strategies for HE, exploring how they can target the mechanisms underlying gut-liver-brain axis dysregulation.

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INTRODUCTION

Hepatic encephalopathy (HE) is one of the most debilitating complications of liver cirrhosis and represents a relevant cause of hospitalization[1]; it is associated with both direct and indirect costs to health services. HE is a predictor of poor prognosis and severely affects patients' quality of life, often entailing a heavy burden for relatives and caregivers[2]. HE consists of a brain dysfunction caused by liver insufficiency and porto-systemic shunting, and it manifests as a wide spectrum of neurological or psychiatric abnormalities, ranging from subclinical alterations to coma[3].

Based on the variable severity of its manifestations, HE has been arbitrarily classified in five stages, from minimal HE (MHE) to grade IV according to the West-Haven criteria[3]. These stages can be further divided into two categories: overt HE (OHE), including grades II-IV, in which diagnosis can be established through a physical examination detecting evident neurologic and neuropsychiatric abnormalities, and covert HE (CHE), including MHE (no clinical evidence of mental dysfunction but presence of abnormalities in psychometric tests) and grade I according to West-Haven criteria (*i.e.*, a trivial lack of awareness, a discreet psychomotor retardation, or a subtle lack of attention)[4,5]. As per International Society for HE and Nitrogen Metabolism consensus, the presence of disorientation in time or asterixis identifies the onset of OHE[4,6].

Although the variety of clinical presentations and the difficulty in detecting MHE make it hard to quantify the exact prevalence of HE, it is estimated that approximately 30%-40% of patients with cirrhosis will develop OHE during their disease course[7,8], whereas MHE or CHE occur in 20%-80% of patients[9]. Subjects with a previous episode of OHE have a 40% cumulative risk of recurrence at 1 year, and subjects with recurrent OHE have a 40% cumulative risk of another episode within 6 months[1,10].

Although the pathogenesis of this condition has not been fully elucidated yet, progress in research has led to the identification of several potential determinants of HE, among which intestinal dysbiosis, gut permeability alterations, inflammation, and oxidative stress seem to play a key role[11]. In particular, HE can be regarded as a model for impaired gut-liver-brain axis functioning: specific microbiota changes in the gut of cirrhotic patients, along with altered intestinal permeability, have been associated with endotoxemia and bacterial translocation, leading to increased inflammatory response both at a systemic level and in the central nervous system (CNS), which finally induces impaired cognition and favors the onset of HE. Although the mechanisms underlying this gut-brain interplay are far from being fully clarified, the importance of the gut in HE pathogenesis is corroborated by the beneficial effects that gut-centric therapies such as lactulose and lactitol, non-absorbable antibiotics such as rifaximin and neomycin, probiotics, and prebiotics exert on patients' cognitive function[12].

In this context, available data suggest that dietary modifications too might exert relevant conditioning on several factors involved in the gut-liver-brain axis, including gut microbiota, intestinal permeability, and inflammation.

This review will give insight into the mechanisms responsible for gut-liver-brain axis dysregulation that leads to HE development in the context of cirrhosis.

Furthermore, we will explore how the different therapeutic approaches investigated so far are supposed to act in this complex network. A special focus will be given to dietary interventions.

PATHOGENESIS

The pathogenesis of HE is a complex entity in which multiple factors cooperate in determining the functional impairment of neuronal cells[13], as illustrated in Figure 1.

In patients with liver cirrhosis, it is believed that high levels of gut-derived toxins and endogenous neurotoxic substances escape from liver catabolism, due to the impaired detoxifying function of the cirrhotic liver and to the presence of porto-systemic shunts, and that these toxins reach the brain through the blood-brain barrier (BBB). In this context, a number of different factors, including gut dysbiosis and small intestine bacterial overgrowth, leaky intestinal barrier, cirrhosis-related systemic inflammation and neuroinflammation, oxidative stress, nitrogen metabolism, changes in neurotransmission, gamma-amino butyric acid (GABA)ergic or benzodiazepine pathway abnormalities, as well as BBB disturbances, appear to contribute to the development of HE[14-16].

Ammonia and other neurotoxic compounds

Increased blood ammonia is a cornerstone in HE development[17-19]. Ammonia, a by-product of nitrogen metabolism, is derived from gut and kidneys[20]. In the gut, both the small intestine and colon are sources of great amounts of ammonia as a product of the enzyme glutaminase and a large number of urease-producing bacteria.

Ammonia-rich blood normally reaches the liver through the portal vein, where it is detoxified through the urea cycle[21,22]. In patients with portosystemic shunts or liver failure, gut-derived blood bypasses the liver, and the liver itself has impaired capacity for detoxification. As a consequence, nitrogenous waste products accumulate in the systemic circulation. Excess ammonia crosses the BBB and is subsequently absorbed and used by astrocytes to synthesize glutamine; intracellular accumulation of excess glutamine causes osmotic and oxidative stress, mitochondrial dysfunction, and, finally, astrocyte swelling. This can lead to cerebral edema (with the extreme consequences of increased intracranial pressure and brain herniation often seen in acute liver failure) as well as to increased GABAergic activity[21,23].

Apart from the gut, also kidneys, urinary tract, and muscles are involved in nitrogen metabolism and contribute in determining ammonia circulating levels. In this setting, muscle tissue is of particular interest because: 1- sarcopenia is a recognized risk factor for HE, due to the reduced utilization of ammonia for glutamine synthesis in the context of muscular tissue deficiency[24-27]; 2- protein catabolism, which is enhanced in fasting conditions, can contribute to hyper-ammonemia through the release of nitrogen compounds.

Nowadays, the relevance of ammonia *per se* in the pathogenesis of HE has been partially questioned, in light of evidence that ammonia levels in chronic liver failure do not reliably correlate with HE severity[28-30] and the identification of the synergistic role of inflammatory mediators and a number of other potentially neurotoxic compounds, including mercaptans, benzodiazepine-like substances, and indole, a tryptophan derivative that is produced by gut microbes and transformed into oxindole in the brain, where it displays sedative properties[31-34].

Inflammation

Inflammation has been suggested to play a synergistic role in HE pathophysiology, increasing the effect of ammonia and thus partially explaining the weak correlation between ammonia circulating levels and HE severity. Inflammation is both systemic and localized to the CNS[35-37]. At a local level, proinflammatory cytokines are produced by the brain in the presence of ammonia, giving rise to neuroinflammation[21,38].

Decompensated cirrhosis is characterized by a chronic systemic inflammatory state that concurs to the maintenance of characteristic clinical features such as generalized vasodilation and hyperdynamic circulation[21,39]. The genesis of systemic inflammation in cirrhosis is multifactorial: an impaired intestinal permeability caused by portal hypertension allows pathological bacterial translocation from the intestinal lumen to the splanchnic and systemic circulation. Translocated bacteria and bacterial products (pathogen-associated molecular patterns) stimulate the immune response, leading to the release of inflammatory cytokines, causing in turn oxidative stress[40,41].

Systemic inflammatory response syndrome and sepsis are recognized as key players in precipitating and exacerbating HE, possibly by rendering the brain more susceptible to concurrent hyperammonemia[23]. HE patients show high levels of

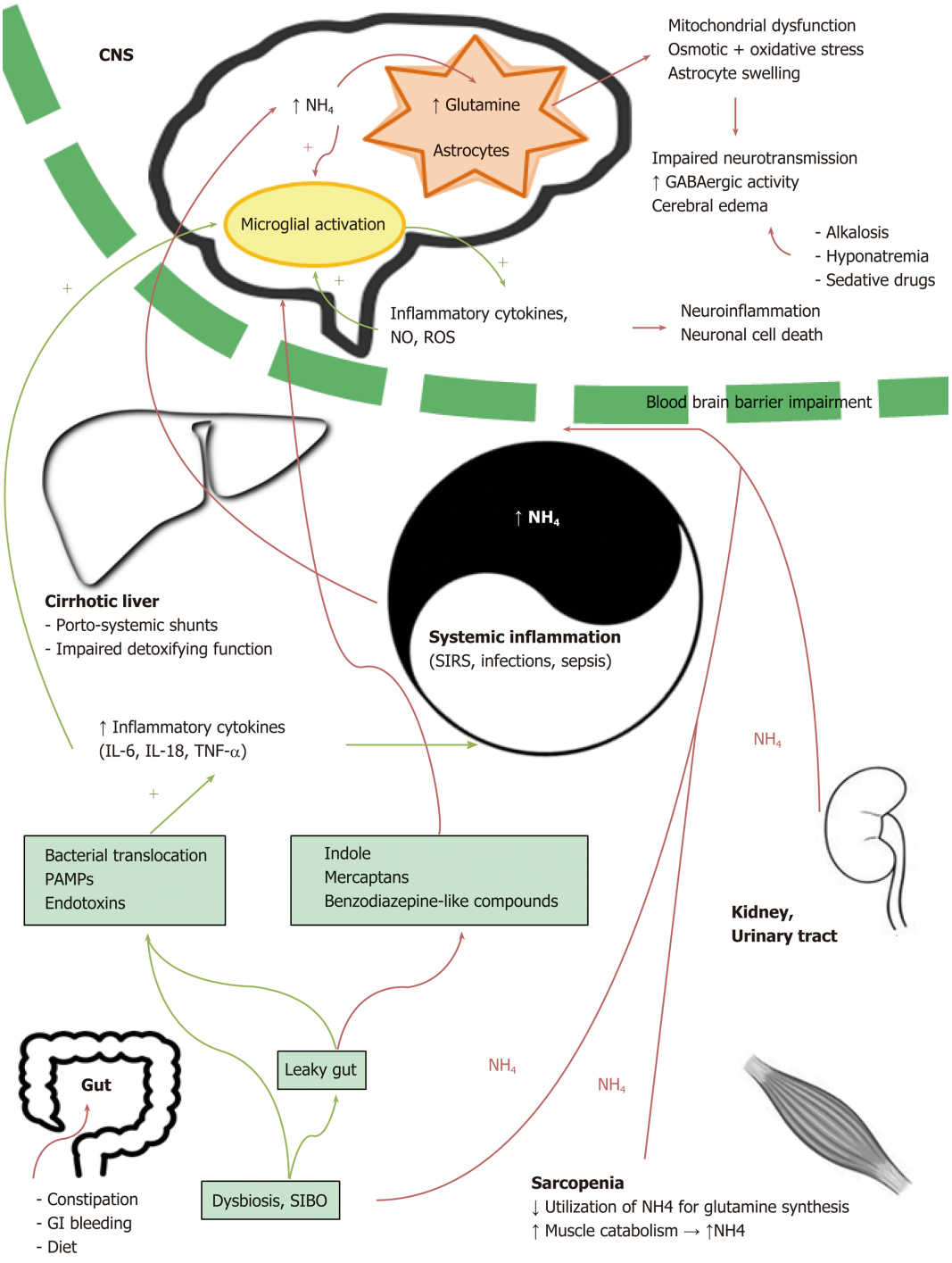

Figure 1 Multifactorial pathogenesis of hepatic encephalopathy. The figure summarizes the pathogenetic mechanisms at all levels of the gut-liver-brain axis underlying the development of hepatic encephalopathy. In this context, the interplay between systemic inflammation and hyperammonemia plays a central role (see text for details). CNS: Central nervous system; GABA: Gamma-aminobutyric acid; GI: Gastrointestinal; IL: Interleukin; NH_4: Ammonia; NO: Nitric oxide; PAMPs: Pathogen associated molecular patterns; ROS: Reactive oxygen species; SIBO: Small intestine bacterial overgrowth; SIRS: Systemic inflammatory response syndrome; TNF-α: Tumor necrosis factor-alpha.

inflammatory cytokines, such as interleukin (IL)-6, IL-18, and tumor necrosis factor alpha (TNF-α).

Tranah *et al*[38] reported that the presence and severity of HE are not associated with ammonia concentration alone but with serum levels of inflammatory cytokines such as TNF-α and IL-6. In another study, induced hyperammonemia in cirrhotic patients resulted in worse neuropsychiatric test scores only when inflammation was present[42].

It is now widely accepted that sepsis can trigger HE in cirrhotic patients by releasing proinflammatory mediators in the context of altered nitrogen metabolism[43,44], thus indicating that systemic inflammation is a critical determinant of the

presence and severity of HE in chronic liver failure[23,45].

Moreover, patients with acute and chronic liver failure are functionally immunosuppressed and prone to infections, which are well-known precipitants of HE. The innate immune response, comprising phagocytic cells such as monocytes and neutrophils, was impaired both in acute liver failure and cirrhosis in different preclinical studies and animal models[43,46,47]. Hyperammonemia itself appears to have a role in worsening immune function. Ammonia-fed rats and cirrhotic patients given amino acid drinks to induce hyperammonemia develop impaired neutrophil phagocytic activity with neutrophils spontaneously producing reactive oxygen species[48].

Hence, on the one hand the aberrant activation of neutrophils contributes to systemic inflammation and bystander damage to host organs, whereas on the other hand their impaired microbicidal capacity predisposes to infections with further worsening of the inflammatory milieu and induction of clinical decompensation of cirrhosis[23,47].

Systemic inflammation can also affect neuroinflammation: proinflammatory cytokines are transported across the BBB from the systemic circulation. However, there is good evidence that inflammatory mediators can also be produced by the brain itself[21].

Microglial cells, which are essentially CNS resident macrophages, can be activated by systemic inflammation and in turn release proinflammatory cytokines. Chronic hyperammonemia is sufficient to induce microglial activation[49], and this activation results in brain-derived proinflammatory cytokines[50], in particular TNF-α, IL-6, and IL-1β[51]. This inflammatory state leads to neuronal death *in vitro* and *in vivo*[52]. In this context, the extent of microglial activation was found to be predictive of the level of HE as well as of the presence of cerebral edema in acute liver failure[53].

Furthermore, BBB contains endothelial cells that can induce the release of proinflammatory mediators when stimulated by systemic inflammation: endothelial cells are provided with TNF-α and IL-1β receptors that convey signals able to induce the synthesis of secondary messengers in the brain, such as nitric oxide and prostanoids[54,55].

Leaky gut and bacterial translocation

The intestinal barrier is a functional unit composed of the intestinal epithelial cells, the immune effectors (immune cells and immunoglobulins), the mucus layer, and the intercellular junctions (tight junctions and gap junctions), which allow selective passage of substances through the paracellular pathway[56]. The paracellular transport regulated by the tight junctions is a dynamic system that can be modulated by several factors, such as neurotransmitters, cytokines, food components, and other signaling molecules such as zonulin, a protein synthesized in the intestinal and liver cells that reversibly increases intestinal permeability[57-59].

Patients with liver cirrhosis exhibit structural and functional changes in the intestinal barrier, the so-called "leaky gut"[60-62], which can lead to increased intestinal permeability to bacteria and their products[63,64]. The impaired expression of tight junction proteins, a common finding in patients with cirrhosis[65,66], is one of the main mechanisms underlying the disruption of the intestinal barrier[67].

Tight junctions are composed by different families of transmembrane proteins, among which occludins, claudins, and junctional adhesion molecules are the most important. The intracellular domains of these proteins interact with cytoplasmic proteins called "zonula occludens", which allow the anchorage of the protein complex to the cytoskeleton[68].

The increased levels of proinflammatory cytokines, particularly TNF-α, as well as other inflammatory mediators were found to be responsible for the decreased expression of occludin and claudin-1 in the intestinal epithelium of cirrhotic patients[69-71]. This downregulation was more significant in the phase of decompensated cirrhosis (Child-Pugh classes B and C)[72-75].

Furthermore, several other factors cooperate in affecting the integrity of the intestinal barrier, such as portal hypertension (by slowing down mucosal blood flow with consequent vascular congestion), gut dysbiosis, short-chain fatty acids (SCFAs), oxidative stress, endotoxemia, and alcohol consumption[76,77], as illustrated in Figure 2.

A recent interesting study conducted by Muñoz *et al*[78] in rat models of cirrhosis demonstrated that the presence of ascites (identifying a phase of decompensated disease) correlates with significant damage of the tight and adherens junctions, increased intestinal permeability, and enhanced bacterial translocation, which can be normalized by antibiotics administration. This reinforced the hypothesis that coexistent dysbiosis and immune dysregulation play a pivotal role in disrupting the intestinal barrier. Hence, the homeostasis of the intestinal barrier is likely to be modulated by a dynamic symbiotic relationship between the gut microbiome and the

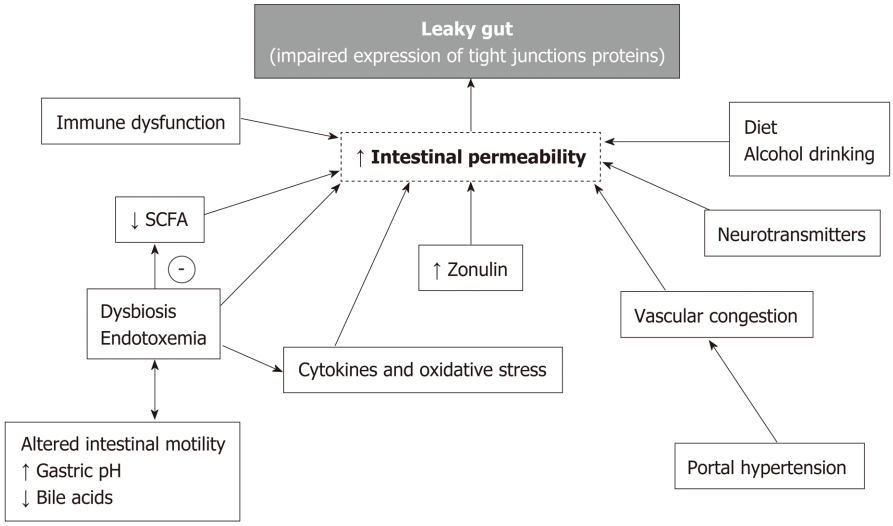

Figure 2 Leaky gut in liver cirrhosis. Multiple factors are involved in the increase of intestinal permeability found in cirrhotic patients. SCFA: Short-chain fatty acids.

immune system[78]. Due to increased gut permeability, bacteria can pass the intestinal barrier and migrate to mesenteric lymph nodes and other organs, a process known as bacterial translocation[79]. This phenomenon is responsible for increased levels of circulating bacterial products and endotoxins, which directly correlate with the severity of liver disease and lead to the development of several complications, especially infections and HE[80,81] .

GUT MICROBIOTA

The human gut contains 10^{14} bacteria, more than ten times the number of somatic cells in the human body[82]. Microorganisms start colonizing the gut after birth, and their density and types vary among different parts of the intestines, among individuals, and in the same individual during periods of illness and following dietary changes[83-85]. In the healthy individual, the host/microbiota relationship is characterized by a homeostatic symbiosis: the host provides nutrients, and the microbiota influences the correct epithelial function and nutrient absorption. Normally, anaerobes are more represented than aerobes, and the majority of species belong to the genera *Bacteroidetes* and *Firmicutes*[86].

The liver receives blood supply from the intestine through the portal circulation and is therefore exposed to gut-derived toxins, including bacteria and bacterial products, which are normally eliminated by the inflammatory response orchestrated by a large number of resident macrophages, dendritic cells, lymphocytes, and natural killer cells[87,88].

In cirrhotic patients with impaired immune response and altered intestinal barrier, it is clear how gut microflora can play a major role in triggering systemic inflammation, even in the absence of overt infection[15,89]. Furthermore, the increase of translocated bacterial products is believed to be responsible for the cognitive impairment found in HE[90].

A growing number of studies is trying to identify the existence of specific "microbiome signatures" related to cirrhosis and its complications, but the heterogeneity in study designs, investigated populations, bacterial taxonomic levels considered, origin of the microbiome samples (fecal microbiota or mucosa samples), the different methodologies used, along with the lack of standardization, make it difficult to obtain clear-cut results. Yet, some common findings in the gut microbiota of patients with cirrhosis can be highlighted, consisting in a higher proportion of *Enterobacteriaceae*, *Alcaligenaceae*, *Streptococcaceae*, *Veillonellaceae*, and *Fusobacteriaceae*, along with a reduction of *Bacteroidetes*, *Ruminococcaceae*, and *Lachnospiraceae* in comparison with healthy controls[91-93].

Of note, *Ruminococcaceae* and *Lachnospiraceae* are butyrate-producing bacteria[77]. Butyrate is a SCFA used as a source of energy by enterocytes and able to influence the intestinal barrier function through the stimulation of tight junctions and mucus production. SCFAs play a role in increasing anti-bacterial peptides and reducing

colonic inflammation; therefore, their reduction may have a detrimental role in the whole setting of systemic inflammation[94,95].

As a result of these findings, further studies were designed to search for associations between gut flora alterations and development of HE or other complications of cirrhosis and to evaluate how gut-centric therapies may help treat them. Hence, specific changes in the gut microbiome have been correlated with cognitive function and systemic inflammation.

In patients with HE, a higher proportion of *Veillonellaceae* was linked to increased circulating inflammatory cytokines (IL-6, TNF-α, IL-2, and IL-13) and poor cognition when compared to cirrhotic patients without HE[95].

Alcaligenaceae abundance was associated with poor cognitive performance[12]. These organisms are Proteobacteria responsible for opportunistic infections that degrade urea to produce ammonia, thus explaining their association with loss of cognitive functions.

In another study by Bajaj *et al*[96], microbiome testing was performed on stool and sigmoid mucosa tissue of cirrhotic patients with concurrent HE, cirrhotic patients with normal cognitive function, and healthy controls. *Blautia, Fecalibacterium, Roseburia*, and *Dorea* were associated with good cognition and decreased inflammation in both HE/non-HE, whereas genera overrepresented in HE (*Enterococcus*, *Megasphaera*, and *Burkholderia*) were linked to poor cognition and inflammation.

Zhang *et al*[97] found an overrepresentation of *Streptococcaceae* and *Veillonellaceae* in stools of cirrhotic patients with and without HE compared with normal individuals. In addition, the abundance of *Streptococcus salivarius* was significantly higher in cirrhotic patients with HE than in those without, and increased levels of this bacteria were correlated with ammonia accumulation in patients with HE.

A recent study by Ahluwalia *et al*[98] aimed to evaluate the contribution of specific gut bacteria to neuronal changes in cirrhotic patients with HE. Cirrhotic patients without HE, cirrhotic patients with HE, and healthy controls underwent stool microbiota analysis, systemic inflammatory assessment, and magnetic resonance imaging analysis. Cirrhotic patients with HE had a higher abundance of *Staphylococcaceae*, *Enterococcaceae*, *Porphyromonadaceae*, and *Lactobacillaceae* compared to controls and cirrhotics without HE. These microbial populations were linked to increased endotoxin and ammonia production as well as with worse cognitive performance. Specific microbial families such as *Enterobacteriaceae* positively correlated with hyperammonemia-associated astrocytic changes diagnosed through magnetic resonance imaging spectroscopy. *Porphyromonadaceae* only correlated with neuronal changes without linkages with ammonia levels.

Other regions of the gastrointestinal tract have been associated with dysbiosis in cirrhotic patients with HE[73]. Bajaj *et al*[99] studied oral and distal gut microbiota in both patients with and without HE. Salivary microbiota in cirrhotic subjects with HE showed an increased proportion of *Enterobacteriaceae* and lower amounts of autochthonous bacteria and *Erysipelothricaceae* compared to non-HE and healthy controls. The alterations of oral microbiota in cirrhotic subjects were correlated with an increased potential for endotoxins synthesis and with the existence of both a local salivary proinflammatory milieu (expressed by higher levels of IL-1β, IL-6, and immunoglobulin A secretion), and a systemic inflammatory status, thus suggesting a contribution of oral microbiota in the overall inflammation found in cirrhosis. Hence, dysbiosis, represented by a reduction in autochthonous bacterial abundance in favor of other microorganisms, is present in saliva as well as in the stools of cirrhotic patients, and this change could reflect a globally impaired mucosal-immune function. As a result, it has been postulated that the identification of specific stool and salivary microbial signatures associated with better cognitive function could potentially be used to predict the absence of MHE thus avoiding cognitive testing[100].

These findings suggest that microbiome composition is strictly correlated with cognition and inflammation in cirrhotic patients, especially in those who develop HE.

Small intestinal bacterial overgrowth

Small intestinal bacterial overgrowth (SIBO), a manifestation of gut microbial dysbiosis, represents a common finding in cirrhosis, affecting up to 59% of patients and correlating with the severity of liver disease[101-103]. Quantitative cultures of proximal jejunal aspirate with bacterial counts ≥ 10^5 colony forming units per milliliter are considered the diagnostic gold standard[104]. However, non-invasive tests such as glucose breath test and lactulose breath test have been developed to investigate SIBO with no need for endoscopic examination and at lower costs[105].

Gram-negative bacteria, and particularly *Escherichia coli* and *Klebsiella pneumoniae*, are found to be overrepresented in SIBO[106,107], and this condition favors bacterial translocation and endotoxemia, thus representing a risk factor for the development of

clinical decompensation events, such as spontaneous bacterial peritonitis or HE[77].

The results of a recent meta-analysis[108] showed an overall prevalence of 41% for SIBO in cirrhosis, significantly higher than the prevalence among control subjects (11%). The prevalence did not differ according to etiology of liver disease, but did vary according to the diagnostic test used (lactulose *vs* glucose breath test *vs* aspirate culture) and according to Child-Pugh class, with higher prevalence in patients with worse liver function. Cirrhotics with SIBO more often had ascites, spontaneous bacterial peritonitis, and MHE compared to those without SIBO [75.6% *vs* 33.5% for MHE; OR 6.28 (95% confidence interval: 2.10–18.80; $P = 0.001$)]. Furthermore, two of the studies included in the meta-analysis evaluated orocecal transit time, demonstrating a significant prolongation in cirrhotics with SIBO compared to those without[109,110].

Therefore, HE appears to be significantly more frequent in cirrhosis when SIBO coexists; in this case, increased amounts of intestinal bacteria in the context of an altered intestinal permeability and disrupted immune function can lead to increased endotoxemia, inflammation, and hyperammonemia, finally eliciting the development of decompensation[111,112].

Future studies are needed to clarify the causes of SIBO in cirrhosis. A cooperation of several factors can be hypothesized, including impaired intestinal motility leading to stasis of luminal content, local and systemic immune dysregulation leading to reduced secretion of luminal immunoglobulins A, the presence of gastric hypochlorhydria (particularly in case of therapy with proton pump inhibitors), and alterations in bile acids metabolism[113].

At present, no clear evidence is available showing that the elimination of SIBO in cirrhosis could lead to clinical improvement of the disease course. Large, randomized controlled trials (RCTs) exploring this issue are required.

THERAPY

As previously described, the accumulation of gut-derived toxic substances in patients with impaired liver function induces a systemic inflammatory response as well as detrimental effects on the CNS, ultimately leading to the development of HE.

Several conditions can precipitate acute episodes of HE, among them: constipation, concomitant infections, gastrointestinal bleeding, administration of sedative drugs, dehydration following liquid losses or excess of diuretics, hyponatremia, and alkalosis. These so-called "precipitating factors" can act at various levels of the gut-liver-brain axis, amplifying the intestinal production of ammonia and absorption of toxins, boosting the inflammatory response or enhancing the negative effects of hyperammonemia on the CNS. Consequently, the initial management of an acute episode of HE should always include an exhaustive search for any precipitating factor and its elimination or correction[3,114]. Secondly, general treatment for HE should be initiated.

Currently, available therapies for HE primarily target the reduction of ammonia and the modulation of gut microbiota. The efficacy of these gut-centric therapeutic approaches further supports the pathogenetic relevance of the alterations of gut microflora and intestinal barrier. See Table 1 for an overview on the available therapeutic approaches for HE, their mechanisms of action, and the corresponding levels of evidence.

NON-DIETARY APPROACH

Non-absorbable disaccharides

At present, non-absorbable disaccharides, such as lactulose and lactitol, represent the first-line standard of care treatment recommended by international guidelines for use in OHE as well as in secondary prophylaxis[3]. The main mechanisms explaining their efficacy in the management of HE can be summarized as a cathartic effect, reducing intestinal transit time and content of toxic compounds, together with the ability to modulate the intestinal flora, and finally resulting in a reduction of ammonia levels[115-117].

In detail, these synthetic disaccharides pass through the intestine without being absorbed and are partially metabolized by gut bacteria, with the production of lactic and acetic acid. The consequent acidification of the gut content inhibits bacterial production of ammonia and converts ammonia into non-absorbable ammonium, trapping it in the intestinal lumen and preventing its passage in the blood[114-116]. Non-absorbable disaccharides can also inhibit glutaminase activity, thus reducing the

Table 1 Therapeutic strategies in hepatic encephalopathy

Therapeutic approach	Mechanism of action		Gut microbiota modulation	Level of evidence according to EASL/AASLD guidelines
Non absorbable disaccharides (lactulose and lactilol)	Decrease serum ammonia levels by:	- Accelerating intestinal transit - Reducing ammonia synthesis in the gut	Yes	- Treatment: GRADE II-1, B, 1 [3] - Secondary prophylaxis: GRADE II-1, A, 1 [3]
Rifaximin	Decreases serum ammonia levels and proinflammatory cytokines release by:	- Modifying intestinal bacterial metabolism and abundance - Inhibiting bacterial translocation	Yes	Secondary prophylaxis: GRADE I, A, 1 [3]
Adequate protein intake (1.2-1.5 g/kg per day)	Decrease serum ammonia levels by:	- Balancing nitrogen metabolism - Preventing sarcopenia	-	Treatment: - GRADE I, A, 1 [3] - GRADE II-2, B, 1 [136]
Dairy proteins	Decrease serum ammonia levels (process unclear)		Yes	Treatment: - GRADE II-3, B, 1 [136]
Vegetable proteins	Decrease serum ammonia levels by: Reduce circulating mercaptans and indoles	- Increasing ammonia detoxification (urea cycle) - Accelerating intestinal transit (high fiber content)	Yes	Treatment: - GRADE II-3, B, 1 [136]
Oral branched-chain amino acids (BCAA)	Unclear. Postulated: Decrease serum ammonia levels by: Rebalance of CNS system neurotransmitters synthesis	- Increasing ammonia detoxification (glutamine synthesis)	-	Treatment: - GRADE I, B, 2 [3] - GRADE I-1, A, 1 [136]
L-ornithine-L-aspartate (LOLA)	Decreases serum ammonia levels by:	- Increasing ammonia detoxification (urea cycle and glutamine synthesis)	-	Treatment: - GRADE I, B, 2 [3]
Zinc	Decrease serum ammonia levels by:	- Increasing ammonia detoxification (urea cycle and glutamine synthesis)	-	No recommendations
Prebiotics	Decrease proinflammatory cytokines release and serum ammonia levels by:	- Reducing intestinal permeability - Reducing luminal pH - Reducing ammonia absorption - Accelerating intestinal transit	Yes	No recommendations
Probiotics	Decrease proinflammatory cytokines release and serum ammonia levels by:	- Reducing intestinal permeability - Reducing luminal pH - Reducing ammonia absorption	Yes	No recommendations
Gluten-casein free diet	Unclear. Postulated: Rebalance of CNS dysfunction by:	- reducing absorption of gluten- and casein-derived peptides - decreasing proinflammatory cytokines production	Yes	No recommendations
Fecal microbiota transplantation	Rebalance of gut microbiota		Yes	No recommendations

Criteria used to classify recommendations (EASL/AASLD guidelines)[3,136]: Level of evidence: I: Randomized, controlled trials, II-1 controlled trials without randomization, II-2) cohort or case-control analytical studies, II-3 multiple time series, dramatic uncontrolled experiments, III opinions of respected authorities, descriptive epidemiology. Quality of evidence: A: High: further research is very unlikely to change our confidence in the estimated effect; B: Moderate: further research is likely to have an important impact on our confidence in the estimated effect and may change the estimate; C: Low: further research is likely to have an important impact on our confidence in the estimated effect and is likely to change the estimate. Any change of estimate is uncertain. Grade of recommendation: 1: Strong: factors influencing the strength of recommendation included the quality of evidence, presumed patient-important outcomes, and costs, 2: Weak: variability in preferences and values or more uncertainty. Recommendation is made with less certainty, higher costs, or resource consumption. CNS: Central nervous system.

intestinal production of ammonia[118]. Besides, lactulose and lactitol act as prebiotics, favoring the growth of beneficial saccharolytic bacteria, such as *Bifidobacteria* and *Lactobacilli*, and counteracting the growth of harmful, ammonia-producing bacteria[15,91,114,115,119].

Moreover, the promotion of microbial growth by non-absorbable disaccharides prompts bacterial uptake of ammonia as a nitrogen source for protein synthesis[120].

Furthermore, it has been demonstrated that lactulose reduces bacterial DNA

translocation, with a consequent decrease in serum ammonia and levels of inflammatory mediators[121].

Rifaximin

In patients experiencing recurrent bouts of HE despite administration of non-absorbable disaccharides, it is recommended to implement secondary prophylaxis by adding rifaximin[3].

Rifaximin is a non-absorbable antibiotic that has been shown to reduce serum ammonia and improve cognitive function in patients with HE, thus preventing recurrences and decreasing hospitalization rates[122,123]. Several studies proved rifaximin efficacy in both prevention of recurrences and treatment of acute bouts of HE, and its beneficial effects on neuropsychiatric and neuromotor abnormalities have been observed[124,125]. Rifaximin is thought to act through a number of mechanisms, including the modulation of gut microbiota, reduction of ammonia circulating levels and bacterial translocation, and reduced release of endotoxins and proinflammatory cytokines with consequent anti-inflammatory effects[126-128]. It also directly affects intestinal barrier and gut bacteria function[129-131].

The effect of rifaximin on the gut-liver-brain axis was investigated by Bajaj *et al*[132], who observed improved cognition and reduced endotoxemia after 8 weeks of rifaximin administration in 20 cirrhotic patients with MHE. Despite only slight modifications of microbiota composition were observed (namely a reduction in *Veillonellaceae* and an increase in *Eubacteriaceae*), serum metabolomics analysis suggested that rifaximin significantly altered bacterial functioning. In fact, there was an increase in serum saturated and unsaturated fatty acids, as well as other bacterial end-products, with a potentially beneficial impact on cognitive functions. The authors postulated that rifaximin might positively affect cognitive function mainly through a beneficial modulation of bacterial metabolism rather than by reducing absolute or relative bacterial abundances.

Rifaximin efficacy appears to be further increased when used in addition to lactulose: a double-blind prospective study by Sharma *et al*[10] revealed a significant decrease in OHE and length of hospital stay with combination therapy compared to lactulose alone. These data reveal how synergistic strategies may enhance treatment efficacy.

Other non-dietary therapies

Several other non-dietary treatments have been proposed for the management of HE in cirrhosis, many of which are still under investigation. They basically aim to lower serum ammonia levels (ornithine phenylacetate, glycerol phenylbutyrate, AST-120, polyethylene glycol) and to scavenge inflammatory and reactive oxygen species (albumin administration and dialysis)[133,134]. At present, the evidence of their efficacy in patients with HE is scarce or limited, and they cannot be recommended in this setting. As modulation of intestinal microbiota or dietary interventions is not the target of these therapies, their literature analysis is beyond the scope of this review.

DIETARY APPROACH

Therapeutic strategies used in the management of HE aimed to treat its main pathogenetic factors: increased ammonia levels, inflammation, and alterations of gut microbiota. Along with pharmaceutical products, diet plays a role of primary importance in addressing this condition. As illustrated in Figure 3, changes in food habits may modulate nitrogen metabolism and exert beneficial effects on gut microbiota, thus interrupting the chain of events that leads to inflammation and development of cognitive impairment[135]. Different nutritional strategies have been proposed in order to correctly manage HE, including modulation of protein intake (regarding both avoidance of protein restriction and selection of specific protein sources), increased fiber intake, and use of foods with prebiotic and probiotic effects.

Current evidence strongly suggests that specific dietary approaches can largely contribute to the treatment and prevention of HE, and several recommendations regarding dietary changes have already been included in the main clinical guidelines.

The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines on nutrition in chronic liver disease[136], the American Association for the Study of Liver Diseases (AASLD) and EASL Practice Guidelines for HE[3], and the ESPEN Guidelines on nutrition in liver disease[137] recommend daily energy intakes of 35-40 kcal/kg and that high-calorie diets should be implemented in cirrhotic patients in conditions of increased energy expenditure (*e.g.*, in cases of acute decompensation). Carbohydrates should make up for 40%-60% of total caloric intake, and complex carbohydrates should be preferred. Lipids, which should account for 25%-50% of

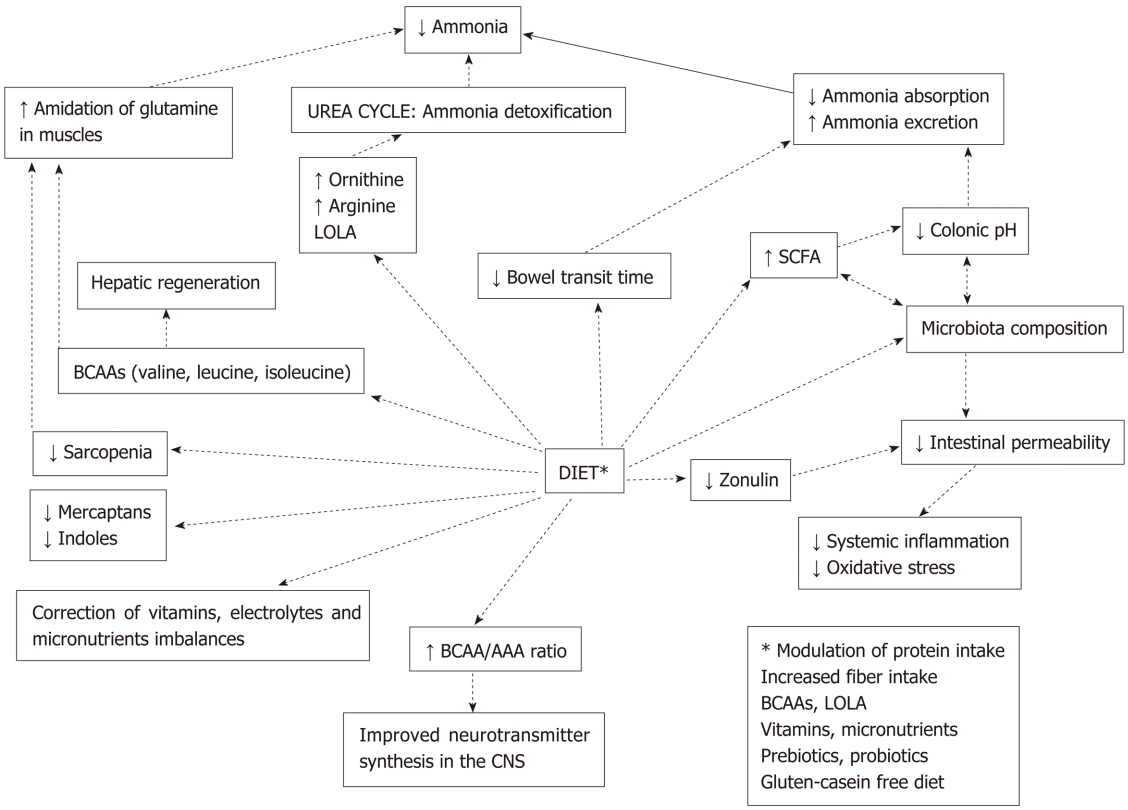

Figure 3 Potential benefits of dietary modulation in hepatic encephalopathy. AAA: Aromatic amino acids; BCAA: Branched-chain amino acids; CNS: Central nervous system; LOLA: L-ornithine–L-aspartate; SCFA: Short-chain fatty acids.

dietary calories, are particularly useful in HE patients as they have been demonstrated to exert beneficial effects on gut flora and on bowel transit time[138].

Proteins

Adequate nutrition is of utmost importance in all cirrhotic patients, who exhibit protein-energy malnutrition and muscle wasting in up to 60% of cases. As muscle tissue contributes to the removal of circulating ammonia by increasing glutamine synthesis, sarcopenia is not only associated with worsening of clinical conditions and increased mortality in cirrhotic patients[139] but also represents an independent risk factor for HE[25,140,141]. Adequate protein intake is therefore extremely important in cirrhotic patients with HE[142], both in terms of timing and quality of nutrient ingestion.

Firstly, it is mandatory to define a pattern of dietary intake in order to grant a correct substrate utilization; this is a very relevant issue, as catabolism of amino acids for glucose production depletes tissues of proteins and increases ammonia levels[143]. Cirrhotic patients should have frequent meals during the day, avoiding fasting for longer than 3-6 h. It has been demonstrated that a late-evening snack, containing approximately 50 g of carbohydrates, has a beneficial effect on substrate utilization and nitrogen production[144], therefore preventing HE and reducing HE severity[2,145,146,147]. It is recommended that breakfast and late-evening snack also include some proteins in order to fulfill energy and protein requirements[136].

As dysregulated nitrogen metabolism plays a key role in the development of HE, protein intake requirement in patients with HE has been widely investigated. Early evidence suggested that episodes of HE could be controlled by reducing protein intake[148,149], but these observations have been largely debunked by several subsequent studies.

In 1995, a study by Morgan *et al*[150] questioned for the first time the real usefulness of protein restriction in HE, demonstrating that patients with alcoholic hepatitis whose diet provided a higher protein intake experienced an improvement in mental status, suggesting that the lack of an adequate protein intake could favor HE. A study found that restriction of protein intake has no beneficial effect on the evolution of episodic HE and that it can worsen the nutritional status of these patients by exacerbating protein breakdown from muscles[151]. Furthermore, they showed that

patients with HE could safely follow a normal protein diet.

Additional studies confirmed that normal protein intake is well tolerated and useful in HE to ensure sufficient substrate for energy synthesis and hepatocyte function[152,153]. Hence, avoidance of protein restriction in patients with HE is now strongly recommended. The International Society for HE and Nitrogen Metabolism[145], the EASL Clinical Practice Guidelines on nutrition in chronic liver disease[136], the AASLD and EASL Practice Guidelines for HE[3], and the ESPEN Guidelines on nutrition in liver disease[137] recommend for patients with HE a daily protein intake of 1.2-1.5 g/kg per day.

The amount of protein is not the only important factor to take into consideration; in cirrhosis, tolerance to dietary proteins (in terms of the development of HE after protein ingestion) seems to vary among different protein sources; dairy and vegetable proteins have been suggested to be better tolerated than animal proteins.

The evidence regarding dairy proteins is limited. An old study by Fenton *et al*[154] described a consistent reduction of plasma ammonia in three patients when meat was replaced by dairy protein and hypothesized that this improvement might be due to gut flora modifications. Bessman *et al*[155] administered intragastrically blood and milk-protein preparations to patients with liver disease, observing significantly higher elevations of circulating ammonia after administration of blood rather than after milk-protein preparations. More recently, a 14 day high-protein casein-vegetable diet was shown to improve cognitive performance and lower serum ammonia levels in 150 patients with OHE, thus confirming the irrationality of dietary protein restriction and the usefulness of a casein-vegetable based diet[152].

The beneficial effects of vegetable proteins have been widely studied among cirrhotic patients. Bianchi *et al*[156] tested the effect of a vegetable versus animal protein diet on nitrogen metabolism and cognitive function in cirrhotic patients with persistent HE, and the results showed that ammonia levels, as well as clinical severity of HE, significantly improved during vegetable protein diet. Uribe *et al*[157] also demonstrated improved mental state and encephalogram results in patients with HE undergoing vegetable protein diet compared to those on animal protein diet. Finally, Maharshi *et al*[158], in a recent RCT, showed that 6 months of 1-1.5 g/d of vegetable protein was effective in treating MHE, preventing OHE episodes, and improving patients' quality of life.

Multiple reasons may explain the superiority of vegetable proteins: they contain lower quantities of methionine and cysteine compared to animal proteins. These amino acids are precursors of mercaptans and indoles, which, as mentioned before, have been implicated in HE development[159]. On the other hand, vegetable-derived proteins contain high ornithine and arginine levels, which are implicated in ammonia detoxification through the urea cycle[160]. Another advantage of vegetable proteins is their high fiber content[145], which favors intestinal transit and consequently a more efficient ammonia excretion. Moreover, fiber digestion operated by intestinal bacteria produces the SCFAs acetic, propionic, and butyric acid, therefore reducing colonic pH, which improves ammonia excretion[161,162]. This may result in favorable changes in microbiota composition, with associated enhanced anti-inflammatory and antioxidant properties[163,164]. Although additional benefits of vegetable protein diets on intestinal microbiota in HE patients have been hypothesized, to our knowledge no studies exploring the effects of this dietary approach on gut microorganisms in HE patients have been published so far.

To summarize, although vegetable proteins may be better than animal proteins for patients with HE and should therefore be encouraged, they can also cause bloating, flatulence, and diarrhea, which may consequently reduce patients' compliance to the dietary regimen. In order to make the diet palatable and tolerable in the long run, a strategy of protein intake from different sources (dairy, vegetable, and high-quality animal proteins) seems the most reasonable one and should be recommended[136,165,166].

Branched-chain amino acids

Another way to prevent excessive protein catabolism and reduce ammonia levels in HE patients is through the administration of branched-chain amino acids (BCAAs): valine, leucine, and isoleucine.

These essential amino acids are used by skeletal muscles for the amidation of glutamine, a process that allows ammonia detoxification[167]. Due to the combination of impaired hepatic function, portosystemic shunting, and skeletal muscle loss, with hyperinsulinemia and hyperglucagonemia, BCAA levels in cirrhotic patients are usually reduced[145,168,169], whereas a concomitant rise in the levels of aromatic amino acids (AAA: phenylalanine, tyrosine, and tryptophan) has been observed[170-172].

Decreased breakdown of AAA due to impaired liver function and increased utilization of BCAAs in the muscle are thought to be the main causes for the observed decrease in the BCAA/AAA ratio, also called the Fischer-ratio[145,167,173]. The consequent

increase of AAA influx in the CNS has been postulated to be responsible for imbalances in neurotransmitter synthesis, contributing to HE[168-170,174].

Oral supplementation of BCAAs in HE patients could therefore improve their clinical condition through the facilitation of ammonia detoxification, and their possible use in these patients has been widely studied[168].

There is accumulating evidence showing that long-term oral supplementation of BCAAs may confer nutritional benefits and improve survival in HE patients, probably due in part to the effect of leucine, which stimulates hepatic regeneration[175] and muscle protein synthesis[176]. Furthermore, BCAAs promote correction of plasmatic amino acid imbalance and counteract the harmful brain influx of AAA across the impaired BBB[145,177].

A meta-analysis[178] performed on nine RCTs demonstrated a significant improvement in the grade of HE with the administration of oral BCAAs compared to other nutritional supplements, but no difference was found in terms of resolution of HE. Another recent Cochrane meta-analysis on 16 RCTs indicated that oral administration of BCAAs had a beneficial impact on HE, without effect on mortality, quality of life, and nutritional status[179]. Considering prophylaxis of HE, several studies showed that oral BCAAs do not prevent development or recurrence of HE in cirrhotic patients[180-182].

Furthermore, it should be mentioned that many of these trials have methodology issues that limit their value and that oral BCAAs supplements are not used in many countries because of their cost (they are not reimbursed) and scarce palatability[137]. As a consequence, even if the use of oral BCAAs should be considered in this clinical setting, there is still a need for additional high-quality RCTs to confirm their efficacy in preventing and treating HE.

L-ornithine–L-aspartate

L-ornithine–L-aspartate (LOLA) is a mixture of two endogenous amino acids with the capacity to fix ammonia in the form of urea or glutamine. They are substrates for the urea cycle and can also activate glutamine production by activating glutamine synthetase in hepatocytes and muscle cells. Therefore, LOLA can be used as a supplement to reduce serum ammonia levels[136,137,183].

The efficacy of LOLA in patients with HE was addressed in three recent reviews and meta-analyses. The first one, a Cochrane review[184], suggested a possible beneficial effect of LOLA on mortality and HE, without increased serious adverse events in comparison with placebo or no intervention and a possible favorable impact on HE when compared with probiotics. The authors, however, considered the beneficial profile of LOLA uncertain, due to the low quality of the available studies. The second study[185] showed that LOLA was significantly more effective compared to placebo/no intervention for improvement of mental state in all types of HE and for lowering of blood ammonia[185]. The last and very recent meta-analysis highlighted the benefit of LOLA in a wide range of clinical presentations of HE, including OHE as well as MHE, where the oral formulation of LOLA was particularly effective[186]. The concomitant reduction of blood ammonia levels was reported in all RCTs that investigated this issue. Network meta-analysis showed that LOLA appears to be comparable (or superior) in efficacy to other ammonia-lowering agents, including non-absorbable disaccharides and probiotics. Furthermore, LOLA seems to be effective also for the treatment of post-transjugular intrahepatic portosystemic shunt HE and secondary HE prophylaxis. The authors concluded supporting the use of LOLA in the treatment of HE.

Vitamins and micronutrients

Generally, patients suffering from liver disease present vitamin deficiencies due to altered hepatic function, reduction of reserves, as well as inadequate dietary intake or malabsorption[187]. Deficiencies of vitamins and electrolytes can potentially cause a variety of neuropsychiatric symptoms, hence mimicking or worsening HE.

Among vitamins potentially affecting cognitive function, cirrhotic patients often present vitamin B deficiency, probably due to intestinal malabsorption and decreased liver storage. Although the consequences of vitamin B deficiency in patients with advanced liver disease are not fully understood (except vitamin B1 deficiency), it is known that this group of vitamins is linked to cognitive function[188,189], and its reduction may cause additional CNS alterations in patients with HE[190].

Patients with cirrhosis may also have reduced levels of micronutrients; among them, zinc has been implicated in the pathogenesis of HE, as glutamine synthetase and ornithine transcarbamylase, which are involved in ammonia detoxification, are both zinc-dependent. Zinc administration has been suggested to improve psychometric tests in some studies[191,192], but overall results are conflicting[193-195].

Furthermore, clinicians should always pay attention to electrolyte imbalances, as

they can both trigger the development of HE and worsen pre-existing abnormalities of mental function. In particular, hyponatremia, hypomagnesemia, and hypercalcemia, if present, should be promptly corrected in cirrhotic patients with altered mental status, bearing in mind the importance of a slow rebalancing in sodium levels, because of the risk of developing central pontine myelinolysis[2,136,196].

Currently, supplementation of vitamins and micronutrients is recommended by the EASL[136] and ESPEN guidelines[137] in patients with documented deficiencies or during the first 2 weeks of nutritional support when the deficiency is clinically suspected.

DIETARY APPROACH, INTESTINAL MICROBIOTA MODULATION, AND GUT-LIVER AXIS

Since the influence of diet on gut microbiota composition in both healthy and unhealthy populations has been abundantly demonstrated[197-199], and the connection between gut, liver, and brain plays a fundamental role in the development of HE in cirrhotic patients[21,69,82], it has been hypothesized that specific dietary approaches targeting the gut-liver-brain axis may be implemented in the therapeutic management of HE.

Prebiotics and probiotics

Prebiotics are food substrates that are selectively used by host microorganisms causing alterations in the composition and activity of gut microbiota and thus conferring a health benefit[200]. Probiotics are live microorganisms that, when ingested in adequate amounts, alter the microflora conferring a favorable effect on the health of the host[201]. Synbiotics are defined as a combination of both pre- and probiotics. They produce beneficial alterations in gut microbiota and may be, at least in theory, helpful in the management of HE thanks to their gut-centric action[145,202]. In fact, the modulation of gut microbiota operated through supplementation of pre- and probiotics decreases pathogenic bacteria and reduces luminal pH, thus lowering ammonia absorption, improving nutritional status of gut epithelium, and decreasing intestinal permeability; all these changes reduce systemic inflammation and oxidative stress and lower circulating ammonia levels[203-205].

Prebiotics

At present, lactulose, lactitol, fructo-oligosaccharides, and galacto-oligosaccharides are the most commonly used prebiotics. Malaguarnera *et al*[206,207] demonstrated that a combination of probiotics and fructo-oligosaccharides was effective in treating MHE, improving neuropsychiatric function when compared both to placebo and lactulose. Liu *et al*[198] showed that the administration of a synbiotic preparation composed by probiotics and four fermentable fibers induced reversal of MHE in 50% of patients. This study also revealed that fermentable fibers alone could be beneficial in a substantial proportion of patients. Soluble fibers have prebiotic properties, as they are usually a substrate for fermentation. According to these data, Sitkin *et al*[208] suggested that dietary fibers supplementation modified gut microbiota and improved psychometric tests in patients with MHE. To summarize, although treatment with prebiotics seems to be promising in cirrhotic patients with HE, their efficacy (except lactulose) has still to be established; and therefore, they cannot be recommended as part of the conventional therapy.

Probiotics

In a recent meta-analysis of 21 trials with 1420 participants, Dalal *et al*[209] compared the effects of probiotics *vs* placebo or no intervention or lactulose in MHE or OHE. The meta-analysis showed that probiotics, when compared to placebo or no intervention, probably improved recovery and may confer an advantage in terms of the development of OHE, quality of life, and plasma ammonia concentrations, with little or no difference on mortality. When compared to lactulose, probiotics did not show any statistically significant advantage in terms of recovery, development of OHE, quality of life, plasma ammonia concentration, or mortality. The authors highlighted that whether probiotics are better than lactulose for HE is uncertain due to the very low quality of the available evidence, and they claimed for new high-quality RCTs to clarify further the efficacy of probiotics on HE. Therefore, at present, the use of probiotics cannot be routinely recommended for treating patients with HE.

Lunia *et al*[210] evaluated the usefulness of probiotics as primary prophylaxis for HE in cirrhotic patients, showing that a 3 month course of probiotics reduced levels of arterial ammonia, improved psychometric tests, and reduced the risk of developing HE compared to placebo. Regarding the setting of secondary prophylaxis for HE, a

clinical trial by Agrawal *et al*[211] compared the efficacy of probiotics and lactulose in this field. Probiotics were revealed to be as effective as lactulose in preventing new episodes of HE. Dhiman *et al*[212] further strengthened these data, demonstrating that probiotics, compared to placebo, reduced the risk of HE-related hospitalization in patients who recovered from a previous episode of HE. The results of these studies are promising, but further standardized trials performed with optimal methodological quality are needed in order to define the role of probiotics in the context of both primary and secondary prophylaxis for HE[213,214].

Probiotic yogurt supplementation

The modulation of gut flora through dietary interventions in cirrhotic patients has been studied by Bajaj *et al*[215], who investigated whether the supplementation of probiotic yogurt in cirrhotic patients could be useful in treating MHE and preventing OHE. Cirrhotic patients were randomized to receive 12 oz of yogurt daily *vs* no treatment for 60 days. The study demonstrated a higher rate of MHE reversal in patients treated with yogurt as well as a better rate of prevention of OHE development. A subsequent study by Liu *et al*[216] displayed that probiotic yogurt could modify intestinal microflora in patients with chronic liver disease, increasing the number of beneficial bacteria and reducing levels of *Escherichia coli*. This research field seems therefore promising, but further evidence is needed to confirm these preliminary results.

Gluten-casein free diet

The gut-liver-brain axis has been widely studied as a possible therapeutic target for other conditions in which gut microbiota alterations and intestinal barrier impairment are thought to have a pathogenetic role, such as celiac disease and autism spectrum disorders (ASD). In these settings, altered intestinal permeability may favor leakage of gut-derived toxic substances, which in turn may trigger systemic inflammation through cytokine production and may reach CNS to induce neurological damage[46,217-219]. This model recalls the mechanisms involved in HE, where gut-derived substances also induce an inflammatory response and can cross the BBB and cause cognitive impairment. In ASD, casein and gluten-derived peptides passing through the altered intestinal barrier have been suggested to play a pathogenetic role[220], potentially eliciting inflammatory responses both at a systemic and CNS level, where these peptides are believed to act as neuropeptides and alter neurological functions[221].

Hence, gluten-casein free diet has been postulated to confer beneficial effects on patients with ASD by reducing both systemic inflammation and circulating opioid peptides levels (β-gliadomorphine and β-caseomorphine). Even if the efficacy of this therapeutic approach remains controversial[217], there is increasing evidence that the elimination or reduction of gluten and casein from the diet may confer some advantages in patients with ASD, in terms of both gastrointestinal and cognitive benefits[222,223]. Furthermore, also in contexts other than celiac disease and ASD, gluten has been shown to impair intestinal permeability through zonulin upregulation, and a gluten-free diet has been shown to influence positively microbiota composition[224-226].

In light of these data, on the basis of the common ground of altered gut-liver-brain axis and increased intestinal permeability, a similar dietary approach could be implemented in the management of cirrhotic patients with HE. This intriguing possibility has been investigated in a pilot study performed by Balzola *et al*[227]. Sixteen patients awaiting liver transplantation for end-stage cirrhosis with chronic HE were enrolled and clinical, neurological, and gastroenterological evaluations were performed. A normoproteic gluten-casein free diet was undertaken, along with maintenance of previously ongoing therapies targeting HE. Clinical and neurological evaluation was performed after 1 and 3 months; cognitive function (arithmetic, memory, and orientation) and memory skills measured with Mini Mental Test and Rey Auditory Verbal Learning Test showed a statistically significant improvement in 14/16 (88%) patients both at 1 and 3 months. Executive functions and attention evaluated by Trial Making Test significantly increased at 3 months. Baseline and 3 month electroencephalograms did not correlate with the improvement of mental status. Only one hospitalization for HE was necessary among the 16 patients during the 3 month follow-up, whilst a mean hospitalization rate of 1 to 3 episodes per month was observed in a control group made of 10 cirrhotic patients with the same clinical background (chronic HE). Notably, a transient HE episode was reported in a patient who accidentally introduced gluten during the study, and a HE recurrence was experienced by one patient who decided to reintroduce gluten after the 3 month follow-up.

Although this experience was very limited, it introduced an element of novelty that, if replicated, could add a simple therapeutic tool in the management of cirrhotic

patients with HE, at least for those affected by the most severe forms. Investigators should therefore address this dietary approach as a potential adjunctive therapy in patients with severe liver disease and HE in order to verify its efficacy.

Fecal microbiota transplantation

Another approach targeting gut dysbiosis in HE patients is fecal microbiota transplantation (FMT): this innovative treatment was investigated by Bajaj *et al*[228], who performed an RCT comparing its efficacy, in terms of cognitive improvement, adverse events, microbiota, and metabolomic changes, versus standard of care in patients with recurrent HE. A suitable stool donor was selected through cross-sectional microbiome data, and patients enrolled in the FMT arm were then administered a 90 mL enema after a 5 day broad-spectrum antibiotic course. After 150 days of follow-up, there was a statistically significant cognitive improvement in the FMT group, together with increased microbial overall diversity and expansion of beneficial taxa. No severe adverse events were registered.

A very recent study by the same authors has strengthened this approach, suggesting long-term (12-15 months) safety and sustained improvement in clinical and cognitive function parameters with prevention of HE recurrence among patients who received FMT[229].

CONCLUSION

HE is a serious complication of cirrhosis that significantly impacts on the quality of life of both patients and caregivers and heavily contributes to hospitalizations and mortality in these patients. The association among HE, malnutrition, sarcopenia, and poor prognosis is nowadays sound, and there is accumulating evidence that in this context intestinal dysbiosis and gut hyperpermeability play a pivotal role, being part of an altered interaction between the gut, the liver, and the brain. The findings discussed in this review show in their entirety and complexity the fundamental implication of the gut-liver-brain axis in the development of HE, as well as the important role that dietary modifications and modulation of microbiota may play in preventing and treating HE. If it is true that further research is surely necessary to achieve stronger scientific evidence in the very complex field of HE, it is equally true that current data suggest that the path taken is the right one.

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REVIEW

Outcomes of staged hepatectomies for liver malignancy

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Abstract

Liver malignancies are the fifth most common cause of death worldwide. Surgical intervention with curative intent is the treatment of choice for liver tumors as it provides long-term survival. However, only 20% of patients with metastatic liver lesions can be managed by curative liver resection. In most of the cases, hepatectomy is not feasible because of insufficient future liver remnant (FLR). Two-stage hepatectomy is advocated to achieve liver resection in a patient who is considered to not be a candidate for resection. Procedures of staged hepatectomy include conventional two-stage hepatectomy, portal vein embolization, and associating liver partition and portal vein ligation for a staged hepatectomy. Technical success is high for each of these procedures but variable between them. All the procedures have been reported as being effective in achieving a satisfactory FLR and completing the second-stage resection. Moreover, the overall survival and disease-free survival rates have improved significantly for patients who were otherwise considered nonresectable; yet, an increase in the morbidity and mortality rates has been observed. We suggest that this type of procedure should be carried out in high-flow centers and through a multidisciplinary approach. An experienced surgeon is key to the success of those interventions.

Key words: Staged hepatectomy; Portal vein embolization; Portal vein ligation; Colorectal liver metastasis; Hepatocellular carcinoma; Associated liver partition and portal vein ligation for staged hepatectomy

Core tip: Surgical intervention with curative intent is the treatment of choice for liver tumors. A variety of techniques have been established to increase the possibility for resectability. Two-staged hepatectomy, with its distinguishing beneficial procedures, is one of the techniques that have been proposed to overcome this clinical challenge. In spite of higher perioperative morbidity and mortality associated with this procedure, the overall survival and disease-free survival rates have increased significantly. Patient

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selection through consensus by a multidisciplinary board panel is the mainstay to successful performance of this procedure.

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INTRODUCTION

Liver malignancies are considered to be the fifth most common cause of death worldwide. Hepatocellular carcinoma (HCC) and colorectal liver metastasis are the main indications of liver resection in the Eastern and Western world, respectively[1]. Surgical intervention with curative intent is the treatment of choice for liver tumors, as it provides for long-term survival[2-4].

Historically, only 20% of patients with metastatic liver lesions have had indications for management by curative liver resection. While in most of the cases, hepatectomy has not been feasible, due to insufficient future liver remnant (FLR)[5-7]. Recently, many options have become available to achieve liver resection with curative intent in those patients initially deemed to have nonresectable liver tumors. These include Portal vein embolization (PVE)/ligation, locoregional therapy, hepatic artery chemo-infusion, and systemic chemotherapy[8-13]. However, these options cannot provide curative resection in all cases, especially in patients with multiple bilobar lesions; in such cases, two-staged hepatectomy (TSH) has been advocated[14].

This new approach is intended to resect the tumor completely in one lobe, with the remaining lobe to undergo resection later. The purpose of this staged resection is to minimize the risk of post hepatectomy liver failure by performing the second resection once liver regeneration is achieved. The second liver resection is curative, when restaging of the tumor after the first resection has excluded tumor progression or metastasis[14].

Prior to 2000, staged hepatectomy was applied to the cases with advanced liver lesions. These were managed initially by laparotomy and portal vein ligation, followed by liver resection of the affected lobe at the later stage. Indeed, the first study on conventional TSH on bilobar liver metastasis from colorectal cancer was reported in 2000[11,14].

We have based this review on our clinical and research experience to highlight the history of staged hepatectomy as well as the current practice, outcome and future direction of this surgical approach. We searched the Medline literature database from 1990 to 2018 using the search terms "staged hepatectomy", "portal vein embolization", "portal vein ligation", "colorectal liver metastasis", "hepatocellular carcinoma", and "associating liver partition and portal vein ligation".

TYPES OF PROCEDURES

As aforementioned, treatment of malignant liver lesions with curative intent is preferred[15]. Since many patients have multiple liver lesions (which often preclude complete resection), multidisciplinary approaches have been proposed to achieve complete resection and decrease postoperative complications[14,15].

The main principle of resectability is the preservation of an adequate FLR with in-flow, out-flow and biliary drainage capability while avoiding post hepatectomy liver failure. The FLR depends on the liver status and volume that can be studied preoperatively. Between 25% and 30% of total liver volume (TLV) in healthy liver is considered adequate. On the other hand, patients with either hepatic dysfunction or liver injury due to chemotherapy require FLR up to 40% of the TLV[8,16-18]. Multiple techniques have been described to augment the FLR; these are PVE/ligation, conventional TSH, and associated liver partition and portal vein ligation for staged hepatectomy (ALPPS).

PVE/ligation

PVE/ligation is one of the strategies developed to increase the number of patients indicated for resection. It can be done radiologically through embolization of the

affected liver lobe's portal vein or surgically by ligating the portal vein with or without clearance of the FLR[19].

In 1980s, Makuuchi *et al*[12] introduced PVE for the induction of FLR hypertrophy. This type of hypertrophy facilitates the removal of extensive liver tumors safely by mitigating the sudden rise in portal pressure that otherwise occurs during surgery. It also prevents perioperative liver dysfunction by increasing the FLR volume. This technique is considered an option in cases of multiple liver malignancy, allowing for the second curative liver resection after an appropriate time of regeneration[20-22].

Four factors are important in deciding which patients will benefit from PVE. These are: ratio of FLR to TLV; extent of liver resection; baseline liver function; and, presence of systemic diseases that might affect the liver hypertrophy, such as diabetes mellitus[23]. Previously, patients with bilobar multiple metastases were not considered candidates for PVE. However, recent studies have confirmed that some of these patients can benefit from PVE in combination with TSH[24]. Patients who are contraindicated for PVE are summarized in Table 1[25,26].

TSH

TSH is a surgical strategy for bilobar liver metastasis, aiming to achieve a curative R0 resection. The main principle of this approach is a planned sequential liver resection that will facilitate complete metastasectomy in those cases in which a major resection in a single surgery would result in FLR insufficient for the patient's survival.

In the first stage, the less affected lobe that will be the FLR is cleared by wedge resections and/or controlled by ablation. Portal vein ligation may be performed during the procedure; otherwise, it can be performed later. The optimal interval time between the two stages has not yet been clarified[27]. Currently, the interval is calculated based on the FLR regeneration and the control of remnant liver tumors. During the regeneration waiting period, interval chemotherapy might be used to control tumor progression[28].

The second stage is then performed, most commonly with hepatectomy of the contralateral lobe. The success of this method depends on the liver regeneration between the two stages, which allows for the second surgical step to be performed with a lower risk of post hepatectomy liver failure.

ALPPS

A new innovative surgical technique for TSH is portal vein ligation and *in situ* splitting of the liver parenchyma. This new approach was developed by Schnitzbauer *et al*[20] from Regensburg, Germany. It is used for patients with marginally resectable or initially nonresectable liver tumors, either primary or metastatic, to induce a rapid increase in FLR[20,29].

In ALPPS, the exact mechanism of the rapid liver regeneration is still not fully understood. Some pathophysiological mechanisms may explain this phenomenon. First, the portal vein ligation will lead to impairment of the bilateral portal flow and subsequently increase portal flow to the FLR; this will result in a redistribution of hepatotropic factors to the FLR. Second, a local regeneration stimulus is initiated after liver partitioning due to surgical trauma[30,31].

In preoperative MRI or CT scan-based volumetric planning, both TLV and FLR are determined and calculated by the radiologist using integrated software techniques. Calculation of the FLR/remaining liver volume (RLV) to TLV ratio (RLV-TLV, expressed as a percentage of the TLV) and the RLV to body weight ratio (RLV-BWR, expressed as a percentage of the body weight) is carried out. The ALPPS procedure is considered when RLV/TLV < 25% or RLV/BWR < 0.5% in patients with a normal liver, and when RLV/TLV < 30% and RLV/BWR < 0.8% is found for patients with diseased liver then the procedure is required[32,33].

ALLPS is a complex surgical procedure that consists of two major surgical stages and one interval phase.

In classical ALPPS, the stage 1 key steps are as follows: (1) Formal laparotomy and abdominal exploration to rule out any extrahepatic disease; (2) Complete liver mobilization, including ligation and division of the retrohepatic veins draining into the inferior vena cava and isolation of and encircling both the right hepatic vein and the middle hepatic vein with vessel loops; (3) Intraoperative ultrasonography to determine resectability and mark the partition plane; (4) Cholecystectomy; (5) If bilobular disease is present, complete tumor wedge resections of the FLR; and (6) Isolation of the right portal vein behind the common hepatic duct, followed by division of the portal supply of the diseased hemiliver. The partition of the liver parenchyma is continued until the retrohepatic vena cava is visualized. The right hepatic arterial in-flow and biliary drainage to the deportalized hemiliver are maintained during this first stage to preserve the liver synthetic function[34].

During the interval phase, the patients are kept in hospital on close monitoring.

Table 1 Contraindications of portal vain embolization

	Contraindications	
	Absolute	**Relative**
Portal vein embolization	Overt clinical portal vein hypertension	Mild portal vein hypertension
	Extensive invasion of portal vein precluding safe catheter manipulation	Tumor extension to the FLR
		Biliary dilatation of the FLR
	Complete lobar portal vein occlusion	Extrahepatic metastatic disease
		Uncorrectable coagulopathy
		Renal insufficiency

FLR: Future liver remnant.

Within 7–10 d after the operation, a contrast-enhanced abdominal CT scan is performed. If adequate FLR (> 30%) has been achieved and the patient is stable, stage 2 of the procedure is scheduled. In stage 2, the diseased deportalized liver is removed by stapling through the hilar plate followed by stapling of the right hepatic vein and then the middle hepatic vein.

Due to the complexity and concerning outcome, classical ALPPS has been modified in many centers to achieve better results. In the associating liver tourniquet and portal vein ligation for staged hepatectomy, a tourniquet is used to compress the future transection plane between the liver lobe that is going to be resect and the FLR, in lieu of *in situ* splitting of the liver parenchyma. The main advantages of this new technique are reduction in operative time in the first stage and in blood loss. In addition, segment IV is not separated from the hilar bifurcation, thereby helping to avoid ischemic necrosis[35].

The right ALPPS modification is used when the first stage consists of a left lateral sectionectomy, ligation of the right portal vein, and limited or non-anatomical multiple resections of the left, right anterior and caudate lobe lesions. *In situ* parenchyma splitting occurs along the right portal fissure. The second stage of this technique consists of completing the right posterior sectionectomy[36].

In rescue ALPPS modification, the first stage consists of *in situ* parenchyma splitting between the right and left liver lobes along the main portal fissure, where the right portal vein has been already embolized radiologically. The second stage consists of completing the resection of the right hemiliver. This technique is considered when the patients are not candidates for the second stage of classical TSH due to insufficient regeneration[36].

During the left ALPPS modification, the first stage consists of anatomical wedge or limited segmentectomy of the right anterior and posterior sections, left portal vein ligation, and *in situ* parenchyma splitting between the right and left lobes along the main portal fissure. The second stage consists of left hemihepatectomy with resection of segment 1[36].

Hybrid ALPPS was proposed by Li *et al*[37] as non-touch technique to treat tumor infiltration of the right portal vein or biliary bifurcation as part of ALPPS. The first stage consists of *in situ* splitting of the hemiliver *via* an anterior approach, followed by right PVE at the first day postoperatively. The final step is completing the second stage of ALPPS. This modified ALPPS could be considered for patients with tumor infiltration of the right portal vein. However, the drawback of this technique is longer operative time in the second stage.

Partial ALPPS was reported by Petrowsky *et al*[38] in 2015. The partial ALPPS differs from classical ALPPS by the performance of partial partitioning (50% to 80%, depending on the hepatic veins and the tumor location). Some reports of partial ALPPS cases have shown zero mortality and favorable postoperative outcome, especially after the first stage.

Different types of monosegment ALPPS hepatectomy have been described by Schadde *et al*[39]. Additionally, a nomenclature was proposed based on the segment of the liver remnant, rather than the segments of resected liver. This variation of the ALPPS technique represents a substantial change to the traditional paradigm of liver resectability, which is defined as the removal of tumors with negative margins and preserving ≥ 2 contiguous liver segments along with their in-flow, out-flow and biliary drainage.

Generally, some modified techniques of the ALPPS procedure have been shown to reduce mortality and morbidity but, due to insufficient data, they are still under

evaluation in current practice.

OUTCOMES

Technical success

For TSH, 76% of patients become candidates for the second stage of the procedure[40,41]. However, some patients fail to complete the second stage due to many contributing factors, disease progression being the most common (13%-35%). Other factors are inadequate liver regeneration (0%-4%) and poor patient condition (3%)[42].

Portal vein occlusion techniques are now routinely used in TSH to achieve microscopically negative resection. The PVE has a high technical success rate, approximately 100%; however, its technical failure has been mentioned in the literature[43,44]. The resection rate post-PVE in healthy liver can reach up to 85%, while in cirrhotic patients this rate is decreased to 70%. Failure of hypertrophy is rare but the degree of it is variable. The FLR hypertrophy ratio after PVE is 8%-25% in normal status liver. On the other hand, the FLR hypertrophy ratio is 6%-20% in cirrhotic livers[24,45].

ALPPS has been reported to increase the FLR volume by 74% in a mean of 9 d. This short interval between the two stages is due to a rapid and effective hypertrophy, as compared to the vascular occlusion techniques. Many studies have demonstrated that 95%-100% of patients who underwent the first stage of ALLPS then completed the second stage. Importantly, this represents a viable treatment choice to patients with otherwise nonresectable tumors[20,46-48]. Furthermore, the R0 resection rate has been reported to be between 86% and 100%[20,46-48].

Knoefel *et al*[49] compared the FLR hypertrophy of patients who underwent ALPPS, PVE, and combined procedures. The rates of FLR hypertrophy after PVE *vs* ALPPS were 43% and 63%, respectively. Moreover, the FLR hypertrophy in the ALLPS patients was achieved in 3 d. Currently, the international ALPPS registry shows a completion rate to second stage of around 100% on 553 patients from 84 centers around the world[50].

In summary, the ALPPS procedure can be an appropriate option to overcome the two main limitations of PVE and TSH. Specifically, these are failure or lengthy time required to achieve adequate liver remnant and the high rate of patient drop-off from completing the second resection.

Efficacy

As mentioned above, the majority of patients can complete the TSH. The remaining patients have a poor prognosis, with a median survival of 20.4 mo. The 3-year survival for patients who complete the second stage is 68% but only 6% for patients who do not. The 5-year survival rate is significantly different between the two groups, 49% and 0%, respectively[51-53].

ALPPS is relatively a new technique, and has shown disease-free survival (DFS) ranging from 73% to 95% at the median of 6 mo. The 1-year DFS is between 46% and 60%[46,48,54,55]. Oldhafer *et al*[56] reported that 86% of ALPPS-treated patients developed tumor recurrence at a median time of 8 mo. Schadde *et al*[46] reported a 1-year recurrence rate in ALPPS of 54%, as compared to 52% for TSH. Cancer-free resection has also been compared between ALLPS and the portal vein occlusion techniques; in one study, 79% of the patients in the ALLPS arm showed cancer-free resection, as compared with 58% in the portal vein occlusion arm.

Complications

Many complications have been reported for PVE, which are classified as percutaneous-related and PVE-related. The percutaneous-related complications are pneumothorax, vascular injury, and hemobilia. The PVE-related complications include non-target canalization and main portal vein thrombosis. Generally, PVE is a safe procedure, having 0% mortality and 2.2% morbidity[57].

The main downside of ALLPS is the associated high morbidity and mortality. ALPPS has shown rates of overall and major complications that are higher than for the TSH procedure[58]. In particular, the postoperative complications reported range between 33% and 64%, as compared to the range of 16% to 25% in TSH[20,48,58]. The higher rate of infections and biliary leaks after the first stage of ALPPS compared to TSH can explain this[59]. The results of another comparison between the two procedures, carried out by Shindoh *et al*[60], are summarized in Table 2.

The 90-d mortality of ALLPS and TSH was compared by Schadde *et al*[46]. He reported that at 15% in ALPPS and 6% in TSH. However, the 90-d mortality of ALLPS is variable in the literature. Schnitzbauer *et al*[20] reported a 12% 90-d mortality and

Table 2 Comparison between associated liver partition and portal vein ligation for staged hepatectomy and conventional two-staged hepatectomy

Complication	ALPPS	Conventional TSH
Major morbidity (Clavien-Dindo IIIA)	40%	33%
Bile leaks	24%	5.8%
Sepsis	20%	0%
Re exploration	28%	2.9%
Liver-related mortality	12%	5.8%

ALPPS: Associated liver partition and portal vein ligation for staged hepatectomy; TSH: Two-staged hepatectomy.

other series reported no 90-d mortalities.

Disease-related outcomes

Colorectal liver metastases (CRLM): Colorectal cancer commonly metastasizes to the lung and liver. Resection is considered the best treatment of liver metastasis. TSH was introduced in 2000 as an effective surgical approach in bilobar CRLM[14]. Since then, it has served to increase the number of patients who can go for liver resection, with an acceptable mortality and morbidity. More recently, in 2012, ALPPS was introduced to treat patients who are borderline or nonresectable CRLM[20].

In TSH, the reported 5- and 10-year survival rates are 40% and 30%, respectively[61]. The 1- and 2-year DFS rates were analyzed by Karoui *et al*[22] and reported to be 85% and 68%, respectively. However, more than 60% of these patients will have recurrence afterwards[61]. The reported 30-d mortality after TSH is 2.5% and the major morbidity rate is 19.6%[62].

In comparison to TSH, ALPPS has shown inferior results. ALPPS registry for patients with CRLM has showed that 1- and 2-year DFS are 59% and 41%, respectively[55]. In total, 86% of the patients who underwent ALPPS for CRLM had a tumor recurrence, with a median time of 8 mo[56].

HCC: Complete surgical resection is the only potentially curative intervention for HCC. Surgical resection improves the survival rate, increasing it to 9-13 mo from the no-intervention survival time of less than 3 mo.

TSH for HCC patients is not thoroughly investigated, as shown by the small body of literature, and hence no conclusion can yet be reached on its benefits in improving overall survival or DFS. On the contrary, ALPPS has been reported in HCC patients, particularly when there is vascular invasion. Torres *et al*[63] reported zero incomplete resection in HCC patients; a 1-year DFS of 87%, 90-d mortality of 12% and rate of high-grade complications (Clavien-Dindo complications IIIb or more) of 25%. Björnsson *et al*[64] reported no 90-d mortality in patients who underwent ALPPS for primary hepatobiliary malignancies (4 out of 10 patients had HCC). Two of the HCC patients were lost to follow-up, while two others had died within 6 mo with unclear reported cause of death. More studies are needed to draw a solid conclusion on the outcome of ALPPS in HCC patients.

CONCLUSION

Staged hepatectomy procedures, including TSH, PVE and ALPPS, are currently well-established and accepted in practice in the field of liver malignancy treatment. Collectively, they have increased the number of patients who are eligible for liver resection from among those who otherwise are labeled as nonresectable. It has been demonstrated, as mentioned earlier, that this advancement has improved the DFS and overall survival as well.

The drawback of these extensive surgical interventions is the higher rate of complication and mortality. It is worth mentioning that these rates have improved from the time they were first advocated. This improvement is probably related to a better selection of patients who are accepted for these procedures. Moreover, the experience level of the healthcare center and its surgeons are paramount factors in these achievements.

We believe that these kinds of advanced intervention techniques and procedures should be carried out in high-flow centers through use of a multidisciplinary approach. More studies and reports are awaited to standardize the future practice and

to minimize the related adverse events.

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ORIGINAL ARTICLE

Basic Study

Proton pump inhibitors increase the severity of hepatic encephalopathy in cirrhotic patients

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Abstract

BACKGROUND

Liver cirrhosis is the late stage of hepatic fibrosis and is characterized by portal hypertension that can clinically lead to decompensation in the form of ascites, esophageal/gastric varices or encephalopathy. The most common sequelae associated with liver cirrhosis are neurologic and neuropsychiatric impairments labeled as hepatic encephalopathy (HE). Well established triggers for HE include infection, gastrointestinal bleeding, constipation, and medications. Alterations to the gut microbiome is one of the leading ammonia producers in the body, and therefore may make patients more susceptible to HE.

AIM

To investigate the relationship between the use of proton pump inhibitors (PPIs) and HE in patients with cirrhosis.

METHODS

This is a single center, retrospective analysis. Patients were included in the study with an admitting diagnosis of HE. The degree of HE was determined from subjective and objective portions of hospital admission notes using the West Haven Criteria. The primary outcome of the study was to evaluate the grade of HE in PPI users *versus* non-users at admission to the hospital and throughout their hospital course. Secondary outcomes included rate of infection, gastrointestinal bleeding within the last 12 mo, mean ammonia level, and model for end-stage liver disease scores at admission.

Intercept, Tobira, Signablock and Gilead. GS is a consultant for TerraFirma, Glympse, Quest Diagnostics, Allergan, Arrow Diagnostics, Salix and GLG. No other potential conflicts of interest relevant to this article were reported.

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RESULTS

The HE grade at admission using the West Haven Criteria was 2.3 in the PPI group compared to 1.7 in the PPI nonuser group (P = 0.001). The average length of hospital stay in PPI group was 8.3 d compared to 6.5 d in PPI nonusers (P = 0.046). Twenty-seven (31.8%) patients in the PPI user group required an Intensive Care Unit admission during their hospital course compared to 6 in the PPI nonuser group (16.7%) (P = 0.138). Finally, 10 (11.8%) patients in the PPI group expired during their hospital stay compared to 1 in the PPI nonuser group (2.8%) (P = 0.220).

CONCLUSION

Chronic PPI use in cirrhotic patients is associated with significantly higher average West Haven Criteria for HE compared to patients that do not use PPIs.

Key words: Cirrhosis; Hepatic encephalopathy; Proton pump inhibitors; Hepatology; Proton pump inhibitor

Core tip: In this study, we investigate whether proton pump inhibitor (PPI) use in hepatic encephalopathy patients predisposes them to more severe stages of hepatic encephalopathy as per West Haven Criteria. We found that chronic PPI use in cirrhotic patients is associated with significantly higher average West Haven Criteria for hepatic encephalopathy compared to patients that did not use PPIs. Our data also indicated that cirrhotic patients on PPIs have longer hospital stays, with increased morbidity and mortality during their hospital stays.

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INTRODUCTION

Liver cirrhosis is a late stage of hepatic fibrosis and is characterized by portal hypertension that can clinically lead to decompensation in the form of ascites, esophageal/gastric varices or encephalopathy. There are multiple etiologies of liver cirrhosis, with Hepatitis C, alcoholic hepatitis/alcoholic liver disease and non-alcoholic fatty liver disease being the most common causes in the developed world[1]. Some of the most common sequelae associated with liver cirrhosis are neurologic and neuropsychiatric impairments labeled as hepatic encephalopathy (HE). Neuropsychiatric changes associated with liver disease were first described by Adams and Foley in the 1940s and 1950s[2]. Since then, our understanding of what HE entails and what precipitates it has only marginally grown. According to the currently accepted definition, HE is a neuropsychiatric disorder that can encompass a broad spectrum of presentations summarized in the West Haven Criteria Severity Scale. HE spans from minimal to Grade I (mild confusion, disordered sleep), through Grades II (lethargy, moderate confusion), III (marked confusion, incoherent speech) and finally Grade IV (coma)[3,4].

While liver cirrhosis can predispose a patient to HE, there are additional triggers that can precipitate it or worsen its severity. Well established triggers include infection, gastrointestinal (GI) bleeding, constipation, and medications such as opioids and benzodiazepines[5-8]. New studies have cited other etiologies, including changes in gut flora and small bowel bacterial overgrowth[9,10]. More recently, there have been studies on the role of proton pump inhibitors (PPIs) in contributing to HE in cirrhotic patients. PPIs are commonly prescribed for many GI diseases, most commonly gastroesophageal reflux disease (commonly known as GERD), peptic ulcer disease, and gastritis[11]. In contrast to previous beliefs, recent data suggests that PPIs have the potential for multiple adverse effects. PPIs act by decreasing gastric acid secretion, which is believed to be protective against acid-related mucosal injury in the

stomach[12]. It was thought that their ability to protect the GI mucosa would mitigate the number of GI bleeds in cirrhotic patients, therefore reducing their risk of HE. However, new studies show that in addition to their direct effects in the stomach, PPIs may affect composition of the gut microbiome while also promoting small bowel bacterial overgrowth[13].

Normally, nitrogenous compounds formed by the gut are drained into the portal system and filtered by the liver[14]. These compounds then enter the urea cycle and are excreted in urine. However, in patients with liver disease, ammonia clearance is compromised due to reduced liver function and increased portosystemic shunting, leading to high levels of ammonia in the blood stream. When ammonia reaches the brain, it is metabolized by astrocytes and transformed from glutamate to glutamine *via* glutamine synthase. Accumulation of glutamine increases intracellular oncotic pressure, leading to cerebral edema. In patients with chronic liver disease, this cerebral edema can be subtle, and at this time, the edema alone does not explain all the findings of HE[15-17]. However, the morphological changes seen with astrocyte swelling are similar to the changes seen in Type II Alzheimer's disease[18]. Therefore, given the current mechanisms, it appears that ammonia levels (and subsequently astrocyte glutamine levels) have an overall neurotoxic effect.

Studies have shown that an increased gastric pH allows for increased gut microflora. In turn this can lead to increased bacterial translocation. Microflora species such as *Salmonella*, *Campylobacter jejuni, Escherichia coli*, *Clostridium difficile*, *Vibrio cholerae* and *Listeria* all appear to proliferate in high gastric pH[13]. In addition, the literature suggests that more severe bacterial proliferation such as small intestinal bacterial overgrowth has also been linked with gastric hypochlorhydria secondary to prolonged PPI use. Overall, it does appear that elevation of gastric pH allows for greater gut bacterial proliferation. Increased proliferation is not without consequence, as the gut microbiome is one of the leading ammonia producers in the body, and therefore may make patients more susceptible to HE, which is what we believe to be the driving force behind our findings. This would partly explain why rifaximin, a poorly absorbable synthetic antibiotic, can lower the risk of HE in cirrhotic patients by affecting the gut microbiota. Given that changes in gut flora may lead to worse HE, the role of PPIs must be reconsidered. This study investigates whether PPI use in HE patients predisposes them to more severe stages of HE as per the West Haven Criteria.

MATERIALS AND METHODS

Patient selection

This retrospective medical chart review was conducted at the UMass Memorial Medical Center. Records for patients who presented with acute HE between January 1, 2012 and January 1, 2016 were reviewed. Patients were included in the study with an admitting diagnosis of HE with and without coma with ICD-9 code 572.2 and ICD-10 codes K72.00 and K72.01.

Eligible patients were ≥ 18 years of age, had prior history of End Stage Liver Disease or cirrhosis as determined by consistent image findings and/or liver biopsy. Patients were on PPIs for a minimum of 30 d prior to hospital admission. Exclusion criteria included pregnancy, current prisoner, failure to sign consent, and concomitant diagnosis of human immunodeficiency virus.

Data collection

Utilizing medical record and data from Electronic Health Records, demographics (age, sex), grade of HE, Model End Stage Liver Disease (MELD) score, Length of stay, etiology of cirrhosis, concomitant infection, ammonia level, history of bleeding in the last 12 mo, etiology of HE, intensive care unit (ICU) stay, and patient expiration, were collected. The degree of HE was determined from subjective and objective portions of hospital admission notes using the West Haven Criteria. Grade I included lack of trivial awareness, presence of euphoria and/or anxiety, shortened attention span and/or altered sleep rhythm. Patients met Grade II if they were lethargic, had personality changes, disorientation to time, dyspraxia and/or asterixis on physical exam. Grade III encephalopathy included confusion, disorientation to space, somnolence or signs of semi-stupor. Finally, Grade IV was defined as coma. The institutional review board at UMass Medical School/UMass Memorial Medical Center approved this study.

Definition of events and study outcomes

The primary outcome of the study was to evaluate the grade of HE in PPI users *versus*

non-users at the time of admission to the hospital and throughout their hospital course. Secondary outcomes included rate of infection, GI bleeding within the last 12 mo, mean ammonia level, and MELD scores at admission.

Statistical analysis

Data was analyzed using R version 3.3.2 GUI 1.68 Mavericks built for Mac computer. The statistical significance of between-cohort differences in categorical variables was tested using the chi-square test and in continuous variables using the two-sample t-test. All tests were two-tailed with a significance level of $P < 0.05$. Multivariate analysis using a linear regression model was applied to primary and secondary endpoints to determine statistically significant differences between PPI users and non-users. The threshold for statistical significance was set at P values < 0.05.

RESULTS

Demographics and clinical characteristics

A total of 103 patients were included in this study from UMass Memorial Medical Center between January 2013 and December 2016. All patients had been diagnosed with liver cirrhosis based on imaging studies (U/S, computer scanning or magnetic resonance imaging) or liver biopsy and evidence of portal hypertension based on clinical signs, imaging or portal pressure measurement. Seventy-five (73%) of these cirrhosis patients were taking PPIs (PPI user), while twenty-eight (27%) patients with cirrhosis were not taking PPIs prior to enrollment (PPI non-user). The mean age of patients included in this study was 58.3 years, with the PPI user group being 59.6 years and in the PPI nonuser group being 55.3 years ($P = 0.044$). With regards to gender, males represented 54 (63.5%) patients in the PPI user group and 17 (47.2%) in PPI nonuser group ($P = 0.143$). Sixty-three (74.1%) patients were on lactulose in the PPI user group compared to 9 (80%) in the PPI nonuser group ($P = 0.599$).

Primary outcomes

The primary outcomes of this study were the grade of HE and hospital course for PPI users compared to non-users. The grade of HE using the West Haven Criteria was 2.3 in the PPI group compared to 1.7 in the PPI nonuser group, which represented a statistically significant difference ($P = 0.001$) (Table 1). With regards to hospital course, several outcomes were analyzed. The average length of hospital stay in the PPI group was 8.3 d compared to 6.5 d in PPI nonusers ($P = 0.046$). Twenty-seven patients (31.8%) in the PPI user group required an ICU admission during their hospital course compared to 6 in the PPI nonuser group (16.7%) ($P = 0.138$). Finally, 10 (11.8%) patients in the PPI group expired during their hospital stay compared to 1 in the PPI nonuser group (2.8%) ($P = 0.220$).

Secondary outcomes

Several secondary outcomes including infections, serum ammonia levels, MELD and GI bleeding were measured to further determine the effects of long-term PPI use in the cirrhotic population. With regards to infections, 5 patients (5.9%) in the PPI group developed *Clostridium difficile* compared to 0 in the PPI nonuser group (0%) ($P = 0.324$). Ten patients (11.8%) of the PPI group developed pneumonia compared to 1 in the PPI nonuser group (2.8%) ($P = 0.220$). Five patients in the PPI group developed spontaneous bacterial peritonitis compared to 4 in the PPI nonuser group (11.1%) ($P = 0.533$). The mean ammonia level of the PPI group on admission to the hospital was significantly higher, 67.8 mg/dL compared to 45.5 mg/dL in the PPI non-user group ($P = 0.095$). The mean MELD for the PPI group was 19.7 compared to 20.3 in the PPI nonuser group ($P = 0.687$). Twenty-six patients (30.6%) in the PPI group were admitted to the hospital for a GI bleed within the year prior to admission compared to 13 (36%) in the PPI nonuser group ($P = 0.703$) (Table 2).

Linear regression model

The multiple linear regression models showed that PPI use was associated with a higher grade of HE in cirrhosis compared to no PPI use. After adjustment for age, sex, MELD score, and lactulose use, the association between PPI use and HE grade was maintained ($P < 0.001$), with a beta of 0.607 and standard error of 0.179. In addition, a higher MELD score was also associated with a higher HE grade, with a beta of 0.024 and standard error of 0.011 ($P = 0.041$) (Table 3).

DISCUSSION

Table 1 Grade of hepatic encephalopathy in proton pump inhibitor users *versus* nonusers

Grade of HE	PPI user	PPI nonuser
	n = 75	*n* = 28
Grade 1	15 (20.0)	11 (39.3)
Grade 2	32 (46.6)	13 (46.4)
Grade 3	18 (24.0)	4 (14.3)
Grade 4	10 (13.4)	0 (0)

n (%), Grade of hepatic encephalopathy (HE) is defined by the West Haven Criteria Severity Scale for HE: Grade I (mild confusion, disordered sleep), II (lethargy moderate confusion), III (marked confusion, incoherent speech), IV (coma). HE: Hepatic encephalopathy; PPI: Proton pump inhibitor.

Because of their effectiveness in suppressing gastric acid secretions, PPIs have become one of the most commonly prescribed drug classes with annual expenditures in 2009 estimated at $13 billion in the United States and $24 billion worldwide[19]. The first PPI available was omeprazole [Prilosec, Prilosec OTC, Zegerid, Zegerid OTC Losec in Canada], which served as a basis for all other PPIs in its mechanism of action by causing irreversible inhibition of H^+/K^+ ATPase, therefore halting hydrogen ion expulsion into the gastric lumen. While many studies have confirmed PPIs to be safe, our study indicates that in cirrhosis patients, the use of PPIs is associated with worsened hospital outcomes.

In this study, we found that hospitalized cirrhotic patients on a PPI had a significantly higher average West Haven Criteria for HE (score of 2.3) compared to patients who were not on a PPI (scored an average of 1.7, $P = 0.001$). Using linear regression models, we showed that patients using PPIs had a higher West Haven Criteria grade HE regardless of age, sex, MELD score, and/or lactulose use. Other statistically significant differences between the PPI user and non-user groups included longer length of hospital stay (8.5 d for PPI users *vs* 6.5 for PPI nonusers, $P = 0.046$). In alignment with patients having a higher grade of HE as well as a longer length of hospital stay, a greater percentage of patients in the PPI user group also had an ICU admission, indicating the greater extent of systemic involvement in this group. A recent meta-analysis by Bian *et al*[20] supports our contention that there is a higher risk of developing HE in PPI users with liver dysfunction.

Prior studies have also indicated that PPI use could worsen HE in cirrhotic patients. A dose response analysis by Tsai *et al*[21] stratified patients based on length of PPI use and showed that longer PPI use led to higher rates of HE. The result remained statistically significant after adjustment of patient comorbidities. Hung *et al*[22] showed that cirrhotic patients on a PPI with HE had higher mortality rates at 30 d, 90 d and one year compared to cirrhotic patients with HE not on PPIs. This study investigates whether PPI use in HE patients predisposes them to more severe stages of HE as defined by the West Haven Criteria. Our analysis shows that patients on a PPI had significantly higher West Haven Criteria scale episode of HE compared to those not on a PPI (2.3 *vs* 1.7, $P = 0.001$). In addition, our study shows that PPIs predispose cirrhotic patients towards worsened encephalopathy regardless of age, sex, MELD score, or lactulose use.

The exact pathophysiology of HE is still not fully understood. Multiple mechanisms of action have been hypothesized and investigated, including the role of ammonia, increased GABA receptors in the brain, and accumulation of endogenous opioids[23]. Overall, it appears that HE is multifactorial, with accumulation of ammonia being a leading cause of overt HE[24]. In fact, studies have shown that HE ammonia levels are increased in 90% of patients. The primary source of ammonia in the body is the GI tract as a byproduct of chronic bacterial colonization, by enterocytes as they transform glutamine into ammonia, and by *H. pylori*, which metabolizes urea into ammonia. However, *H. pylori*'s role in HE is still unclear[25,26].

One of the secondary endpoints in this study was determining the risk for infection in patients with cirrhosis on a PPI. Our data shows that patients on a PPI may have higher rates of *C. difficile* infection, pneumonia and spontaneous bacterial peritonitis. However, these results were not statistically significant with *P*-values of 0.324, 0.220 and 0.533, respectively. This is thought to be due to this study's small sample size of 103 patients. A recent meta-analysis by Lambert *et al*[27] again demonstrated the association of community acquired pneumonia and *Clostridium difficile*-associated diarrhea (CDAD) with the use of PPI. The most likely pathogenesis of the development of these infections has been attributed to direct acid suppression in the

Table 2 Participant characteristics

Variables	Total, *n* = 103	PPI user, *n* = 75	PPI nonuser, *n* = 28	*P* value
Age, yr, Mean ± SD	58.3 (10.8)	59.6 (10.6)	55.3 (10.7)	0.044[a]
Sex, male, *n* (%)	71 (58.7)	54 (63.5)	17 (47.2)	0.143
On lactulose, *n* (%)	92 (76)	63 (74.1)	29 (80)	0.599
Bleeding in last 12 mo, *n* (%)	39 (32.2)	26 (30.6)	13 (36.1)	0.703
Infection, *n* (%)				
Clostridium difficile colitis	5 (4.1)	5 (5.9)	0 (0)	0.324
Pneumonia	11 (9.1)	10 (11.8)	1 (2.8)	0.220
Spontaneous bacterial peritonitis	9 (7.4)	5 (5.9)	4 (11.1)	0.533
Serum ammonia level, Mean ± SD	61.1 (67.2)	67.8 (67.8)	45.5 (64.2)	0.095
Grade of hepatic encephalopathy, Mean ± SD	2.1 (0.9)	2.3 (0.9)	1.7 (0.7)	0.001[b]
MELD score, Mean ± SD	19.9 (7.2)	19.7 (7.4)	20.3 (6.7)	0.687
Length of stay in d, Mean ± SD	8.5 (7.0)	8.3 (7.9)	6.5 (3.7)	0.046[a]
Required ICU, *n* (%)	33 (27.3)	27 (31.8)	6 (16.7)	0.138
Expired, *n* (%)	11 (9.1)	10 (11.8)	1 (2.8)	0.220

[a]$P < 0.05$,
[b]$P < 0.01$. MELD: Model for end-stage liver disease; ICU: Intensive care unit.

stomach and small bowel. With regards to CDAD, Janarthanan *et al*[28] suggested that the alkaline status of the stomach (pH > 5) likely predisposes the patient to enhanced survival of *C. difficile* vegetative spores. A recent study in 2018 by Naito *et al*[29] confirms our notion that continued PPI use leads to intestinal dysbiosis. Using 16S rRNA gene sequencing, PPIs were found to significantly increase certain enteric microbe taxonomy, including *Streptococcaceae* and *Enterococcaceae*, which are risk factors for CDAD, and to decrease *Faecalibacterium*, a commensal anti-inflammatory microbe present in human models.

Our paper has several limitations. First, because it is a retrospective review, information collection is incomplete, particularly regarding follow-up evaluation. Secondly because, we had an uneven distribution of the number of patients in the PPI use and non-use groups. Finally, due to the small sample size used for our study, several of our secondary outcomes were not statistically significant, including several infections and ICU admission rate, likely secondary to a lack of power. Again, as with any retrospective study, it is important to note that this type of study is unable to define exact causality. Further randomized, controlled, prospective studies are needed to help confirm the observation seen in our study.

In conclusion, PPIs are commonly prescribed for many GI diseases including GERD, peptic ulcer disease, and gastritis. They are often used without regard for their adverse effects. Our study demonstrates that PPI use in cirrhotic patients is associated with more severe degree of HE compared to those not on a PPI. Our data also showed that PPI use in this population was associated with a longer hospital stay and higher percentage of patients requiring an ICU admission. We suggest reducing PPI use in the cirrhotic population as a means to reduce episodes of HE. Further randomized-controlled, prospective studies are needed to help confirm this observation.

Table 3 Linear regression models, grade of hepatic encephalopathy

Variables	B ± SE	*P* value
Model 1, demographic variables		
Age	-0.001 ± 0.001	0.871
Sex	0.062 ± 0.167	0.710
PPI use	0.607 ± 0.180	0.001[b]
Model 2, medical comorbidities		
Age	0.002 ± 0.008	0.787
Sex	0.043 ± 0.166	0.797
MELD Score	0.020 ± 0.011	0.079
PPI Use	0.607 ± 0.179	< 0.001[b]
Model 3, other medications		
Age	0.004 ± 0.008	0.647
Sex	0.033 ± 0.164	0.839
MELD Score	0.024 ± 0.011	0.041[a]
Lactulose	0.324 ± 0.189	0.089
PPI use	0.625 ± 0.178	< 0.001[b]

[a]$P < 0.05$,
[b]$P < 0.01$. B ± SE: beta ± standard error. MELD: Model for end-stage liver disease; PPI: Proton pump inhibitor.

ARTICLE HIGHLIGHTS

Research background

Proton pump inhibitors (PPIs) are a recent hot topic in both internal medicine and gastroenterology, mostly because of their widespread use. Studies are quickly demonstrating that these medications may not come without risk, as recent studies have demonstrated a clear association between PPI and conditions like osteoporosis, pneumonia, *Clostridium difficile*, and some even postulate an association with dementia. While many effects of PPIs are still in question, it has also been shown that PPIs work by acid suppression, which can disrupt the gut microbiome. Patients with cirrhosis are at risk to develop hepatic encephalopathy (HE), primarily through ammonia produced by typical gut flora, and could subsequently be at risk for changes in this condition if the microbiome is altered in any way.

Research motivation

The main topic we are trying to address is whether PPI overuse can lead to additional effects aside from those previously mentioned and described in the literature. One particularly vulnerable population is those with cirrhosis, as ammonia production is affected by the gut microbiome. Solving this problem would allow future therapeutics to focus on the gut-liver-microbiome axis to prevent or lessen the severity of HE.

Research objectives

The main objective we want to demonstrate is the effect of PPI on the degree of HE. We hope to draw an association between PPIs and HE to encourage further prospective research studies on the side effects of PPIs, the gut microbiome in relation to HE, and to further aid in hospital outcomes for patients with cirrhosis.

Research methods

This is a retrospective analysis of patients with liver cirrhosis who were admitted with an ICD-9 and/or ICD-9 diagnosis of HE. Once these patients were identified, a chart analysis was performed to determine if these patients were on a PPI for > 30 d prior to their hospital admission. Those who were on a PPI for > 30 d were compared to patients who were not on a PPI at all in relation to their hospital stay. A linear regression model was applied to all patients to confirm the absence of any confounding variables.

Research results

During our analysis, we found that patients on a PPI who were admitted with HE subsequently had a significantly longer hospital stay, significantly worse grade of HE, and a larger percentage of those had intensive care unit (commonly known as ICU) admissions during their hospital stay. These findings suggest that patients should be assessed for the need for PPIs at every visit. This also points to the gap in knowledge between PPI and HE, especially if future research is able to demonstrate changes in the gut microbiome in patients on PPIs.

Research conclusions

In summary, in this retrospective medical chart review, PPI use was shown to be associated with worsened HE, greater length of hospital stays, and higher rate of ICU admissions in cirrhotic patients. To our knowledge, this is the first study that demonstrated that PPI use is associated with worse grades of HE, whereas prior studies by Tsai *et al* and Hung *et al* demonstrated higher risk of HE and overall higher mortality, respectively, in an Asian population. We propose that PPI use might affect cirrhotic patients by altering gastric pH, leading to the proliferation of gut micro-biome, thereby increasing ammonia production and bacterial translation. Considering the recent increased prevalence of PPIs, this study provides clinically relevant information regarding their potential risks in the cirrhotic population.

Research perspectives

As a retrospective review, our study is limited by incomplete data collection and uneven distribution of PPI user and non-user groups. However, the observation that PPI users experience worsen HE and longer hospital stays is clinically important. Future randomized-controlled studies will help confirm this observation and guide clinicians in a shift away from the use of PPI in cirrhotic patients.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Efficacy of long-term rifaximin treatment for hepatic encephalopathy in the Japanese

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Abstract

BACKGROUND

Hepatic encephalopathy (HE) is a complication of liver cirrhosis and can result in neuropsychological and neuromuscular dysfunctions in patients. Rifaximin, an antibiotic, has been reported to decrease the occurrence of overt HE and also improve cognitive function in studies from Europe and the United States of America. There is not enough evidence of the relationship between the long-term use of rifaximin and its clinical effects in the Japanese.

AIM

To determine the clinical effects of long-term rifaximin therapy in decompensated liver cirrhosis patients, with overt HE or hyperammonemia.

METHODS

In this single-center retrospective observational cohort study, we reviewed the data of 38 patients who had taken rifaximin at the dose of 1200 mg/d for more than 24 wk. The primary outcome measured was the efficacy of long-term rifaximin use, and secondary outcome measured was the safety of its long-term use as determined by its influence on portosystemic shunts as well as *Escherichia coli*-related infections. Moreover, we compared the prognosis between the rifaximin group and control cases, matched for hepatic elasticity assessed by magnetic resonance ela-stography, age, and Child-Pugh classification.

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RESULTS

Of the 38 patients included in the study, 12 (31.6%) had overt HE, 27 (71.1%) had complications of esophageal varices, and 9 (23.7%) had hepatocellular carcinoma (HCC). The control group was matched for age, Child-Pugh classification, liver stiffness, and presence of HCC. The median of serum ammonia level before treatment was 104 μg/dL (59-297), and 2 wk after treatment, it significantly decreased to 85 μg/dL (34-153) (P = 0.002). A significantly low value of 80.5 μg/dL (44-150) was maintained 24 wk after treatment. The long-term use of rifaximin did not cause a decline in liver function. Diarrhea occurred in 2 patients, who improved with the administration of probiotics, and there were no cases of aborted rifaximin therapy owing to adverse events. In patients with Child C, the survival was short, but there was no significant difference compared with that of the control group.

CONCLUSION

Rifaximin therapy improves overt HE. The long-term use of rifaximin in the Japanese is effective and safe.

Key words: Hepatic encephalopathy; Rifaximin; Hepatic cirrhosis; Spontaneous portosystemic shunt; Magnetic resonance elastography; Child-Pugh classification

Core tip: This study evaluated the efficacy and safety of long-term rifaximin therapy for hepatic encephalopathy in the Japanese population. Serum ammonia level was maintained at a significantly low median value of 80.5 μg/dL (range, 44-150 μg/dL) at 24 wk after treatment, and the long-term use of rifaximin did not worsen liver function.

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INTRODUCTION

Hepatic encephalopathy (HE) is a complication seen in liver cirrhosis patients, which causes neuropsychological and neuromuscular dysfunction in varying degrees[1].

HE is classified into two types: minimal HE that presents as a slight abnormality and cannot be proved except by a neuromuscular examination, and overt HE that causes obvious disorders of consciousness and motor activity[1,2].

It has been reported that once overt HE occurs, the prognosis is so poor that the mortality rate within 1 year is 64% and that within 5 years is 85%. Overt HE as well as minimal HE, which are present in 80% of liver cirrhosis patients, can impair the prognosis and increase the risk of hospitalization[3].

Conventionally in Japan, the first-choice treatment for HE is synthesized disaccharides or branched-chain amino acids, and poorly absorbed antibiotics have not been approved by the health insurance. Rifaximin has been available for treatment since November 2016. Rifaximin is an oral antibiotic which decreases the ammonia-producing enteric bacteria[4,5].

Studies in Europe and the United States of America have reported on the clinical effects of rifaximin. It prevents the occurrence and recurrence of overt HE and decreases the rate of hospitalization because of HE as a long-term effect[6,7]. Also, a study on minimal HE reported that the use of rifaximin 550 mg for 8 wk improved cognitive function test performance as well as white matter integrity, as found on functional magnetic resonance imaging[8]. A meta-analysis that compared rifaximin with synthesized disaccharides reported that both were effective to the same extent in improving HE[9]. A randomized, double-blind controlled trial that compared the results of treatment with rifaximin plus lactulose therapy with lactulose therapy alone showed that significantly more cases experienced a complete recovery from HE in the rifaximin plus lactulose group than that in the lactulose only group. Moreover,

rifaximin plus lactulose decreased the mortality rate and death from sepsis, and shortened hospitalization[10]. Various factors influence the development of HE[11], and spontaneous portosystemic shunts are also related to its occurrence. Moreover, it has been noted that larger the size of the shunt, the higher the possibility for HE to recur[12].

The efficacy of rifaximin therapy for 10 wk has been proved in the Japanese population[13]. However, there are no reports on the relationship between the long-term use of rifaximin and its clinical effects and the size of the shunts. In this retrospective cohort study, we clarified the efficacy and safety of long-term use of rifaximin, and investigated the influence of shunts, *Escherichia coli (E. coli)*-related infection, and the prognosis in the rifaximin group compared with that in the control group comprising cases of liver cirrhosis matched for magnetic resonance elastography (MRE), age, Child-Pugh classification, and the presence of hepatocellular carcinoma (HCC). We evaluated the risk factors due to which the serum ammonia levels remained above 80 μg/dL at 24 wk after initiating rifaximin treatment.

MATERIALS AND METHODS

Patients

This was a single-center retrospective observational cohort study. The subjects were 38 consecutive patients with liver cirrhosis who were administered rifaximin continuously for more than 24 wk at our institute between April 2017 and July 2018. The primary outcome evaluated was the efficacy of long-term rifaximin use, and the secondary outcomes were the influences of spontaneous portosystemic shunts and *E. coli*-related infection. It was reported that liver stiffness measured by MRE was related to the recurrence of HCC, liver decompensation, and overall survival, and we compared the clinical prognosis between the rifaximin and control groups, pair-matched from 451 patients with chronic hepatic disease who were not given rifaximin, and measured the MRE from September 2016 to April 2018 using the covariates for liver stiffness based on MRE, age, Child-Pugh classification, and the presence of HCC.

The mortality rate as well as the incidence of complications of liver cirrhosis is influenced by whether the maximum size of the portosystemic shunt is over 8 mm or under 8 mm[14].

Moreover, the smallest size of a symptomatic portosystemic shunt reported in the literature is 8 mm[15]. Based on these reports, we classified the portosystemic shunts over 8 mm into the large group, and those under 8 mm into the small group. There was no set standard for initiating treatment with rifaximin.

This study was approved by the Ethics Committee of the Southern-Tohoku General Hospital and was conducted according to the guidelines of the 1975 Declaration of Helsinki, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

Assessment of MRE

3.0-T imagers (GE Healthcare, Milwaukee, WI) were used for all MRE examinations, and the condition was set as the report[16-18]. We fused the T2 image and the elastogram and measured the stiffness in 3 regions where the wave image was good and the region of interest (ROI) was set as broad as possible avoiding the liver surface, vessels, tumor, cross-hatching, hot spots, and dark spots.

MRE was measured at the beginning of the rifaximin treatment.

Assessment of portosystemic shunts

Contrast-enhanced CT images taken during the follow-up of HCC were assessed. The instrument used was the 64-detector MDCT Light Speed VCT (GE Healthcare, UK Ltd.) and the medium was Iomeprol 350 1.8 mL/kg (630 mg I/kg); the time taken for injecting the medium was 30 s, and the range was from the supradiaphragmatic to the subpubic region with 0.5-mm slices in the portal phase. The measurement was assessed with 5-mm slices in the axial dimension, and coronal reconstructed images in 40-mm partial maximum intensity projection (MIP). When a case had a plural system of shunts, the maximum diameter of the largest shunt was measured.

Statistical analysis

Fisher's exact test or the Kruskal-Wallis exact test was used to compare categorical variables, and the Mann-Whitney *U*-test was used to compare median values of continuous variables. Wilcoxon signed-rank test was used to compare paired continuous variables. To evaluate the overall survival in the rifaximin group, the control group was chosen using MRE as the measure of comparison. As the outcome

parameters may be influenced by patient selection in the rifaximin group and/or the control group, we performed propensity score matching. A multivariable logistic regression model was used to develop the propensity score, which included the following parameters: MRE, age, Child-Pugh classification, and the presence of HCC. Death was calculated using the Kaplan-Meier method and compared using the log-rank test. Survival duration was measured from the time of index date until either death or the date of the last follow-up visit for patients who remained alive. Statistical comparisons were performed using the IBM SPSS software program (IBM Corp., Armonk, NY, United States) and *P*–values of < 0.05 by a two-tailed test were considered statistically significant.

RESULTS

Patient characteristics

The background of the patients is summarized in Table 1. Rifaximin treatment was given to 38 patients with decompensated liver cirrhosis. Of these, 12 patients (31.6%) had a history of overt HE, 27 patients (71.1%) had complications associated with esophageal varices, and 9 patients (23.7%) had HCC. In 27 cases (71.1%), rifaximin was added to lactulose, and in 24 cases, branched-chain amino acid supplementation was used in combination. We compared the control group matched for age, Child-Pugh classification, liver stiffness measured by MRE, and the presence of HCC with the rifaximin group.

Outcomes

Serum ammonia levels were significantly decreased 2 wk after the beginning of rifaximin compared to the pretreatment levels (P = 0.002), and it remained significantly lower for up to 60 wk. Decrease was noted at all the points after the start of the treatment when compared with the ones before it.

There were 3 recurrence cases of overt encephalopathy: 2 occurred during drainage of the hepatic hydrothorax and 1 followed the self-interruption of rifaximin therapy.

There were no cases of spontaneous bacterial peritonitis (SBP) during the process (Figure 1).

Safety of long-term use of rifaximin

There were no significant differences in the blood albumin level, total bilirubin, prothrombin time, and platelet count 24 wk after the treatment. Rifaximin did not worsen the functionality of the liver. Adverse events included 2 cases of diarrhea (5.3%), which improved following the intake of probiotics. There were no cases of treatment interruption due to adverse events.

Portosystemic shunts

There were 2 cases without any shunts (5.3%), 8 cases with one systemic shunt (21.1%), 15 cases (39.5%) with two, 12 cases (31.6%) with three, and 1 case (2.6%) with four systemic shunts.

Effect of rifaximin based on shunt diameter

There were 17 (44.7%) cases with shunts over 8 mm who had a median serum ammonia value of 112 (93-297) μg/dL before rifaximin treatment, which decreased to 86 (54-150) μg/dL after 24 wk, with a significant difference (P = 0.002). On the other hand, there were 19 cases (50%) with shunts below 8 mm whose median serum ammonia value was 99 (59-184) μg/dL before the treatment and 67 (44-148) μg/dL after the treatment, with a significant difference (P = 0.037).

Effect of rifaximin based on number of systemic shunts

There were 28 cases (73.7%) with multiple shunts with a median serum ammonia value of 104 (69-297) μg/dL before treatment, which decreased to 80.5 (44-150) μg/dL after 24 wk, with a significant difference (P < 0.001).

There were 10 cases (26.3%) with no or only one shunt with a median serum ammonia value of 111.5 (59-184) μg/dL before treatment, which decreased to 81 (45-148) μg/dL after 24 wk (P = 0.375).

Risk factors for insufficient improvement of serum ammonia levels after rifaximin treatment

Univariate analyses revealed that the insufficient improvement of serum ammonia level (below 80 μg/dL at 24 wk) was associated with two variables: maximum shunt diameter and the albumin-bilirubin (ALBI) score. These factors were evaluated in the multivariate analyses, which revealed that the insufficient improvement in the serum

Table 1 Patient baseline characteristics

	Rifaximin (*n* = 38)	Control (*n* = 38)	*P* value
Age (yr)	69 (40-85)	69.5 (42-90)	0.934
Sex (female/male)	17 (44.7%)/21 (55.3%)	11 (28.9%)/27 (71.1%)	0.234
BMI (kg/m^2)	24.78(18.67-35.88)	23.1 (15.5-30.1)	0.035
Etiology (HBV/HCV/Alcohol/NASH)	7 (18.4%)/5 (13.2%)/13 (34.2%)/5 (13.2%)	4 (10.5%)/14 (36.8%)/9 (23.7%)/3 (7.9%)	0.107
Child-Pugh A/B/C	15 (39.5%)/15 (39.5%)/8 (21.1%)	20 (52.6%)/ 17 (44.7%)/1 (2.6%)	0.051
ALBI Grade	1(2.6%) /27 (71.1%) /10 (26.3%)	22 (57.9%)/15 (39.5%)/1(2.6%)	< 0.001
MELD Score[1]	6 (2-17)	9 (0-22)	0.205
ALT (IU/L)[1]	24.5 (8.0-105.0)	31.5 (8.0-1490)	0.109
AST (IU/L)[1]	39.5 (20.0-136.0)	45 (15-1041)	0.426
GGTP (IU/L)[1]	35.5 (11.0-558.0)	64 (14-506)	0.055
Serum ammonia	104 (59-297)	52 (23-89)	< 0.001
Albumin (g/dL)[1]	3.0 (2.1-4.4)	4.05 (2.4-5.0)	< 0.001
Total bilirubin (mg/dL)[1]	1.65 (0.4-4.7)	1.0 (0.4-8.6)	0.002
Platelet count (×103/μL)[1]	8.7 (4.8-27.9)	12.3 (2.3-30.2)	0.023
Prothrombin activity (%)	60 (38-133)	69 (14-108)	0.066
AFP (ng/mL)[1]	4.3 (1.0-3375)	6.25 (1.3-385.5)	0.065
DCP (mAU/mL)[1]	37 (15-3178)	25 (11-11688)	0.383
Fibrosis 4 index[1]	5.61 (1.71-15.1)	4.25 (1.2-33.4)	0.117
MRE (kPa)[1]	5.04 (3.00-11.11)	4.86 (3.36-12.50)	0.748
SWE (kPa)[1]	23.1 (8.3-56)	16.8 (7.0-36.8)	< 0.001
History of overt hepatic encephalopathy	12 (31.6%)	0 (0%)	< 0.001
HCC	9 (23.7%)	12 (31.6%)	0.442
Ascites	11 (29.0%)	10 (26.3%)	0.834
Esophageal varices	27 (71.1%)	23 (60.5%)	0.469
Max diameter of portosystemic shunts (mm)	7.6 (1.7-18.5)	4.2 (1.6-10.9)	< 0.001
Number of portosystemic shunts (0,1/2-)	8 (22.2%)/28 (77.8%)	24 (63.2%)/14 (36.8%)	0.001
Lactulose	29 (76.3%)	6 (15.8%)	< 0.001
BCAA	24 (63.2%)	14 (36.8%)	0.038
Sarcopenia	17 (44.7%)	17 (44.7%)	1
Follow up period (wk)[1]	61 (24-91)	91 (5-120)	< 0.001

[1]Values are expressed as median (range). BMI: Body mass index; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis; ALBI score: Albumin bilirubin score; MELD score: Model for End-Stage Liver Disease score; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGTP: Gamma-glutamyl transpeptidase; AFP: Alpha fetoprotein; DCP: Des-gamma carboxyprothrombin; MRE: Magnetic resonance elastography; SWE: Shear wave elastography; HCC: Hepatocellular carcinoma; BCAA: Branched-chain amino acid.

ammonia level was independently associated with the maximum shunt diameter of ≥ 8.0 mm (risk ratio = 5.52, P = 0.040) (Table 2).

The cutoff values for predicting the insufficient improvement in ammonia levels after rifaximin treatment were determined by receiver operator characteristics analysis.

Survival

The median follow-up period was 62 wk for the Rifaximin group and 91 wk for the control group.

There were 4 deaths in the observation period: 2 cases of liver failure caused by pleurodesis for pleural effusion and 2 cases of liver failure brought about by worsened cirrhosis. When classifying according to Child-Pugh, the survival time span of Child C was significantly shortened. (Figure 2A)

There were 5 deaths in the control group. Hepatic insufficiency due to worsening of the HCC was the cause of death in 2 patients. Disseminated intravascular coagulation due to thoracic empyema by intestinal bacteria was the cause of death in 1 patient and cardiovascular disease was the cause of death in 2 patients.

Altogether, there were no significant differences when we compared the rifaximin group with the control group (P = 0.897) (Figure 2B).

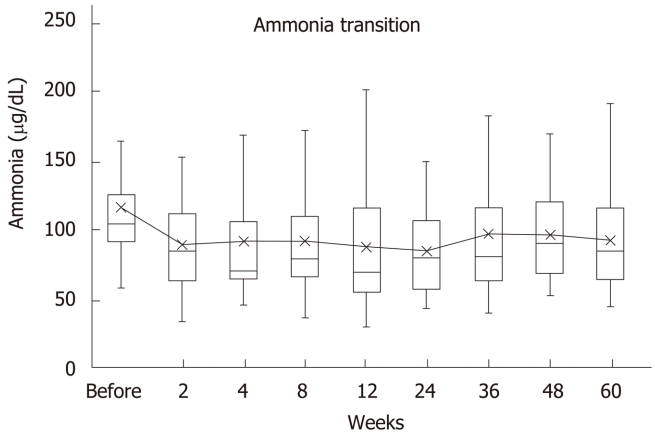

Figure 1 Serum ammonia levels. Before rifaximin treatment, median of blood ammonia level was 104 μg/dL (range, 59-297 μg/dL), whereas two weeks after the treatment it decreased to 85 μg/dL (34-153), showing a significant difference (P = 0.002). The median values were 71 μg/ dL (47-341) after 4 wk, 79.5 μg/ dL (37-215) after 8 wk, 70 μg/ dL (31-221) after 12 wk, 80.5 μg/dL (44-150) after 24 wk, 81 μg/dL (20-298) after 36 wk, 91 μg/dL (53-169) after 48 wk, and eventually 85 μg/dL (45-192) after 60 wk. Decrease was noted at all the points after the start of the treatment when compared with the ones before it.

DISCUSSION

HE is one of the major complications of liver cirrhosis, and in Japan, rifaximin has been available for treating HE since November 2016. This study assessed the efficacy and safety of long-term rifaximin use in the Japanese population. In addition, we also investigated the differences in the kind of portosystemic shunts and the infections related to *E. coli*. The serum ammonia level significantly decreased 2 wk after initiating treatment compared to the pretreatment level, and the decrease was maintained for as long as 60 wk. Moreover, there was recurrence of overt HE in cases of drainage of hepatic pleural effusion and self-cessation of rifaximin therapy, but not in other cases. This suggests that the long-term use of rifaximin has good efficacy and rifaximin can be used continuously for a long time without discontinuation.

As to adverse events, 2 cases (5.3%) had diarrhea which improved with probiotics, and there were no cases of cessation of rifaximin therapy due to side effects. The efficacy and safety of long-term use of rifaximin has been proved in the West, but there has been no reported evidence in Japan thus far. Therefore, the result of this study, that the long-term use of rifaximin is effective and safe, is important.

HE is said to be precipitated by events such as infections and enteric bleeding. In this study, there was recurrence of overt HE in 3 cases, 2 of which occurred on draining the hepatic pleural effusion; it was considered that the recurrences occurred due to dehydration and poor general health conditions, including infections and intestinal bleeding. West Haven criteria is used to assess HE[19]. However the West Haven test is not sufficient to identify subclinical encephalopathy and the Trail Making Test was more useful to detect minimal encephalopathy[20]. In this retrospective study, we could not evaluate the Trail Making Test.

It was reported that the prevalence of large portosystemic shunts was 70% among patients suffering from chronic HE and 14% among the control group. In other words, there is a strong association between HE and the development of portosystemic shunts. One previous study reported that the grade of portosystemic collateral veins was concordant with ammonia levels[21]. This finding was consistent with our results. There have been no studies to date investigating the relationship between portosystemic shunts and rifaximin therapy. This study revealed that rifaximin could improve the elevated serum ammonia levels and HE of patients who had large portosystemic shunts as well as that of those who had not developed shunts. It is expected that rifaximin can be a useful treatment for HE. In this study, some patients did not show a decrease in their serum ammonia levels to below 80 μg/dL and multivariate analyses revealed that this was independently associated with a maximum shunt diameter of ≥ 8.0 mm. This result suggests that although rifaximin improved overt HE, minimal HE may remain in cases with large shunts and that the grade of portosystemic shunts affected the serum level of ammonia as stated in the previous report[21]. Interventional radiology or surgical treatment should be considered for those patients who do not show adequate improvement of HE on

Table 2 Risk factors for insufficient improvement of serum ammonia after rifaximin treatment

Category	HR (95%CI)	*P* value
Univariate analysis		
Age (yr at MRE)		
< 70	1	
≥ 70	2.2 (0.56-8.7)	0.26
Sex		
female	1	
male	0.491 (0.128-1.88)	0.30
BMI (kg/m^2)		
< 28	1	
≥ 28	0.42 (0.094-1.85)	0.25
AST (IU/L)		
< 36	1	
≥ 36	0.372 (0.094-1.47)	0.16
ALT (IU/L)		
< 30	1	
≥ 30	0.43 (0.11-1.7)	0.23
GGTP (IU/L)[a]		
< 42	1	
≥ 42	0.71 (0.18-2.87)	0.64
Albumin (g/dL)		
≥ 3.0	1	
< 3.0	3.3 (0.83-13)	0.091
Total bilirubin (mg/dL)		
< 1.4	1	
≥ 1.4	3.0 (0.76-12)	0.12
Platelet count (×10^3/μL)		
≥ 6.8	1	
< 6.8	4.1 (0.67-25)	0.13
Prothrombin activity (%)		
< 58	1	0.13
≥ 58	3.0 (0.72-13)	
AFP (ng/mL)		
< 6.5	1	
≥ 6.5	3.25 (0.55-19)	0.20
DCP (mAU/mL)		
< 18	1	
≥ 18	3.6 (0.57-23)	0.17
Ascites		
Absent	1	0.16
Present	2.5 (0.70-8.8)	
Esophageal varices		
Absent	1	0.42
Present	1.8 (0.43-7.5)	
History of hepatic encephalopathy		
Absent	1	0.11
Present	3.6 (0.77-16)	
HCC[1]		
Absent	1	0.72
Present	0.75 (0.16-3.6)	
Sarcopenia		
Absent	1	0.49
Present	1.64 (0.41-6.6)	

Child-Pugh Score		0.061
< 7	1	
≥ 7	3.9 (0.94-16)	
ALBI Score		
< -1.6	1	
≥ -1.6	5.3 (1.1-25)	0.033
MELD Score		
< 12	1	0.13
≥ 12	3.0 (0.72-13)	
Fibrosis 4 index		
< 8.3	1	
≥ 8.3	0.42 (0.094-1.9)	0.25
MRE (kPa)		
< 5.0	1	0.43
≥ 5.0	1.63 (0.40-8.3)	
SWE (kPa)		
< 17.7	1	
≥ 17.7	3.0 (0.65-14)	0.16
Max diameter of portosystemic shunt		
< 8.0	1	
≥ 8.0	6.8 (1.4-33)	0.017
Number of portosystemic shunt systems		
0,1	1	0.68
2-4	1.36 (0.32-5.9)	
Multivariate analysis		
Max diameter of portosystemic shunt		
< 8.0	1	
≥ 8.0	5.5 (1.1-28)	0.039
ALBI Score		0.16
< -1.6	1	
≥ -1.6	3.3 (0.62-18)	

BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGTP: Gamma-glutamyl transpeptidase; AFP: Alpha fetoprotein; DCP: Des-gamma carboxyprothrombin; HCC: Hepatocellular carcinoma; MELD Score: Model for End-Stage Liver Disease score; ALBI Score: Albumin-bilirubin score; MRE: Magnetic resonance elastography; SWE: Shear wave elastography.

rifaximin therapy.

It has been suggested that in liver cirrhosis patients, the function of the gut microbiome is altered, and advanced liver disease has a significantly decreased the cirrhosis dysbiosis ratio (CDR); altered gut microbiota was observed in patients with severe liver cirrhosis. It was reported that the mortality was highest in these patients[22].

There were no SBP cases reported in both rifaximin and control groups in this study, but one patient died of thoracic empyema caused by intestinal bacteria.

This suggests that rifaximin suppressed *E. coli*-related infections as previously reported[23].

Also, no significant difference was recognized between the rifaximin group and the control group in the survival rates. Among the rifaximin group, the survival rate was significantly different based on the Child-Pugh classification, and especially cases of Child-Pugh C group had the shortest survival. We can therefore infer that rifaximin cannot treat liver cirrhosis itself, and that is the limitation of this treatment. Previous report indicated that spleno-renal shunts are burdened by an increased incidence of HCC[24]. We also need to be aware of the existence of HCC.

One of the limitations of this study was that we did not examine each test to diagnose minimal HE, and the improvement of minimal HE cannot be proved. Also, there was no set standard for initiating rifaximin treatment; it was started based on the judgement of the attending physicians. This was a retrospective study of a few cases in a single institution, and it is necessary to evaluate a larger number of cases in a multiple-center study to assess survival. It is also necessary to consider HCC,

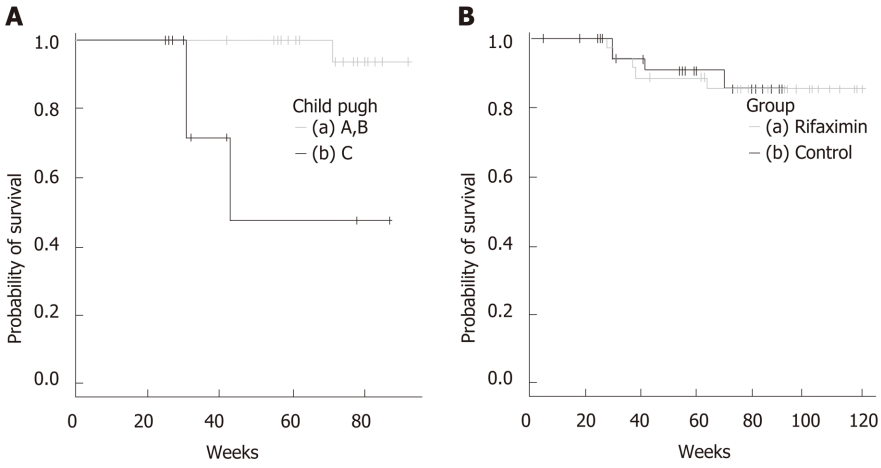

Figure 2 Kaplan-Meir survival curves. A: Results from log-rank test showed significant differences in Child-Pugh A and B *vs* Child-Pugh C. a: Survival data of patients with Child-Pugh A and B (n = 30), b: Survival data of patients with Child-Pugh C (n = 8). Median survival time was 43 wk; B: Results from log-rank test showed no significant differences of rifaximin group *vs* control group. a: Survival data of rifaximin group (n = 38), b: Survival data of control group (n = 34).

infections, malnutrition, and cardiovascular disease, *etc*. as causes of death in patients with liver cirrhosis.

In conclusion, the long-term use of rifaximin was found to be safe and effective in the Japanese population.

ARTICLE HIGHLIGHTS

Research background

Hepatic encephalopathy (HE) is a complication of liver cirrhosis. Rifaximin, an antibiotic, has been reported to decrease the occurrence of overt HE and improve cognitive function in studies from Europe and the United States of America. There is not enough evidence of the relationship between the long-term use of rifaximin and its clinical effects in the Japanese.

Research objectives

To determine the clinical effects of long-term rifaximin therapy in decompensated liver cirrhosis patients, with overt HE or hyperammonemia. We evaluated the relationship between the long-term use of rifaximin and its clinical effects and the size of the shunts.

Research methods

In this single-center retrospective observational cohort study, we reviewed the data of 38 patients who had taken rifaximin at the dose of 1200 mg/d for more than 24 wk. The primary outcome measured was the efficacy of long-term rifaximin use, and the secondary outcome measured was the safety of its long-term use as determined by its influence on portosystemic shunts.

Research results

Rifaximin did not worsen the functionality of the liver 24 wk after the treatment. Adverse events included 2 cases of diarrhea (5.3%), which improved following the intake of probiotics. There were no cases of treatment interruption due to adverse events. Serum ammonia levels were significantly decreased 2 wk after the beginning of rifaximin compared to the pretreatment levels (P = 0.002), and it remained significantly lower for up to 60 wk. Multivariate analyses revealed that the insufficient improvement in the serum ammonia level was independently associated with the maximum shunt diameter of ≥ 8.0 mm (risk ratio = 5.52, P = 0.040).

Research conclusions

The long-term use of rifaximin was found to be safe and effective in the Japanese population.

Research perspectives

In this retrospective study, we did not examine each test to diagnose minimal HE, and the improvement of minimal HE cannot be proved. Tests as the Trail Making Test should be evaluated to minimal HE in the future investigation.

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ORIGINAL ARTICLE

Retrospective Study

Validation of modified albumin-bilirubin-TNM score as a prognostic model to evaluate patients with hepatocellular carcinoma

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Abstract

BACKGROUND

An ideal staging system for hepatocellular carcinoma (HCC) should rely on the hepatic reserve function and tumor burden. With the improvement in diagnostic and treatment strategies for HCC, in addition to recent treatment of viral hepatitis, finding a suitable assessment tool for hepatic reserve has become mandatory.

AIM

To validate a recently proposed modified albumin-bilirubin-TNM (mALBI-T) grade as a prognostic model for patients with HCC in Egypt.

METHODS

For patients diagnosed with HCC, Child-Turcotte-Pugh (CTP) score, Barcelona Clinic Liver Cancer (BCLC) stage, albumin-bilirubin (ALBI), plateltet-albumin–bilirubin (PALBI), ALBI-based BCLC, ALBI-T and mALBI-T grades were estimated. Patients were followed from time of diagnosis to date of death or date of data collection if they remained alive. Overall survival and received treatments were determined. Survival data were analyzed.

RESULTS

A total of 1910 patients were included (mean age, 57 years; 1575 males). At presentation, 50.6% had CTP A, 36.1% had CTP B and 13.4 % had CTP C; 12% had ALBI grade 1, 62.3% had ALBI grade 2 and 24.7% had ALBI grade 3. Overall median survival was 13 mo; survival was better in patients with ALBI 1 than in those with ALBI 2 and 3 (28.6 *vs* 14 and 5.8 mo, respectively, $P < 0.001$). Patients with ALBI-T grades 0 and 1 had better survival than those with ALBI-T grades 2, 3, 4 and 5 ($P < 0.001$). The modified ALBI-T showed better stratification and significant improvement in prediction of survival.

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CONCLUSION
ALBI-T grade is a superior prognostic tool that selects patients with HCC who have better liver reservoir and tumor stage. mALBI-T is a better prognostic model in patients with HCC.

Key words: Staging; Hepatocellular carcinoma; Albumin-bilirubin grade; Scores

Core tip: In this study, we validated the modified version of the albumin-bilirubin-TNM grade (mALBI-T) in a retrospective Egyptian cohort. In addition, this study offered a comparative analysis of eight different established and novel scoring systems. We concluded that the modified ALBI-T score is superior to the other scoring systems and offers a better prognostic tool.

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for > 5% of all human cancers and is a major health problem worldwide, being the fifth most common malignancy in men and the eighth in women. The estimated annual incidence of HCC exceeds 840000, and it is the third most common cause of cancer-related death in the world[1].

It is widely perceived that survival in HCC depends on tumor stage, underlying liver function, and the performance status of the patient[2]. The Child-Turcotte-Pugh (CTP) classification of liver function relies on individual parameters scored based on arbitrarily defined, predetermined cutoff points that lead to misjudging of the liver condition of the patients[3]. This was an incentive for the development of a novel scoring system, the Albumin-Bilirubin(ALBI) grade by Johnson *et al*[4] incorporating albumin and bilirubin only, which are numerical parameters readily available in the basic investigations for patients with liver disease. ALBI grade does not comprise the subjective parameters which are included in CTP classification, for example, grading of ascites and encephalopathy. The cut-off points of the predictive equation of ALBI grade allocate patients according to the calculated score as follows: score of -2.60 or less is ALBI grade 1, those with a score more than -1.39 is ALBI grade 3, while those in between are classified as ALBI grade 2. Nevertheless, patients with ALBI grade 2 had a wide range of hepatic function, the same drawback of Child-Pugh class B. So, other grades based on ALBI grade were proposed[5-8]. Hiraoka *et al*[8] proposed the ALBI-T grade which icorporated TNM staging system (LCSG fifth edition) into the previously discussed ALBI grading system. It showed better performance and superiority as a prognostic scoring system regarding survival prediction in HCC patients.

Hiraoka *et al*[9] proposed to divide ALBI grade into four levels, namely, the modified ALBI (mALBI) grade, using an additional cut-off value for ALBI score (–2.270), based on an indocyanine green retention test (ICG-R15) of 30%, which is statistically defined as the cut-off value for minimal anatomical surgical resection for HCC in Makuuchi criteria. In the current work, the prognostic value of the modified albumin-bilirubin-TNM (mALBI-T) grade proposed by Hiraoka *et al*[9] was evaluated as a tool to predict survival in HCC patients and compared to other ALBI-based grades.

MATERIALS AND METHODS

All patients diagnosed with HCC according to the AASLD guidelines[10] with complete data and contact details, from the HCC clinic at National Liver Institute, Menoufia University, were included in this study. The study was granted ethical committee approval prior to data retrieval. We had access to a data set of a cohort of

1910 patients diagnosed with HCC who fulfilled the inclusion criteria.

The diagnosis of HCC was based on the presence of an arterial hypervascular focal lesion > 2 cm with rapid wash-out with a single imaging modality (triphasic spiral computed tomography (CT), magnetic resonance imaging or angiography) or two imaging modalities demonstrating the before mentioned feature for lesions < 2 cm[10]. All patients included in the study were subjected to history taking and complete clinical examination with special emphasis on the etiology of liver disease, history of ascites or encephalopathy, performance status based on the Eastern Co-operative Oncology Group (ECOG) performance scale, demographic data and contact details. Laboratory investigations included serum albumin, total bilirubin, alpha fetoprotein (AFP) and international normalized ratio (INR). Ultrasound and multislice triphasic CT of the abdomen with contrast were done to detect the number of nodules, nodule type (unifocal, multifocal, or diffuse), maximum tumor diameter (cm), location (right/left/both lobes), portal vein thrombosis (PVT) and extrahepatic or lymph node metastasis. Any patient with incomplete data or inaccessible contact was excluded from the analysis.

Staging and grading systems

The following staging and grading systems were estimated at the time of HCC diagnosis: CTP score, Barcelona Clinic Liver Cancer (BCLC) stage and TNM stage[4,10-12]. During data preparation and analysis, the following scores were estimated based on baseline data using the following formulas.

ALBI grade[4]: The cut points of this linear predictor place patients with a calculated score of -2.60 or less into ALBI grade 1, those with a score higher than -1.39 into ALBI grade 3, and those in between into ALBI grade 2. The equation for the linear predictor is (log10 bilirubin x 0.66) + (albumin x -0.085), where bilirubin is in mcmol/L and albumin is in g/L[5].

PALBI grade[6]: ALBI score was calculated as [2.02*Log10 bilirubin] + [-0.37*[log10 bilirubin]2] + [-0.04*albumin] + [-3.48* log10 platelets] + [1.01 *(log10 platelets2)]. PALBI grade was assigned based on 50% and 85% Cox cut points: PALBI 1, ≤ -2.53; PALBI 2, -2.53 to -2.09; PALBI 3, >-2.09[7].

ALBI-BCLC grade[7]: It was calculated by replacing the CTP grades A, B and C used in the BCLC system with the ALBI grades 1, 2 and 3 respectively[8].

ALBI-T grade[8]: ALBI-T score was obtained by adding the TNM stage from the LCSGJ 5th edition to the ALBI grade and then subtracting 2. Patients were graded from 0 to 5[9].

mALBI-T grade[9]: The mALBI-T score was proposed by Hiraoka *et al*[9] as they added a new cut-off point for ALBI grade 2 at -2.27 based on an ICG-R15 of 30%, resulting in four stages of ALBI grade. This modification was introduced to ALBI-T grade where TNM stage was added to modified ALBI grade and subtracting 2. This led to mALBI-T stages from 0 to 6. Consequently, patients were followed from the time of diagnosis to the date of death or date of data collection if they remained alive.

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 25.0 (IBM, United States). Overall survival and the received treatment were determined. Survival data were analyzed using Kaplan Meir Survival curves and log rank test, then the area under the receiver operating characteristic curve (AUC) was determined for each staging and scoring system. Results were considered statistically significant when *P*-values were lower than 0.05. *P*-values less than 0.01 were considered highly significant. Of note, all reported *P*-values are uncorrected and two-tailed.

RESULTS

Patients and tumor characteristics

A total of 1910 patients with HCC were included, of whom 1575 (82.5%) were male. The mean age of the patients was 57 years. The underlying cause of cirrhosis was hepatitis C virus infection in 71.6% of cases. The right lobe was the site of HCC in about 1204 (63%) patients. PVT was detected in 345 (18%) patients, and extra-hepatic metastasis was detected in (7.74%) 148. EGOG performance status was zero in 1154 (60.3%) patients. The baseline clinical features of the included patients are shown in Table 1.

Among these basic features, we can see that hepatitis B virus infection as an

Table 1 Characteristics of the included patients

Variable	Patients
	***n* = 1912**
Male gender, *n* (%)	1575 (82.4)
Age (yr, mean ± SD)	57(± 7.2)
Etiology	
Hepatitis C	1369 (71.6)
Hepatitis B	468 (24.5)
Other	75 (3.9)
Albumin (g/L, mean ± SD)	31.4(± 4.6)
Bilirubin [μmol/L, median (IQR)]	24.5 (13.9-29.1)
Platelet count (x 10^9/L, mean ± SD)	96 (± 37.4)
AFP [μg/L, median (IQR)]	43 (10-184.3)
Tumor size [cm, median (IQR)]	5.6 (1.8-8.4)
Solitary tumor, *n* (%)	369 (19.3)
PVT	345 (18)
Metastasis	148 (7.7)
ECOG grade 0, *n* (%)	1154 (60.4)
Treatment	
Liver resection	67 (3.5)
Liver transplantation	43 (2.3)
Ablative procedures (PEI, RFA and microwave)	249 (13.1)
TACE	1036 (54.1)
Combined treatment (TACE + ablation)	8 (0.4)
Systemic therapy	40 (2.1)
Conservative	469 (24.5)
Overall survival, months (95%CI)	17.9 (12.1-13.8)
Overall deaths, *n* (%)	1219 (63.8)

AFP: Alpha fetoprotein; PVT: Portal vein thrombosis; TACE: Transarterial chemoembolization; PEI: Percutaneius ethanol injectuion; RFA: Radiofrequency ablation; IQR: Interquartile range.

etiology is a predictor of poor survival in the multivariate analysis (Table 2). Also, single tumor and good liver function (*e.g.*, low bilirubin and high albumin) were good predictors of survival in our cohort (Table 2).

Patient classification according to different scoring systems

Patients were classified according to CTP class, ALBI grade, PALBI, TNM, BCLC staging system, ALBI-based BCLC, ALBI-T and mALBI-T (Table 3).

Survival according to different scoring systems

Each of the investigated scoring systems showed significant stratification of the median survival across its different grades. The overall median survival was 13 mo for the whole cohort. The median survival for CTP A patients were 19 mo, better than that for patients with CTP B and C (11 and 3.8 mo, respectively) ($P < 0.001$). Indeed, the median survival was better in patients with ALBI grade 1 than in those with ALBI grades 2 and 3 (28.6, 14 and 5.8 mo, respectively, $P < 0.001$). Also, patients with PALBI grade 1 had a better median survival (34.6 mo) than in those with PALBI grades 2, 3 and 4 (27.5, 23.9 and 7.5 mo, respectively, $P < 0.001$).

The ALBI-based BCLC system showed better performance to stratify the included patients (Table 3). The median survival was [undefined (mean = 33.8 mo), 30.8, 14.2, 11 and 5 mo, $P < 0.001$] for stages 0, A, B, C and D compared to (25.4, 19.8, 16, 8 and 4 mo, $P < 0.001$) for stages 0, A, B, C and D, respectively, in the CTP-based BCLC staging system.

Incorporating TNM staging into ALBI grade (ALBI-T) provided better performance and ability to discriminate median survival of patients across different stages ($P < 0.001$). Furthermore, ALBI-T grades 0 and 1 patients had better median survival than those with ALBI-T grades 2, 3, 4 and 5 (42, 28.9, 17, 8, 5 and 3 mo, respectively $P < 0.001$). Notably, the mALBI-T showed significant improvement in the median survival

Table 2 Multivariable Cox regression anaylysis

Variable	Hazard ratio (95%CI)	*P*-value
Tumour number		
Solitary	1	
Multiple	1.456 (1.236, 1.750)	<0.01
Log_{10} tumour size (cm)	3.517 (2.678, 4.567)	<0.001
Baseline log_{10} AFP	1.302 (1.208, 1.411)	<0.001
Baseline albumin	0.993 (0.966, 0.998)	0.031
Baseline log_{10} bilirubin	1.581 (1.139, 2.194)	<0.001
Vascular invasion		
No	1	
Yes	1.612 (1.251, 2.045)	0.001
Aetiology		
Hepatitis C virus	1	
hepatitis B virus	1.260 (1.020, 1.407)	<0.05
Others	0.914 (0.887, 0.991)	0.043

AFP: Alpha fetoprotein.

in patients with ALBI-T grades. Also, mALBI-T grades 2, 3 and 4 showed improved survival (20, 14 and 8 mo, respectively, $P < 0.001$), as shown in Figure 1. However, other stages remained the same as they were not affected by the sub-classification of ALBI grade 2.

Accuracy of different scoring systems in predicting survival

The AUC was determined for each grade (Figure 2). Of note, the ALBI-based BCLC had significantly higher AUC in comparison to CTP-based BCLC (AUC = 0.749 *vs* 0.713, respectively). However, the mALBI-T grade showed the highest AUC compared to all other studied scoring systems (AUC = 0.848, $P < 0.001$).

DISCUSSION

The most widely used staging system for stratifying patients with HCC is the BCLC system. It was proposed according to the recommendations of many randomized controlled trials and cohort studies which precisely included the tumor stage, patient's performance status, and liver function into an algorithm which allocates patients to different treatment plans according to the evidence-based results[11]. The BCLC system helps to allocate treatment modalities and properly identify suitable patients for clinical trials. However, it is important to know that the liver function is assessed in the BCLC system according to the subjective CTP grade which is considered one of the limitations of this widely used staging system[10].

Residual hepatic function plays an important role in predicting the outcome of patients with HCC. On this premise, Johnson *et al*[4] conducted the pioneer study that coined the term ALBI grade on 6000 patients from Europe, Japan, China and the United States with various stages of HCC. Simply, this model depended on two laboratory variables (bilirubin and albumin) and omitted the subjective CTP variables ascites and hepatic encephalopathy. Questions have been raised regarding INR inclusion in ALBI grade. However, INR as a parameter is considered a source of bias in patients taking oral anti-coagulants, but it is impossible to stop treatment just to calculate a score. Since residual hepatic function plays an important role in predicting the outcome of patients with HCC, six models evaluating the severity of cirrhosis (CTP, ALBI, PALBI, ALBI-based BCLC and ALBI-T grading systems) were investigated in this study in addition to the recently proposed modification of ALBI-T.

Patients with CTP class A had a significantly better median survival than those with CTP classes B and C. This is in agreement with several reports[12-14]. Similarly, patients with ALBI grade 1 had a significantly better median survival than those with ALBI grades 2 and 3 in this study, in accordance with other reports[15,16]. ALBI grade has become a core for further prognostic models, and Roayaie *et al*[6] proposed PALBI grade, where they added the platelet count in a new linear predictive equation to the ALBI grade parameters. In this study, patients with PALBI grade 1 had significantly

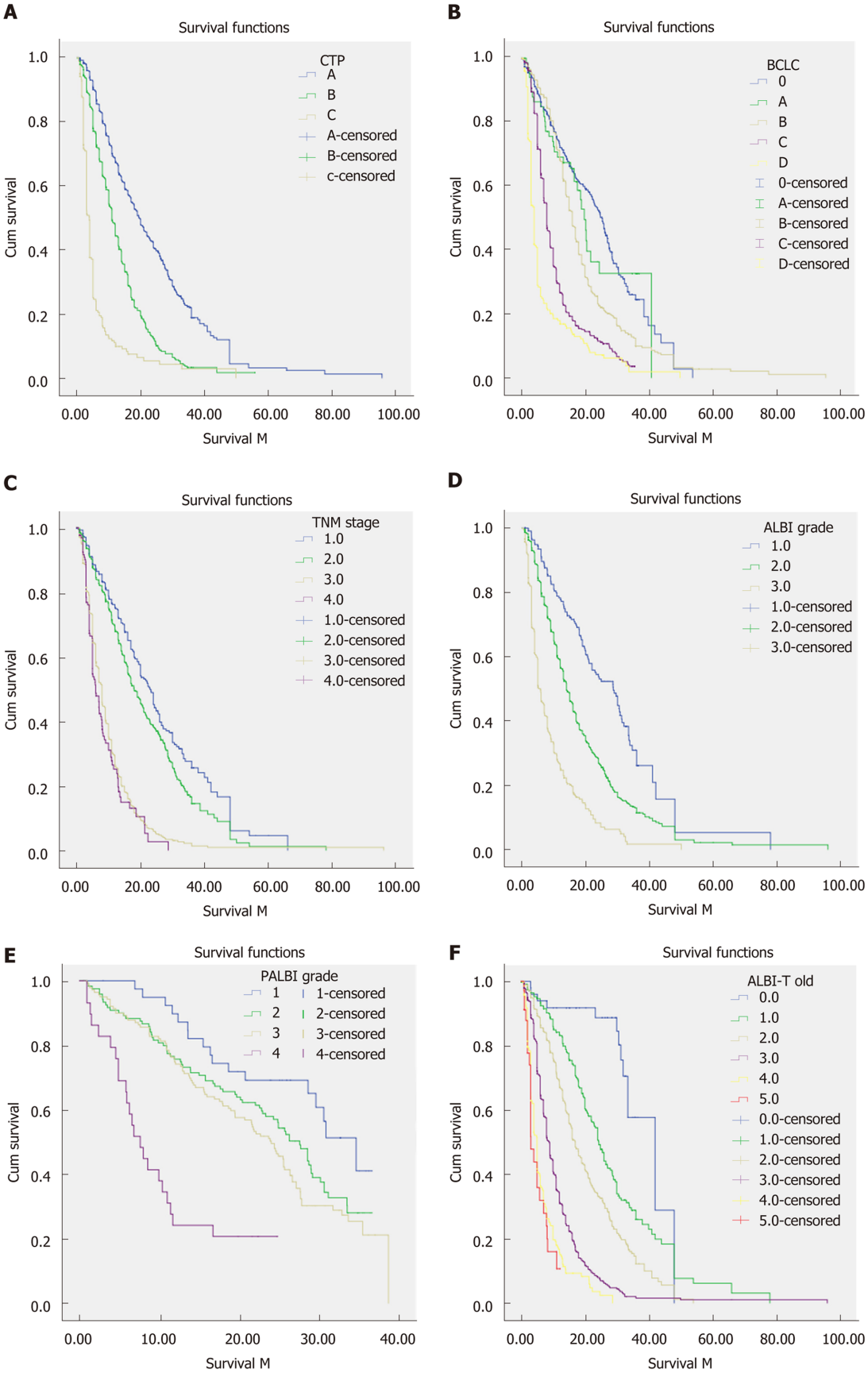
Table 3 Distribution and median survival of patients with hepatocellular carcinoma according to the studied staging systems

Staging/grading system	Stage/grade	Percentage of patients	Median survival (mo)	*P*-value
CTP	A	50.6	19	< 0.001
	B	36.1	11	
	C	13.4	3.8	
BCLC	0	3.6	25.4	< 0.001
	A	27.5	19.8	
	B	26.6	16	
	C	28.9	8	
	D	13.4	4	
TNM	I	19.5	23	< 0.001
	II	36.6	18	
	III	35.4	8	
	IV	8.5	6	
ALBI	1	12	28.6	< 0.001
	2	63.2	14	
	3	24.7	5.8	
PALBI	1	10.1	34.6	< 0.001
	2	29	27.5	
	3	33.9	23.9	
	4	27	7.5	
ALBI-based BCLC	0	1.5	Undefined	< 0.001
	A	5.9	30.8	
	B	67.2	14.2	
	C	2.2	11	
	D	23.2	5	
ALBI-T	0	2.6	42	< 0.001
	1	18.1	28.9	
	2	30.4	17	
	3	31.1	8	
	4	16	5	
	5	1.8	3	
Modified ALBI-T	0	2.6	42	< 0.001
	1	9.1	28.9	
	2	18.5	20	
	3	27.9	14	
	4	25.5	8	
	5	14.6	5	
	6	1.8	3	

CTP: Child-Turcotte-Pugh score; ALBI: Albumin-bilirubin score; TNM: Tumor-node-metastasis staging system; BCLC: Barcelona Clinic Liver Cancer staging system.

better median survival than those with PALBI grade 2, 3 and 4 ($P < 0.001$).

Both ALBI and PALBI grades showed precise ability to stratify HCC patients into different stages according to their survival. The PALBI grade was superior to CTP classification and ALBI grade in terms of mortality prediction (AUC = 0.734, 0.643 and 0.709, respectively). For CTP grade A patients, the PALBI grade had significantly higher AUC in comparison to ALBI grade at 1-year and 3-years intervals (Table 4). This is in line with the endpoints reported by Roayaie *et al*[6] who concluded that PALBI was independently associated with survival when controlled for age, tumor related factors and performance status, and can stratify patients with HCC undergoing curative therapies more precisely than CTP class[6]. Other studies reported the same results using different treatment modalities for HCC[17,18]. PALBI grade was found to perform better than ALBI grade, as incorporating platelet count reflects the role of portal hypertension. However, not all HCC patients have portal hypertension and some fallacies may result from platelet counting, as pseudo thrombocytopenia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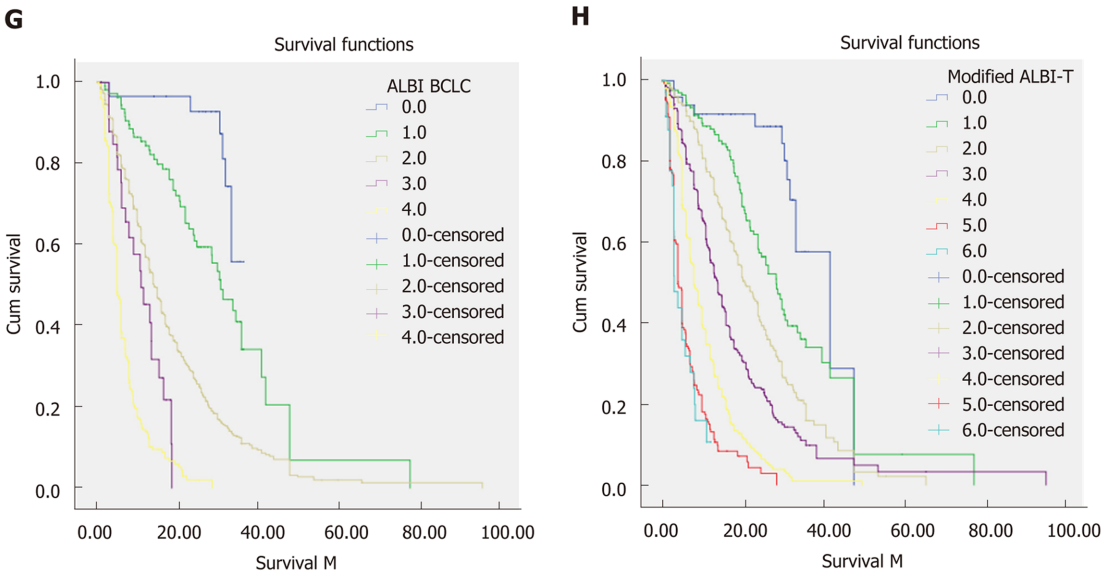

Figure 1 Survival curves of patients with hepatocellular carcinoma. A: Child-Turcotte-Pugh score; B: Barcelona Clinic Liver Cancer (BCLC) staging system; C: TNM staging system; D: Albumin-bilirubin (ALBI) grade; E: Plateltet-ALBI grade; F: ALBI-TNM score; G: ALBI-BCLC score; H: Modified ALB-T score.

resulting from adding EDITA to blood samples that lead to aggregate formation inside automated cell counters. In addition, thrombocytosis can be found as a paraneoplastic event in patients with HCC and can affect the results of the score.

ALBI grade succeeded to overcome the weaknesses of CTP classification, and this led to further trials to substitute CTP score in the BCLC staging system by Chan *et al*[7] or incorporating it to the TNM staging system to result in ALBI-T which was proposed by Hiraoka *et al*[8]. The ALBI-based BCLC staging system showed more prognostic power than CTP-based BCLC in the studied cohort. The ALBI-based BCLC had significantly higher AUC compared with CTP-based BCLC (AUC = 0.749 *vs* 0.713, respectively). In contrast, an international multicenter study found that the ALBI-based BCLC system yields the same prognostic discrimination as the CTP-based BCLC system, regardless of treatment modalities. The authors concluded that the substitution of CTP class by ALBI grade does not improve the overall prognostic performance of the BCLC system[7]. However, other authors found that the ALBI grade can estimates objective hepatic reserve across each BCLC stage[19].

ALBI-T grade was proposed through evaluation of the prognosis of 2,584 Japanese patients with HCC and validated in a nationwide survey of 46,681 patients in Japan[8,9]. In this study, patients with ALBI-T grade 0 had a median survival of 42 mo and patients with ALBI-T grade 1 was found to have a better median survival (28.9 mo) than those with ALBI-T grades 2, 3, 4 and 5 (17, 8, 5 and 3 mo, respectively). Comparable results were reported by other investigators[9,20-22].

ALBI-T grade was able to percisely predict survival in comparison to other scoring systems, due to its incorporation of TNM stage, which is suitable for assessment of small HCC tumors, and ALBI grade, which is an accurate objective tool for assessment of liver function. This could be emphasized by the multivariate Cox regression analysis showing that the parameters of the ALBI score performed well and it was a good predictor of survival (Table 2). Future scores would perform better if they include other parameters that evolved in our analsysis as a good predictor of survival such as vascular invasion and etiology.

The mALBI-T grade showed more detailed stratification ability and better predictive value for prognosis of Egyptian patients with HCC, compared to those shown by other staging and scoring systems including ALBI-T. When comparing the modified model to other staging systems, the mALBI-T had the highest AUC (0.848), while the AUC for ALBI-T was 0.801.

The retrospective nature and the short follow-up period are the main limitations to the present study. Despite this, the mALBI-T grade, with its detailed stratification of hepatic reserve function, may provide more accurate prediction of prognosis and overall survival of patients with HCC than other available grading systems.

Table 4 AUROC values for all scores at 1 and 3 years in addition to overall survival

Grade	1-yr AUROC	3-yr AUROC	Overall AUROC
TNM	0.639	0.626	0.620
CTP	0.672	0.649	0.643
ALBI	0.741	0.728	0.709
Modified ALBI	0.755	0.732	0.719
PALBI	0.753	0.741	0.734
BCLC	0.758	0.743	0.713
ALBI-based BCLC	0.762	0.751	0.749
ALBI-T	0.822	0.809	0.801
Modified ALBI-T	0.851	0.848	0.848

CTP: Child-Turcotte-Pugh score; ALBI: Albumin-bilirubin score; TNM: Tumor-node-metastasis staging system; BCLC: Barcelona Clinic Liver Cancer staging system.

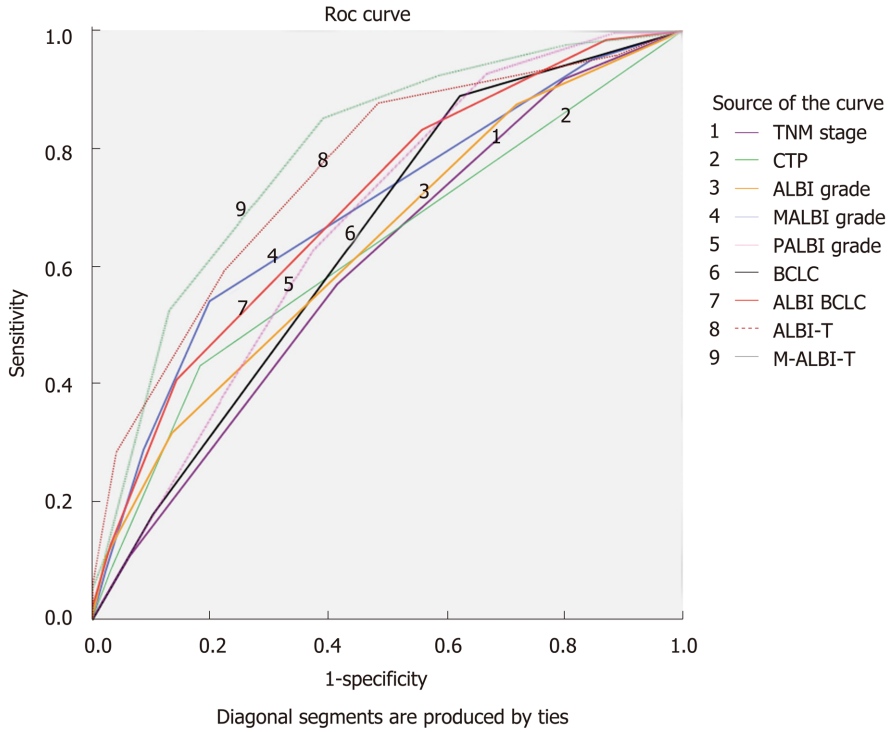

Figure 2 ROC curves for overall survival of patients with hepatocellular carcinoma according to the studied staging systems.

ARTICLE HIGHLIGHTS

Research background

It has been an urge to find a proper staging system for patients with hepatocellular carcinoma (HCC). The significance of this study is owed to comparing on a large scale a wide selection of scoring systems that included established [Child-Turcotte-Pugh (CTP), TNM and Barcelona Clinic Liver Cancer (BCLC)] and novel scoring systems (albumin-bilirubin (ALBI), plateltet-albumin–bilirubin (PALBI), ALBI-BCLC, ALBI-T and modified albumin-bilirubin-TNM (mALBI-T)].

Research objectives

Linear predictive equations based scoring systems (such as ALBI and PALBI) offer better performance than those based on predetermined cut-off points (CTP, TNM and BCLC). The best scoring system would be a linear function that incorporates liver function paramters and other tumor specific paramters such as alpha fetoprotein (AFP), tumor size and number.

Researh methods

We applied the tested scoring systems on our retrospective cohort and compared their

performance using survival curves and AUROCS to predict survival in addition to patient stratification according to different scores.

Research results

Our findings proved that the modified version of ALBI-T score offered better stratification than the original score and is a better prognostic tool. It remains to be explored whether treatment modality specific scores would result in better performance than general scores or not and whether we need such specific scores or not.

Research conclusions

This study offers a validation of the mALBI-T score in a large scale Egyptian cohort. mALBI-T performs better than the other investigated scoring systems such as ALBI and BCLC.

Research perspectives

The best model for HCC patients should assess liver function, tumor number, size and other tumor specific paramters such as AFP. We suggest developing scores that include treatment response such as mRECIST criteria or recurrence status. Linear equations based scores are more precise and future scores should abandon old techniques that use predetermined points.

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ORIGINAL ARTICLE

Retrospective Study

Risk factors for ribavirin treatment failure in Asian organ transplant recipients with chronic hepatitis E infection

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Abstract

BACKGROUND

Hepatitis E virus (HEV) infection is a cause of chronic hepatitis in immunosuppressed patients. Sustained virologic response rates to a 12-wk course of ribavirin therapy were reported to be > 70% in the West. This study describes the outcome of HEV treatment in a transplant center in Singapore.

AIM

To study the outcome of ribavirin treatment in a series of chronic HEV patients, and the cause of treatment failure.

METHODS

We studied all of the transplant recipients who were diagnosed with HEV

Informed consent statement: All involved subjects provided consent for study participation.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

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infection between 2012 to 2015. The outcome of therapy and virologic relapse are monitored for three years after the end of therapy.

RESULTS

Ten transplant recipients (4 liver, 5 kidney, and 1 bone marrow transplantation) with positive HEV RNA were studied. Nine patients received at least 12 wk of ribavirin therapy, and the remaining patient resolved after reducing immunosuppression therapy. Two subjects had prolonged viremia that lasted more than one year, despite continuous ribavirin therapy. Four ribavirin-treated patients (44.4%) had HEV RNA relapse after achieving a virologic response by the end of treatment. The overall failure rate is 66.7%. Being a kidney transplant recipient is the strongest risk factor for not achieving an initial sustained virologic response (0/5 treated, Chi-Square test, $P < 0.05$). The most common side effect of ribavirin is anemia (100%) (haemoglobin reduction of 3-6.2 g/dL). Seven patients required either a blood transfusion or erythropoietin therapy.

CONCLUSION

The sustained virologic response rate of 12-wk ribavirin therapy for HEV infection in this Asian series was lower than expected. Kidney transplant recipients had a higher rate of treatment failure due to higher immunosuppression requirements and adverse effects.

Key words: Toxicity; Antiviral agents; Hepatitis E virus; Virus classification; Systemic immunity; Immune responses; Persistent infection

Core tip: Hepatitis E virus (HEV) infection is a cause of chronic hepatitis in immunosuppressed patients. Sustained virologic response (SVR) rate to a 12-wk course of ribavirin therapy was reported to be > 70% in the West. This study describes the outcome of HEV treatment in a transplant centre in Singapore. Ten transplant recipients (liver, kidney, bone marrow transplantation) with positive HEV RNA were studied. The SVR rate of 12-wk ribavirin therapy for HEV infection in this Asian series was lower than expected; an overall failure rate of 66.7%. Kidney transplant recipients had a higher treatment failure due to higher immunosuppression requirements and adverse effects.

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INTRODUCTION

In the last decade, our understanding of hepatitis E virus (HEV) infection has changed from that of an acute self-limiting disease into one that has "two-faces". In immunosuppressed or immunocompromised hosts, some HEV genotypes may cause chronic hepatitis and sometimes accelerated liver decompensation, especially in post-organ transplant recipients[1]. While it was previously thought to be limited to developing countries with a few sporadic cases in industrialized nations secondary to migration, it has been clear that autochthonous infection is also found in developed countries[2,3]. A Singapore study done with the Communicable Diseases Division of the Ministry of Health showed that between 2000-2011, 45.5% (n = 481) of reported acute hepatitis E cases diagnosed by ELISA for Anti-HEV immunoglobulin M were considered to be autochthonous[4]. Singapore is unique, in that it is an industrialized country with a population of 5.6 million that serves as a port and travel hub for many countries deemed endemic for HEV. The ethnic Chinese in Singapore make up a majority of the population (76%), while ethnic Malays make up 15%, and ethnic Indians 7%.

The characteristics of genotypes 1-4 HEV have been well-described, with genotype

1 and 2 causing waterborne outbreaks and only infecting humans, and genotypes 3 and 4 recognized as zoonotic infections carried by mainly domestic pigs, boar, and deer[2]. More recently, additional genotypes have been identified. Genotypes 5 and 6 HEV are found to occur in wild boar in Japan, and genotype 7 have been identified in dromedary camels in Dubai[5]. Chronic infections of HEV have been predominantly caused by HEV genotype 3, although case reports on chronic infection due to HEV genotypes 4 and 7 in liver transplant recipients[6] have been published.

Studies from France have reported a rate of chronicity of about 58% in post-transplant patients diagnosed with HEV[3]. Ribavirin is considered a safe and effective treatment option for chronic HEV. A study from the same group in France reported a sustained virologic response (SVR) rate of 78% (n = 59)[7], while a study in Germany gave an SVR rate of 75% (n = 4) in a group of liver transplant patients given ribavirin[8].

While a reasonable strategy for a trial of decreasing immunosuppression doses and eventual starting of ribavirin has been suggested, there have been some issues with regards to the management of HEV in transplant patients, and no guidelines have been established. In 2013, Kamar *et al*[9,10] proposed that carriage of HEV for more than 3 mo can be called the threshold for identifying chronicity. Their group further suggests that persistence of HEV replication for 3 mo to be the cutoff point for considering treating HEV with ribavirin. Others, however, disagree and believe that HEV conforms to the convention of a 6-mo cut-off, as in hepatitis B and C[11]. A systematic review has concluded that ribavirin is an effective and reasonably safe first-line option for the treatment of chronic hepatitis E, especially in organ transplant recipients[12].

Most clinical studies on chronic HEV cohorts come from studies based in Europe and the United States. There is a need to characterize and investigate the chronic HEV infection in Asian populations, including those in Southeast Asia.

We report on the first 10 consecutive cases of hepatitis E diagnosed in immunosuppressed post-transplant patients in an organ transplant center in Singapore. The aim is to describe and characterize hepatitis E infection in this multi-ethnic Asian series, and how the outcome differs from the experience of our Western counterparts.

MATERIALS AND METHODS

Methodology

This retrospective case series study includes all the immunosuppressed patients, solid organ, and haematological transplant recipients who were diagnosed with hepatitis E virus infection in the National University Hospital, a tertiary referral center in Singapore, between May 2012 to September 2015. All patients with newly diagnosed HEV infection based on positive HEV RNA assay, who were on immunosuppression therapy for prevention of graft rejection, were followed up. In one patient, the time of infection was determined with the use of stored, frozen serum. During the study period, the subjects who had transaminitis that were caused by persistent HEV replication were treated with ribavirin. The study was approved by the Domain-Specific Review Board of the National Healthcare Group (DSRB Number 2016/00250), and all the patients gave written informed consent.

Medical records including all available laboratory assays for HEV, including RNA and serology, were reviewed. Pertinent data, like the type of organ transplantation, time of organ transplantation and types and doses of immunosuppression at the time of HEV infection diagnosis, were included. The management of the HEV infection was also reviewed, including the length of time from onset of unexplained transaminitis, to diagnosis and subsequent resumption of ribavirin. The outcome of ribavirin therapy and virologic relapse were monitored for three years after the end of therapy.

All samples were analysed at the Molecular Diagnostic Center at the National University Hospital. HEV RNA was detected and quantified with the use of a commercial real-time PCR assay for HEV (RealStar® HEV RT-PCR Kit 2.0, Altona Diagnostics, Hamburg, Germany). The limit of detection for HEV RNA was 10 IU/μL. HEV genotyping was determined by sequencing and analysis as described in a previous study[5]. An SVR is defined as an undetectable level of HEV RNA in the serum at least 6 mo after completion of ribavirin therapy. A non-responder is defined as persistently detectable HEV RNA after 12-wk of treatment with ribavirin. Relapse is defined as recurrent HEV RNA-positive viremia after the completion of a 12-wk ribavirin therapy, despite an initial response and HEV RNA negativity at the end of treatment.

Statistical analysis

Proportions were compared with the use of the Fisher's exact test. Quantitative variables were compared with the use of the non-parametric Wilcoxon test. Independent factors associated with non-responder or relapse after initial SVR were analysed with the use of SPSS version 21 software. In this analysis, non-responder/relapser (as defined above) were compared with those with a durable SVR for three years. A *P* value (two-sided) of less than 0.05 was considered as statistically significant.

RESULTS

Patient characteristics

The data of the first 10 HEV RNA-positive patients who had received a solid organ transplant (5 kidney transplant recipients, four liver-transplant recipients, and one bone marrow transplant recipients) were analysed. They were all diagnosed with HEV infections between 2012 to 2015, based on positive HEV RNA assays. Nine patients (Singapore resident) were infected with HEV genotype 3, and the remaining patient who came from United Arab Emirates had HEV genotype 7 (a ribavirin responder). All patients were investigated for HEV after presenting with transaminitis, and all the other viral causes and other primary hepatic causes of transaminitis were ruled out.

The demographics and clinically relevant characteristics of the HEV-infected patients are summarized in Table 1. The time between organ transplantation and the diagnosis of HEV infection ranged from 2 wk to 23 years after organ transplantation. Immunosuppression was tacrolimus-based in 6 patients (60%), and tacrolimus levels at the time of diagnosis of HEV infection were 2.8-9.5 μg/mL. Most patients (80%) had mild to moderate elevation of liver enzymes (< 400 IU/mL) at the time of diagnosis. One patient, who was 2 wk post-liver transplantation, had extremely high transaminase levels (ALT 8127 U/L and AST 2591 U/L) at the time HEV RNA was found to be positive. This result was later deemed mainly due to liver reperfusion injury. The liver enzyme and HEV RNA levels of this patient spontaneously resolved within the next 12 wk, with the progressive reduction of immunosuppression therapy.

Virologic and biochemical responses with ribavirin therapy

In the remaining nine patients, a decision to start ribavirin therapy was made by the clinician based on the trajectory of the HEV viral load and extent of HEV-related liver injury (Figure 1). The median time between the onset of transaminitis due to HEV and the initiation of ribavirin therapy was 3 mo (range, 1 to 9). In 7 patients, the period of observation was under 3 mo.

The median starting dose of ribavirin was 600 mg per day (range, 400 to 800), which was equivalent to 9.7 mg per kilogram of body weight per day (range, 2.7 to 13.5). The dosages were subsequently adjusted based on the estimated glomerular filtration rate (GFR). In more than half of patients (5 out of 9), dose reductions of ribavirin were necessary due to clinically significant anemia.

All the patients had positive HEV RNA at the start of ribavirin therapy (Figure 2). Peak viral load ranged from 1.78×10^4 to 3.36×10^7 IU/mL. At the end of the first month, HEV RNA levels were assessed in 7 patients, and the level remained detectable in all patients (range, 1.4 to 5.5 log IU/mL). At mo 3 and the end of therapy, 7 of the 9 patients undetectable HEV RNA, and two patients remained detectable with prolonged viremia despite continuous ribavirin therapy. Both these patients were given reduced doses of ribavirin due to side effects (mainly symptomatic anemia) before achieving SVR (1.5 and 3.7 years, respectively). One of them was later found to be non-compliant to therapy due to fear of adverse effects. After resuming ribavirin therapy, four patients (44%, all are kidney transplant recipients) had good responses during treatment, but developed HEV recurrence after initial complete virologic response at the end of therapy.

At the beginning of ribavirin therapy, all nine patients had an elevation of liver enzymes. Eight patients had transaminitis greater than two times the normal upper limit. All treated subjects had a resolution of transaminitis within 10 wk of starting ribavirin. Eight patients had normal liver tests after just 4 wk of therapy.

Factors associated with a sustained virologic response

The overall failure rate of achieving sustained viral clearance for a 12-wk course of ribavirin was 66.67% (delayed virologic response plus viral recurrence after completing treatment). All four patients with viremia recurrence were kidney transplant recipients. One of them had a late relapse, which occurred one year after

Table 1 Demographics and clinical characteristics of 10 patients

Variables	Value
Age, yr	
Mean	47
Range	34-59
Male gender, *n* (%)	8 (80)
Type of organ transplant, *n* (%)	
Liver	4 (40)
Kidney	5 (50)
Bone Marrow	1 (10)
Time from transplant to unexplained transaminitis in mo	
Median	41.5
Range	0.5-276
Time from transplant to diagnosis of HEV in mo	
Median	43.0
Range	0.5-276
Immunosuppressive therapy at the diagnosis of HEV, *n* (%)	
Tacrolimus	6 (60)
Cyclosporin	1 (10)
Mycophenolate	6 (60)
Azathioprine	3 (30)
Prednisolone	7 (70)
Sirolimus	1 (10)
BEAM-R	1 (10)
(carmustine, etoposide, cytarabine, and melphalan – rituximab)	
Peak alanine transaminase, as U/L	
Median	213
Range	133-8127
Peak aspartate aminotransferase, as U/L	
Median	203
Range	84-2591
Baseline estimated glomerular filtration rate, as mL/min	
Median	63
Range	27-108
Baseline creatinine, as μmol/L	
Median	141
Range	66-323

HEV: Hepatitis E virus.

the end of therapy. During the HEV recurrence, transient transaminitis was noted. An HEV reinfection cannot be excluded. The rest of the other relapsers did not have accompanying transaminitis during the recurrence of HEV viremia. All 4 of them responded to the second course of ribavirin (12-16 wk) and achieved durable SVR.

We analysed the factors that could potentially influence the rate of SVR (Table 2). Given the limited sample size, the only statistically significant predictive factor found to be associated with SVR was being a kidney transplant recipient (0 of 5 treated, χ^2 test, $P < 0.05$). The most common side effect of ribavirin was anemia, with many patients requiring dose adjustments, erythropoietin or blood transfusions. Of the nine treated patients, all had a decrease in haemoglobin (Hb) levels (range, 3 to 6.2 g/dL). Five patients required blood transfusions, and seven required erythropoietin injections during ribavirin therapy.

DISCUSSION

Since the landmark paper by Kamar *et al*[7] in 2014, a 12-wk course of ribavirin

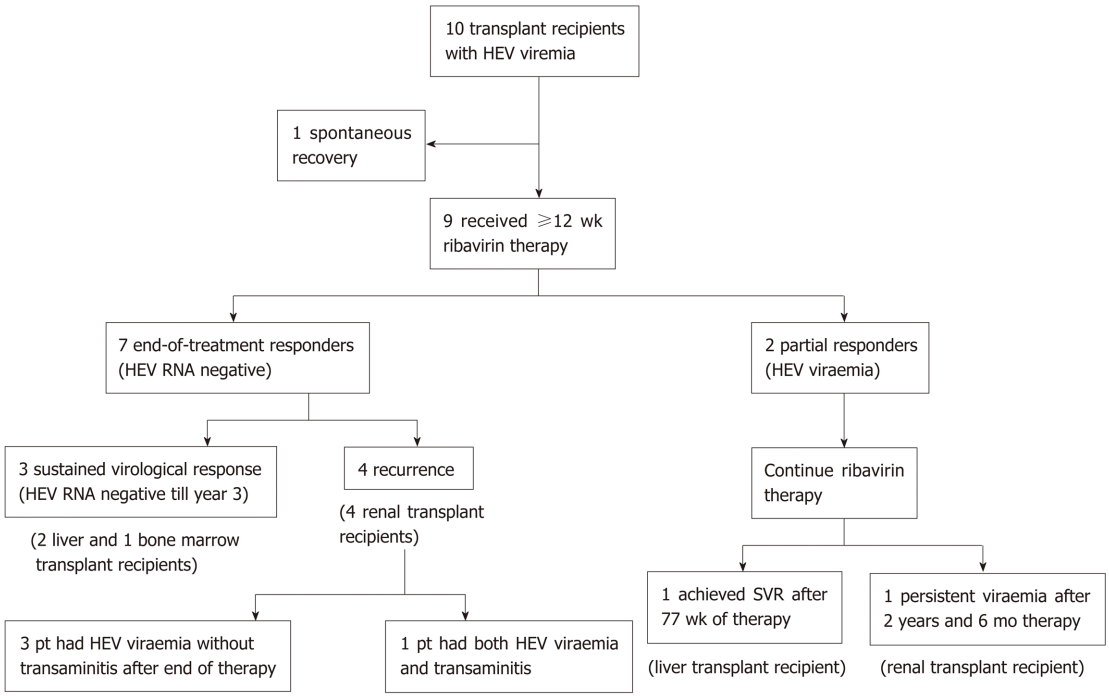

Figure 1 Outcomes of ribavirin therapy in transplant recipients with hepatitis E virus infection. HEV: Hepatitis E virus.

monotherapy has been widely used to treat chronic hepatitis E virus infection in transplant recipients, if a reduction in immunosuppression fails to eradicate the virus. Our study aims to assess the effects of ribavirin therapy in this Asian series, and compare the results with prior studies. Our study has two main findings: the SVR rate of ribavirin therapy for chronic HEV infection in our immunosuppressed Asian hosts may be lower than reported earlier, and kidney transplant recipients are associated with a higher risk of HEV recurrence or partial response to ribavirin monotherapy.

In this study, all five consecutive kidney transplant recipients (50%) had either partial response (HEV remains detectable) or had a recurrence of HEV after an initial virologic response. Statistically and clinically, this is unlikely to be coincidental. In Kamar *et al*[7] 2014, 42 out of 59 patients (71%) had a kidney transplant or kidney and pancreas transplantation, and they responded to treatment almost immediately from the beginning. The ability of the patients to tolerate the full dose of ribavirin therapy is one of the main reasons. Kidney transplant patients tend to have lower GFR than the other patients, and thus develop more haemolytic anemia on the same dose of ribavirin. On average, our kidney transplant patients received 7.8 mg/kg body weight of ribavirin, which was comparable to 8.1 mg/kg body weight in the study, but still significantly lower than the liver and bone marrow transplant recipients in our study (10.3 mg/kg). Our kidney transplant recipients were also slightly older (48 *vs* 42 years), had more years between transplantation and HEV infection, roughly double the peak viral load, and were on higher doses of calcineurin inhibitors than liver transplant recipients.

At the end of the 12-wk therapy, one patient (a kidney transplant recipient) continued to have high viremia of > 7 log IU/mL, whose HEV viremia persisted for another 3 years, during which he continued to receive reduced-dose, maintenance ribavirin therapy due to his renal graft impairment. Another patient had borderline HEV RNA levels at the end of treatment. Her HEV infection subsequently recurred due to non-adherence to ribavirin therapy. Hence the rate of virologic response at the end of therapy was 78%. Adding the number of virological recurrences (n = 4), the overall failure rate of a 12-wk ribavirin therapy for this study is 66.7%, far higher than that reported in the Western population. For those who had a recurrence of HEV, a second course of ribavirin for 12 wk resulted in a complete virologic response at the end of therapy and SVR at wk 24. In retrospect, a longer course of ribavirin therapy may be considered in kidney transplant patients who could not tolerate a full dose of ribavirin therapy.

It needs to be pointed out that in this study, most patients received ribavirin therapy between 1 mo to 3 mo after the first HEV RNA positive result on clinical grounds, based on the discretion of the physicians. Thus, these were not technically

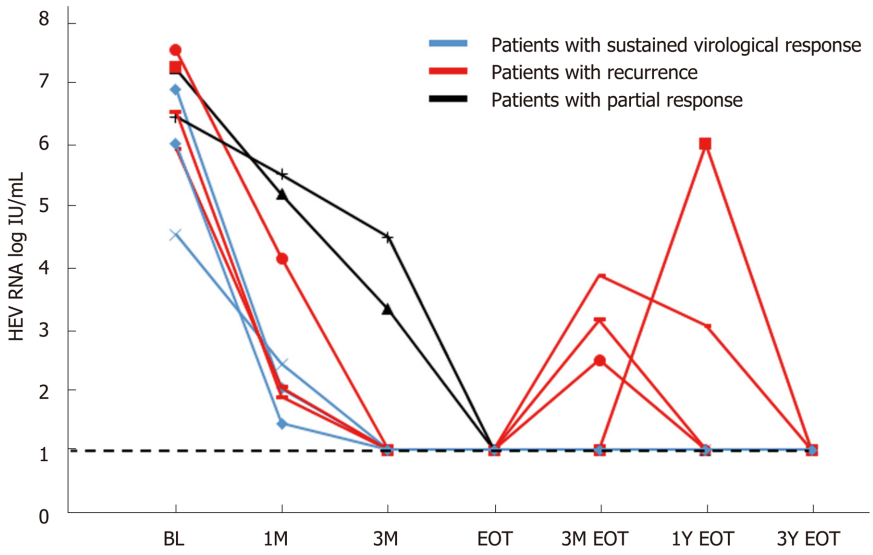

Figure 2 Hepatitis E virus concentration during ribavirin therapy. BL: Baseline; EOT: End of treatment.

chronic HEV infection by the original definition that requires viral persistence of 6 mo or more. It is possible that some of these patients (the three patients who had SVR) may spontaneously clear the virus if ribavirin had not been started. However, we are focusing on the group of immunosuppressed subjects who had persistent viremia or HEV recurrence beyond 6 mo, thus qualifying them as chronic HEV infections. It is interesting to note that as HEV infection in transplant recipients are diagnosed increasingly earlier, and there is little reason to continue observing deranged liver function tests when treatment is available, the definition and cut-off for treatment of chronic/persistent HEV infection in this group are due for revision.

Anemia was the main limiting side effect. The mean hemoglobin fall was 3.4 g/dL for the patients who were treated with ribavirin. Three patients (33.3%) required a transient interruption of ribavirin therapy due to severe anemia. Five patients need transfusions, and seven patients require erythropoietin.

This uncontrolled case series has several limitations. (1) The number of patients studied was small; (2) The immunosuppression regimen was not controlled; and (3) The dosage of ribavirin and the monitoring protocol for each patient were individualized based on clinical assessment.

In conclusion, this Asian single-centre case series shows that the SVR rate of HEV infection treated with a 12-wk course of ribavirin may be lower than reported earlier. Kidney transplant recipients are at higher risk of relapse, possibly due to higher immunosuppression requirements and reduced tolerance for higher ribavirin dosages. A 3-mo regimen seems to be sufficient for the remaining organ transplant recipients. Whether a longer therapy for all Asian kidney transplant recipients, and whether the extended therapy should be guided by the on-treatment response, remains uncertain. Larger prospective studies are required to determine the most beneficial dose and duration of ribavirin therapy.

Table 2 Comparison of clinical, biochemical and virological factors between the sustained responders and relapsers/delayed responders of ribavirin therapy

	Sustained responders, *n* = 4	Relapsers/ delayed responders, *n* = 5
Type of transplant	3 liver transplants, 1 bone marrow transplant	Kidney transplant
Mean age in yr	42 (15–58)	48 (17–66)
Males	3	4
Mean Time from transplant to HEV infection	40.3 mo (7-132 mo)	130.7 mo (3.5-204 mo)
Mean HEV viral load peak, as IU/mL	6.0×10^6	1.2×10^7
Mean daily dose of Prednisolone at time of infection, *n* = 6	13.5 mg (*n* = 2)	9.6 mg (*n* = 4)
Mean level of Tacrolimus at time of infection, as μg/L for *n* = 5	5.5 (6.4, 7.4, 2.8) *n* = 3	8.6 (9.5, 7.7) *n* = 2
Mean peak ALT, as U/L	402 U/L (223-766)	169 U/L (133–202)
Mean peak AST, as U/L	374 U/L (186-818)	132 U/L (84-219)
Lowest eGFR, as mL/min	49 (34 to 85)	32 (11-68)
Mean ribavirin dose, as mg/kg body weight, at initiation	10.3 mg/kg body weight (6.9- 13.5)	7.8 mg/kg body weight (2.7-11.9)
Haemoglobin drop	3.3 g/dL	3.5 g/dL
Resolution of Transaminitis after starting treatment	15 d (12-21)	35 d (14-70)
Mean time to viral clearance after starting ribavirin	8.5 wk (8-11)	9.3 wk (5-12), *n* = 4
HEV recurrence	None in all 4 patients (including the non-adherent subject after she became compliant to ribavirin Rx)	Yes in 4 patients + 1 patient with persistent viremia

HEV: Hepatitis E virus; eGFR: Estimated glomerular filtration rate; ALT: Alanine transaminase; AST: Aspartate aminotransferase.

ARTICLE HIGHLIGHTS

Research background

There has been an increase in the amount of literature and our understanding of Hepatitis E virus (HEV) infection since the landmark paper by Kamar *et al* in 2014. This study describes the outcome of HEV treatment in a transplant center in Singapore, where immunosuppressed Asian hosts appear to have lower sustained virologic response (SVR) rates after a 12-wk course of ribavirin than reported earlier.

Research motivation

Singapore is a unique industrialized country where, although ethnic Chinese make up 76% of the population, it is a thriving hub with many international visitors. Seven genotypes of HEV have been described so far, and studies have reported a rate of chronicity of 50%-60%. Ribavirin is considered an effective treatment option for chronic HEV in post-transplant patients, where success rates > 75% have been reported in France and Germany alongside a reduction in immunosuppression dose. Since most clinical studies come from Europe and the United States, there is a pressing need to characterize and investigate the state of chronic HEV infection in Asian populations.

Research objectives

Our report describes the first 10 consecutive cases of hepatitis E diagnosed in post-transplant patients in an organ transplant center in Singapore.

Research methods

This is a retrospective case series that studied all newly diagnosed HEV infections in post-transplant patients from May 2012 to September 2015. Subjects who had transaminitis that were caused by persistent HEV replication were treated with ribavirin, and the results were collected and tabulated. Data from the first 10 HEV RNA-positive patients who had received a solid organ transplant (5 kidney transplant recipients, 4 liver-transplant recipients, and 1 bone marrow transplant recipient) were analysed.

Research results

One of the patients was from United Arab Emirates, and the other nine were Singapore residents. The median starting dose of ribavirin was 600 mg per day. The dosages were subsequently adjusted based on the estimated GFR. In more than half of patients (5 out of 9), dose reductions of ribavirin were necessary due to clinically significant anemia. The overall failure rate of achieving sustained viral clearance for a 12-wk course of ribavirin was 66.67%

(delayed virologic response plus viral recurrence after completing treatment) – far higher than that reported in Western populations. All four patients with viremia recurrence were kidney transplant recipients, which was found to be the only statistically significant predictive factor. The most common side effect of ribavirin was anemia.

Research conclusions

This study proposes that kidney transplant recipients, particularly those with poorer renal function, are more susceptible to the adverse effects of ribavirin. Asian patients with lower body weight may be even more likely to suffer from the side effects. This Asian single-centre case series shows that the SVR rate of HEV infection treated with a 12-wk course of ribavirin may be lower than reported earlier. Kidney transplant recipients are at higher risk of relapse, possibly due to higher immunosuppression requirements and reduced tolerance for higher ribavirin dosages.

Research perspectives

More effective therapy for chronic HEV infection may be needed, including more accurate markers to predict ribavirin response. A large, prospective, controlled study comparing kidney transplants and other groups of chronic HEV patients will be useful to confirm the results of this study and minimise bias.

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