The Evolving Landscape of Liver Cirrhosis Management

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Preface

Liver cirrhosis is the irreversible fibrosis of the liver, the end stage of a final shared pathway in chronic damage to a major vital organ. It is the 9th leading cause of death in Japan and the 13th leading cause of death globally, with worldwide mortality having increased by 45.6% from 1990 to 2013. Currently, the patients with compensated cirrhosis take a risk of death that is 4.7 times as high as the risk in the healthy population, and those with decompensated cirrhosis take a risk that is 9.7 times as high. The average life expectancy of a patient with compensated cirrhosis is 10–13 years, and the average life expectancy may be as low as 2 years if there is decompensation.

Recent developments of antiviral therapies, including nucleotide analogues and direct-acting antivirals for hepatitis B and C, lead to significant leap forward in clinical medicine for liver cirrhosis; however, a critical issue is an increase in the number of non-B, non-C cirrhosis cases in Japan. Cirrhosis is not a single disease entity but is based on various etiologies such as alcoholic and nonalcoholic steatohepatitis, autoimmune hepatitis, and congenital hepatic disorders, and it has serious complications, which exacerbate the disease prognosis. With continuous hepatocyte destruction and collagen deposition, the liver is shrunken in size and distorted in shape, forming multiple nodules of liver cells separated by broad fibrotic bands, which disturbs intrahepatic blood circulation and induces portal hypertension with extensive portacaval shunts. The pathophysiological features of cirrhosis involve progressive liver injury and fibrosis resulting in portal hypertension and decompensation, including ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, variceal hemorrhage, and hepatocellular carcinoma. The aim of this book is to review the overall progress of clinical management in liver cirrhosis including etiology and diagnosis (Chaps. 1–5), nutritional management (Chap. 6), microbiome (Chap. 7), complications (Chaps. 8–13), and novel and prospective therapies (Chaps. 9–17).

We hope that this book will be helpful in facilitating clinical and research activities on liver cirrhosis. Finally, we would like to thank all of the authors for their contributions as well as Springer Japan for their efforts in publishing this book.

Kashihara, Japan — Hitoshi Yoshiji
Kashihara, Japan — Kosuke Kaji

Contents

Chapter 1
Liver Cirrhosis with Steatohepatitis: Nonalcoholic Steatohepatitis and Alcoholic Steatohepatitis

Teruki Miyake and Yoichi Hiasa

Abstract Nonalcoholic steatohepatitis is a phenotype of metabolic diseases in the liver, associated with eating disorders and lack of exercise. In contrast, alcoholic steatohepatitis develops due to alcohol abuse. Although the causes are different, each type of steatohepatitis exhibits the same histological features, such as steatosis, lobular and portal inflammation, hepatocellular ballooning, and perisinusoidal and pericellular fibrosis. Untreated nonalcoholic and alcoholic steatohepatitis can progress to cirrhosis, and advanced fibrosis is a predictor of poor prognosis. Therefore, it is important to elucidate the pathophysiology and make appropriate diagnoses and initiate treatment. Various factors are involved in each pathological conditions. To diagnose these diseases, a more user-friendly diagnostic assessment is needed. Currently, predictive models combined with several indicators and imaging assessments are used. Further, several treatments are attempted for patients in clinical practice and clinical trials, however the efficacy is not sufficient. In this chapter, we reviewed the epidemiology, pathophysiology, diagnosis, and treatment of cirrhosis due to both nonalcoholic and alcoholic steatohepatitis.

Keywords Nonalcoholic steatohepatitis · Alcoholic steatohepatitis · Cirrhosis Epidemiology · Pathophysiology · Diagnosis · Genetic factor · Treatment

The causes of steatohepatitis are divided into alcoholic and nonalcoholic. Alcoholic fatty liver disease has long been widely recognized. However, eating habit disorder and lack of exercise have recently increased nonalcoholic fatty liver disease (NAFLD), which is a phenotype of metabolic diseases in the liver. These two fatty liver diseases show the same histological features, such as steatosis, lobular and portal inflammation, hepatocellular ballooning, and perisinusoidal and pericellular

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fibrosis [1, 2], but their causes, treatments, and prognoses are different. In this chapter, we reviewed the epidemiology, pathophysiology, diagnosis, and treatment of nonalcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH).

1.1 Epidemiology

NAFLD is the most common liver disease worldwide. A meta-analysis including 8,515,431 subjects from 22 countries estimated that the global prevalence of NAFLD was 25.24% (95% confidence interval (CI): 22.10–28.65) and the global prevalence of NASH among patients with biopsy-confirmed NAFLD was 59.1% (95% CI: 47.55–69.73). Moreover, NASH prevalence estimates among patients with NAFLD without an indication for biopsy were 6.67% (95% CI: 2.17–18.73) for Asia and 29.85% (95% CI: 22.72–38.12) for North America [3]. The incidence of advanced fibrosis in NASH was 67.95 in 1000 person-years (95% CI: 46.84–98.59), and 40.76% (95% CI: 34.69–47.13) of patients with NASH developed fibrosis with an average annual progression rate of 0.09% (95% CI: 0.06–0.12) [3]. Patients with NASH had 5.29 per 1000 person-years (95% CI: 0.75–37.56) incidence of hepatocellular carcinoma (HCC). The liver-specific mortality incidence rate was 11.77 per 1000 person-years (range, 7.10–19.53), and the overall mortality incidence rate was 25.56 per 1000 person-years (range, 6.29–103.80) [3]. The characteristics of NAFLD are different from those of other liver diseases because NAFLD frequently complicates various metabolic diseases. A meta-analysis and systematic review of 16 observational or retrospective studies showed that patients with NAFLD were at higher risk for fatal and nonfatal cardiovascular events than those without NAFLD (random effect odds ratio (OR): 1.64; 95% CI: 1.26–2.13) [4]. In addition, patients with NASH were at elevated risk for fatal and nonfatal cardiovascular events (random effect OR: 2.58; 95% CI: 2.58–3.75) [4]. Alcoholic liver disease (ALD) remains a major disease of the liver worldwide, particularly in Europe and the USA [5]. The definition of ALD in Europe is slightly different from that in the USA and Japan. Although alcoholic steatohepatitis is defined by the European Association for the Study of the Liver [6], it is considered as a subtype of alcoholic hepatitis in Japan and the USA [7]. Protein calorie malnutrition was previously common in patients with alcoholic liver cirrhosis (LC) [8]. However, these patients have recently become polarized in overnutrition and malnutrition cases [9], and obesity and metabolic diseases are the risk factors for the development of alcoholic LC. The amount of alcohol consumption is associated with the development of fatty liver and LC [10–14]. Fatty liver develops in approximately 90% of individuals who consume more than 60 g/day of alcohol [10]. The risk for developing cirrhosis increases with 60 g/day or more alcohol consumption for 10 years or longer (the amount of alcohol consumption is lower and drinking period is shorter in women than in men) [11, 12, 15], and 6–41% of total drinkers develop cirrhosis at this level [11, 13, 16]. An epidemiologic study estimated 14% and 8% increases in cirrhosis in men and women, respectively, as the consumption of 1 L alcohol increases per capita [17]. In patients with alcoholic

LC, the cumulative rate of HCC onset is 6.8–23.2% at 10 years [18–20] and that of survival is 41.9–53.8% at 10 years [18, 19]. However, alcoholic LC sometimes develops from alcoholic liver fibrosis without alcoholic hepatitis [7, 8, 21]. On the other hand, the influence of alcohol differs depending on race, gender, and genetic polymorphisms, among others, and alcohol consumption at lower doses and with shorter duration affects progression to cirrhosis [22–31]. In particular, in Japan, genetic polymorphisms of alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2) affect susceptibility to alcoholism [22], and the age-adjusted odds ratios (AORs; 95% CI) for LC (1.58 [1.19–2.09]) are higher in ADH1B∗2 allele carriers than in ADH1B∗1/∗1 carriers, and the AORs for LC (1.43 [1.01–2.02]) are higher in ALDH2∗1/∗1 carriers than in ALDH2∗1/∗2 carriers. Additionally, the ADH1B∗2-associated age-AORs increase according to the severity of the liver disease (Child–Pugh class A, 1.81 (1.24–2.63); Child–Pugh class B/C, 3.17 (1.98–5.07)) compared with non-LC and no/mild fibrosis [22].

1.2 Pathophysiology of NASH (Fig. 1.1)

NASH is affected variously, and its pathological condition is completed. Overnutrition activates de novo lipogenesis and accumulates visceral adipose tissue. Accumulated visceral adipose tissue supplies excess free fatty acid to the liver via the portal vein, and it is the main source of hepatic triglycerides [32–34].

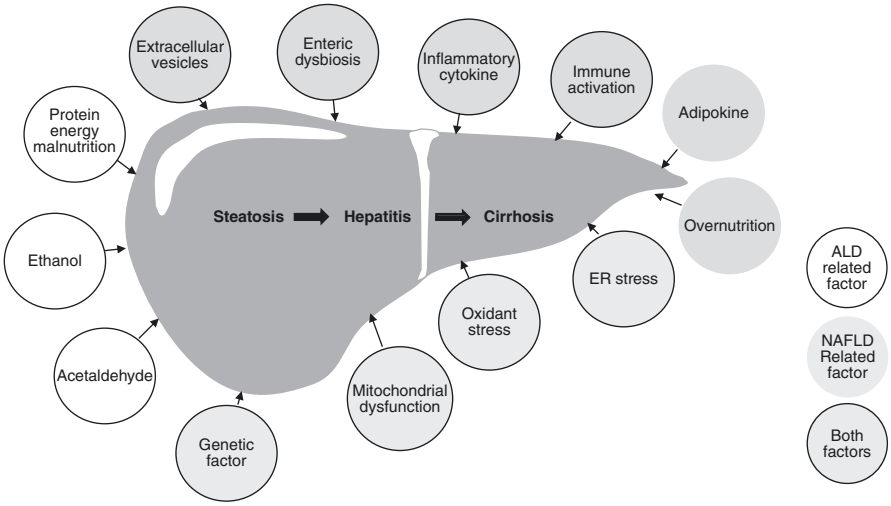

Fig. 1.1 The pathophysiologies of nonalcoholic fatty liver disease and alcoholic liver disease. Various factors directly or indirectly affect the liver, and influence the progress to fatty liver, steatohepatitis, and cirrhosis. Abbreviations: *ALD* alcoholic liver disease, *NAFLD* nonalcoholic fatty liver disease, *ER stress* endoplasmic reticulum

Unbalanced fatty acid intake, de novo lipogenesis, fatty acid oxidation, and export of very low-density lipoprotein exacerbate steatosis, hepatic inflammation, and hepatocellular ballooning. In the accumulated visceral adipose tissue, enlarged adipocyte causes abnormal secretion of adipokines, such as decrease in adiponectin and increase in leptin, which decrease fatty acid oxidation and insulin sensitivity; chemokines, such as monocyte chemotactic protein-1; and inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 [35–38]. This abnormal secretion switches macrophage phenotype from anti-inflammatory M2 polarization to proinflammatory M1 polarization, which exacerbates inflammation further and induces peripheral and hepatic insulin resistance and hyperinsulinemia [39, 40]. Hyperinsulinemia activates sterol regulatory element-binding protein 1c (SREBP-1) and increases de novo lipogenesis [41]. Accumulated visceral adipose tissue does not only supply excessive fatty acid and alter inflammatory cytokine and adipokine secretion to the liver via portal vein, but also accelerate fatty acid synthesis through whole-body insulin resistance. Excess accumulated fatty acid is metabolized mainly in the mitochondria. Lipid accumulation beyond metabolic capacity induces mitochondria dysfunction, worsens lipid metabolism, and is a trigger for peroxisomal and microsomal oxidation [42]. Oxidative balance leads to the production of reactive oxygen species (ROS) and to liver injury [42–46]. Additionally, lipid overload induces endoplasmic reticulum (ER) stress, which triggers unfolded protein response. Inadequate response to ER stress may cause fat accumulation, insulin resistance, inflammation, autophagy, and apoptosis, and is associated with the development of NASH [47–52]. Gut microbiota contributes to various functions, such as digestion, vitamin synthesis, and immune system development. It also helps to protect from pathogens and maintain intestinal homeostasis and metabolic functions [53]. Therefore, dysbiosis can be considered a predisposing factor for the development and progression of NAFLD [53, 54]. Short-chain fatty acids are products of carbohydrate fermentation by gut microbes [53]. They activate metabolism by increasing the secretion of peptide YY and incretin, and activating AMP-activated protein kinase (AMPK) [55–57]. They also improve barrier function to prevent the passage of bacterial toxins into the circulation. However, dysbiosis inhibits the production of short-chain fatty acids [54] and causes increased intestinal permeability and translocation of bacteria or bacterial products into the portal circulation, followed by activation of proinflammatory pathways after binding with several receptors in the liver [58–60] and progression to chronic liver injury. Toll-like receptors (TLRs) and nucleotide oligomerization domain-like receptors (NLRs) recognize pathogen-associated molecular patterns, such as bacterial peptidoglycans or lipopolysaccharides (LPS), double-stranded DNA and RNA [61], and damage-associated molecular patterns (DAMPs) [62], as a product of cell stress/death [61, 63–65]. They are associated with the relationship between dysbiosis and hepatic inflammation. NLRs also mediate intracellular signaling and activate inflammasomes. Intracellular cascade promotes secretion of the biologically active cytokines IL-1b and IL-18 and induces inflammation and cell death [66–71]. Additionally, dysbiosis inhibits the synthesis of angiopoietin-related protein 4 and decreases lipoprotein lipase activity resulting in decreased

release of free fatty acids from very low-density lipoprotein particles to the liver [72]. On the other hand, hepatocytes release extracellular vesicles (EVs), which are nanoparticles of different sizes, into the intracellular milieu. EVs are sub-classified into exosomes, microparticles, and apoptotic bodies according to their size and release mechanism [66, 73]. Lipotoxic effects cause EV release into the extracellular environment, inducing inflammation, fibrosis, and angiogenesis [74–76]. Additionally, specific lipid types, such as saturated fatty acid, trans-fatty acid, free cholesterol, lysophosphatidylcholine, and ceramide, also induce ER stress [77], stimulate macrophage via TLR4 [78, 79], directly result in inflammasome activation [80], cause ROS generation [81] and mitochondrial dysfunction [81–83], or activate hepatic stellate cells (HSCs) [84, 85]. Regenerative responses for liver injury of various causes promote progressive scarring, and repetition of regeneration leads to cirrhosis.

1.3 Pathophysiology of ASH (Fig. 1.1)

Ethanol is metabolized into acetaldehyde mainly in the liver by oxidative system pathways, such as ADH1 in the cytosol, cytochrome P450 in microsomes, and catalase in peroxisomes (Fig. 1.2) [86]. Acetaldehyde is metabolized into acetate by ALDH2 in the mitochondria (Fig. 1.2). ADH and ALDH reactions use nicotinamide adeninedinucleotide (NAD)+ as a cofactor and induce NADH. NADH is mainly reoxidized

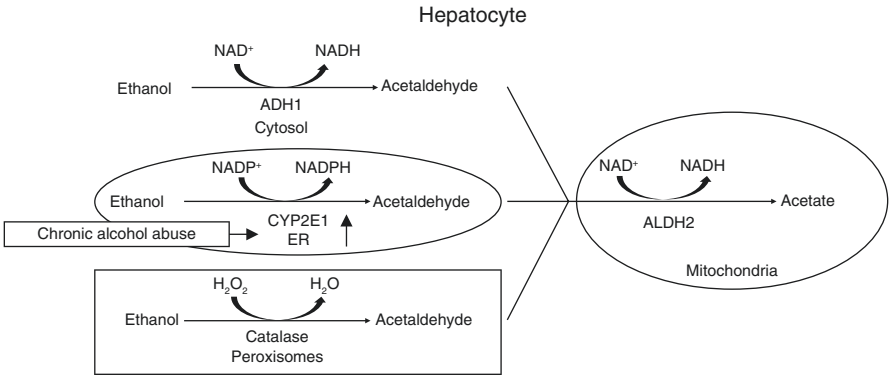

Fig. 1.2 Ethanol metabolism in a hepatocyte. In hepatocytes, ethanol is mainly metabolized to acetaldehyde by the action of alcohol dehydrogenase 1 (ADH1) in the cytosol, cytochrome P450 in microsomes, and catalase in peroxisomes. Subsequently, acetaldehyde is metabolized to acetate by the action of aldehyde dehydrogenase 2 (ALDH2) in the mitochondria. In cases of low blood alcohol concentration, ADH metabolizes more than 80% of absorbed alcohol, and CYP2E1 plays a minor role. However, in cases of chronic alcohol abuse, CYP2E1 is induced and associated with 50% of alcohol metabolism. Abbreviations: *NAD* nicotinamide adenine dinucleotide, *ADH1* alcohol dehydrogenase 1, *NADP* nicotinamide adenine dinucleotide phosphate, *CYP2E1 Cytochrome P450 2E1, ER* endoplasmic reticulum, *ALDH2* aldehyde dehydrogenase 2

to NAD+ by the mitochondrial electron transfer chain [87, 88] and constitutes ROS [87]. In case of low blood alcohol concentration, ADH metabolizes more than 80% of absorbed alcohol, and Cytochrome P450 2E1 (CYP2E1) which is an nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme, has a small role [89]. On the other hand, in case of chronic alcohol abuse, CYP2E1 is induced and accounts for 50% of alcohol metabolism [90–94], and catalytic reaction of CYP2E1 also generates a significant amount of ROS (Fig. 1.2) [95, 96]. The catalase pathway is not significant in the liver. Alcohol consumption inhibits the enzyme associated with fatty acid oxidation because alcohol exposure directly or indirectly increases NADH and decreases AMPK [97–100]. Alcohol exposure also inhibits peroxisome proliferator-activated receptor (PPAR)α via upregulation of CYP2E1-derived oxidative stress [101], adenosine [102], and acetaldehyde, or via downregulation of adiponectin [103] and zinc [104], which exacerbates fat accumulation in the liver [105]. In ALD, SREBP-1c expression is upregulated by increasing acetaldehyde [106], LPS signaling via TLR4 [107–110], TNF-α [111, 112], circadian gene Per-1 [113], adenosine [102], endocannabinoids [114], early growth response 1 [115], epinephrine [116], c-Jun N-terminal protein kinase [117], and ER stress response [118], and by decreasing AMPK [99], Sirtuin1 [119], adiponectin [120], and signal transducer and activator of transcription 3 [121]. Furthermore, autophagy is important in removing lipid droplets in hepatocytes [122]. Although short-term alcohol consumption activates autophagy, long-term alcohol consumption inhibits autophagy [122–124]. These disorders, which are induced by alcohol consumption, exacerbate fat accumulation in the liver. ROS and acetaldehyde, which are produced by alcohol metabolism, form a variety of protein and DNA adducts that promote lipid peroxidation, mitochondrial glutathione depletion, and mitochondrial damage, and cause hepatocyte injury [125, 126]. Alcohol-mediated hepatotoxicity induces hepatocyte apoptosis, which leads to the release of various DAMPs [127]. DAMPs bind to pattern recognition receptors, initiate inflammation [127], and activate inflammasomes [128, 129]. Further, alcohol consumption induces bacterial overgrowth [130], and enteric dysbiosis increases LPS influx from the gut to the liver [131, 132]. Increase of LPS stimulates the Kupffer cells and HSCs via TLR4. Activated Kupffer cells produce proinflammatory cytokines and oxidant stress. Moreover, acetaldehyde and LPS [133–135] stimulate parenchymal and nonparenchymal cells to produce IL-8, chemokine CXC ligand 1 (Gro-α), and IL-17, and contribute to neutrophil infiltration and activation [136–138] along with activated Kupffer cells. Activated C1q, C3, and C5 components by alcohol consumption also stimulate Kupffer cells to produce TNF-α [129, 137–138]. This activation of innate immunity also causes liver injury. On the other hand, various proteins modified by oxidant stress and acetaldehyde, among others, serve as antigens to activate adaptive immune response [139–142]. Activation of adaptive immunity is also involved in the pathogenesis of ALD [139–142]. Additionally, acetaldehyde activates HSCs via activation of multiple signaling pathways and transcriptional factors, and is one of the main causes of alcohol fibrogenesis in the liver [143–145]. DAMPs also directly activate HSCs and trigger fibrosis progression [146]. Activated HSCs are regulated by interferon-γ production [147–149]. The cross talk between natural killer cells and activated HSCs induces interferon-γ production by natural killer cells, which results

in cell cycle arrest, apoptosis, and cytotoxicity of HSCs [147–149]. Oxidative stress induced by long-term alcohol consumption suppresses antifibrotic effects by blocking NK cell killing of activated HSCs [150] and promotes fibrosis in the liver.

1.4 Diagnosis

Advanced fibrosis is associated with liver-related illness, liver transplantation, and liver-related death in patients with NAFLD and AFLD. Therefore, it is important to enclose advanced fibrosis. Liver biopsy is currently the gold standard for determining the stage and assessing the severity of NASH and ASH. However, it is invasive, expensive, and inconvenient, and a more easy-to-use diagnostic assessment is desired.

Many clinical biological variables, such as age, body mass index, alanine aminotransferase, bilirubin, platelet, prothrombin time, albumin, fibrosis marker, and diabetes, are associated with advanced fibrosis. However, a single indicator is not sufficient for diagnosis because of the many false-positive and false-negative cases. Therefore, predictive models combined with several indicators are used.

In advanced NAFLD, previous reports showed that aspartate aminotransferase (AST) platelet ratio index score [151, 152], AST/ALT [151, 153, 154], BARD score [151, 155, 156], BARDI score [157], enhanced liver fibrosis test [158–160], FIB-4 index [151, 156, 161], FibroTest [162–164], FibroMeter [159], Hepascore [165], NAFLD fibrosis score [151, 156, 161, 166], FIB-C3 [167], FIBROSpect test [168], and the model combining serum hyaluronic acid, cytokeratin (CK)-18, and tissue inhibitor of metalloproteinase-1 (TIMP-1) have high area under the receiver operating characteristic curve for predicting advanced NASH, and are useful models for predicting advanced NASH [169]. The terminal peptides of procollagen III [170] and pro-C3 [171] also present high diagnostic rate, but they are single markers. Although the aforementioned tests have not been sufficiently validated for ALD and values are needed to set a cut-off for ALD, the tests seem to be efficient in AFLD and NAFLD [159, 162, 172–175].

Imaging assessment helps to diagnose advanced NAFLD and AFLD. The findings of a small, shrunken liver, hepatic nodularity, abnormal tortuous vessels from intra-abdominal varices, ascites, and so on are consistent with cirrhosis [176–178]. For evaluation of steatosis, conventional ultrasonography is widely used. Conventional ultrasonography does not require specific techniques and is convenient. It roughly assesses the severity of steatosis, but the assessment is affected by patient obesity and performance of the technique. Quantification of fatty deposition in the liver is evaluated by computed tomography (CT) [179], magnetic resonance spectroscopy (MRS) [180–182], magnetic resonance imaging-proton density fat fraction (MRI-PDFF) [183], and controlled attenuation parameter (CAP) using vibration-controlled transient elastography (VCTE) [184–186]. MRS is the gold standard for quantification of fat content in the liver, but it is expensive and requires a specialist and a special device. Although MRI-PDFF, CT, and CAP are relatively convenient, they are expensive, expose patients to ionizing radiation, require an

additional device, or are unable to assess severely obese patients [185]. Similarly, in the evaluation of liver fibrosis, several modalities, such as ultrasonography, CT, and MRI, are used [187]. Additionally, VCTE [186–193], strain elastography [194], acoustic resonance forced impulse imaging [193, 195], and shear wave elastography [193] are techniques that adapt ultrasound imaging to produce liver stiffness measurement. Magnetic resonance elastography also evaluates the severity of liver fibrosis and is better than ultrasound imaging for liver fibrosis detection [190, 193, 195–197]. However, as shown in steatosis evaluation, several limitations exist.

1.5 Genetic Factor

Polymorphisms in patatin-like phospholipase domain-containing 3 (PNPLA3) and transmembrane 6 superfamily, member 2 (TM6SF2) promote NASH development and are risk factors for liver-related disease such as cirrhosis and HCC [198–202]. PNPLA3 encodes adiponutrin, a lipase that regulates both triglyceride and retinoid metabolism. PNPLA3 polymorphisms are strongly associated with hepatic steatosis, steatohepatitis, fibrosis, and cancer. In patients with ALD, PNPLA3 genetic polymorphism is also associated with increased risk for alcoholic hepatitis, alcoholic cirrhosis, and HCC among drinkers [198, 203].

1.6 Treatment

In clinical practice, several treatments have been attempted on patients with NASH. Weight loss for overweight or obese individuals by lifestyle intervention resolves histological steatohepatitis and improves liver fibrosis [204, 205]. In particular, ≥5% or ≥7% weight loss improves steatohepatitis, and ≥10% weight loss results in steatohepatitis resolution and fibrosis regression [204]. Bariatric surgery, which could control body weight, is also useful for treating NASH. After surgery, a high proportion (85%) of patients show improvement in NASH including advanced NASH, and 33.8% of patients exhibit reduction of fibrotic stage by histologic analysis [206]. Although weight reduction is effective for patients with advanced NASH with sufficient residual function of the liver, enough nutrition is necessary for decompensated cirrhosis patient caused by NASH in order to maintain liver function. For nutritional therapy, please refer to the other chapter.

On the other hand, several pharmacotherapies are used for treating NASH. Vitamin E, an antioxidant, demonstrates improvements in various features of NASH, such as steatosis, lobular inflammation, and ballooning [207, 208]. Pioglitazone, an insulin sensitizer, improves steatosis, lobular inflammation, ballooning, and fibrosis [209–211]. Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone, whic possesses multifunction. GLP-1 promotes insulin secretion, reduces glucagon secretion in a glucose-dependent manner, suppresses appetite, delays gastric emptying, and induces weight loss and insulin sensitivity [207, 208]. Administration of liraglutide (GLP-1

receptor agonist) is associated with greater resolution of NASH (especially steatosis) and less progression of fibrosis compared with placebo [212, 213]. However, further studies are warranted to determine whether these treatments are effective for patients with NASH with cirrhosis. Additionally, although numerous clinical trials for the pharmacotherapies for NASH have been attempted, or are still in progress, a majority of clinical trials aimed at NASH of stages 0–3, not cirrhosis, and clinical trials on cirrhosis are limited.

Clinical trials of Emricasan [214], galectin-3 protein inhibitor [215], pegylated fibroblast growth factor (FGF21) analog [216], obeticholic acid [217], non-bile farnesoid X receptor (FXR) agonist [218], acetyl-CoA carboxylase (ACC) inhibitors (GS-0976) [218], apoptosis signal-regulating kinase (ASK)-1 inhibitor [219], and combinations using two drugs among non-bile FXR agonist, ACC inhibitors, and ASK-1 inhibitor [218] for LC of NASH are still in progress. These trials assessed the effect for HVPG (hepatic venous pressure gradient), event-free survival, change of fibrosis, and portal hypertension.

However, once a patient with NASH has progressed to decompensated cirrhosis, improvement through diet therapy or drug therapy is difficult to achieve. Liver transplantation is a useful treatment for decompensated NASH. Recently, liver transplantation for NASH is increasing and has the same treatment outcome as other diseases [220–223]. The 1-, 3-, and 5-year survival rates after liver transplantation for patients with NASH are 87.6%, 82.2%, and 76.7%, respectively [220]. Hence, management assessment is important to prevent NASH recurrence after liver transplantation.

On the other hand, with regard to patients with ASH, alcohol abstinence is the most important therapeutic intervention [224]. It improves histological feature and decreases portal pressure, improving survival for all stages in patients with ALD [224–227]. However, for alcoholics, continuous abstinence is difficult, and many patients resume drinking [228]. Therefore, to sustain alcohol abstinence, a combination of psychosocial intervention, pharmacological therapy, and medical management is the most effective management strategy for alcohol use disorder (AUD) patients with ALD [229]. Currently, some medications are approved in most countries to promote abstinence [230]. However, the use of most of these drugs is not supported in patients with advanced liver disease [6, 231] because of liver metabolism and/or possible liver toxicity. Only the efficacy and safety of baclofen have been confirmed for AUD patients with LC in a randomized controlled trial in AUD patients with advanced liver disease. Baclofen shows significant efficacy in promoting total alcohol abstinence and in reducing alcohol lapse and relapse [232]. Clinical trials of nalmefene are still in progress [233].

Nutritional therapy is more important in alcoholic cirrhosis than in other liver diseases, because of the presence of not only protein energy malnutrition but also deficiencies of vitamins and trace minerals such as vitamins A and D, thiamine, folate, pyridoxine, and zinc [234, 235]. Therefore, in addition to nutritional support for LC, adequate supplementation is required considering the multiple micronutrient deficiencies in patients with alcoholic cirrhosis [234]. For detailed liver nutritional therapy, please refer to the other chapter. Liver transplantation is a useful treatment for decompensated alcoholic cirrhosis. The European Liver Transplant Registry data showed better survival rate of liver transplantation for ALD (at 84%, 78%, 73%, and 58%

after 1, 3, 5, and 10 years, respectively) than for hepatitis C virus (HCV) and hepatitis B virus (HBV)-related liver disease and cryptogenic cirrhosis [236]. However, in alcoholic cirrhosis, a 6-month period of alcohol abstinence is recommended to allow sufficient clinical improvement to render liver transplantation unnecessary, or to reduce the risk of post-transplant recidivism although a 6-month period of abstinence as predictor of post-transplantation abstinence is poor [6, 237, 238].

1.7 Conclusion

Cirrhosis of nonalcoholic and alcoholic steatohepatitis is an important problem worldwide. However, its onset and progression have not been suppressed and its treatment has not been sufficiently established. To improve a patient prognosis, screening for complications, such as esophageal varices and liver cancer, is also necessary, and further efforts are needed to overcome the disease in the future.

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Chapter 2
Liver Cirrhosis with Autoimmune Liver Diseases: AIH and PBC

Kazumichi Abe, Atsushi Takahashi, and Hiromasa Ohira

Abstract Autoimmune liver diseases (AILDs) are a chronic inflammatory disorder of unknown etiology that may proceed to cirrhosis, although some patients already have cirrhosis at the time of diagnosis. AILDs patients with cirrhosis have higher risks of morbidity and mortality and, in the decompensated phase, complications of portal hypertension and hepatocellular carcinoma (HCC). Management of these patients requires knowledge of the fibrosis stage, since liver fibrosis is closely associated with prognosis. The aim of this report is to provide a current overview of liver cirrhosis in AILDs.

Keywords Autoimmune hepatitis · Primary biliary cholangitis · Liver cirrhosis

2.1 Introduction

Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are classically viewed as distinct autoimmune liver diseases (AILDs). AIH manifests as chronic liver inflammation of an unknown cause. It generally affects young- to middle-aged women and is associated with the presence of autoantibodies and hypergammaglobulinemia [1]. PBC is a progressive AILD characterized by portal inflammation, immune-mediated destruction of the intrahepatic bile ducts, and the presence of highly specific anti-mitochondrial antibodies in serum [2, 3]. AILD is thought to be triggered by environmental factors in genetically susceptible individuals. Genome-wide association and murine model studies have expanded our knowledge of AILD; however, the factors associated with cirrhosis development are unclear. The aim of this report is to provide a current overview of the clinical features, diagnosis, and prognosis of liver cirrhosis with AILDs.

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2.2 Liver Cirrhosis in AILDs

2.2.1 Liver Cirrhosis in AIH

A diagnosis of AIH must be considered in all individuals with acute or chronic hepatitis, although some patients develop cirrhosis at the onset of AIH. In past studies, approximately 6.4–34% of AIH patients were found to already have cirrhosis at presentation, and approximately 3.8–40% of AIH patients developed cirrhosis related to relapse during the follow-up periods [4–9]. As shown in Table 2.1, Roberts reported that thirty-seven patients (29%) had histological cirrhosis at entry, whereas 36 of the 91 patients without cirrhosis (40%) developed it over 39 months. Development of cirrhosis was predicted by lower serum albumin levels at presentation. The frequencies of remission, relapse after drug withdrawal, and treatment failure were comparable in patients with and without cirrhosis at entry. The overall

Table 2.1 Long-term outcome of AIH patients with liver cirrhosis

Author	Roberts [5]	Feld [10]	Kirstein [14]	Ngu [15]	Migita [11]	Abe [12]	Yoshizawa [13]
Year	1996	2005	2011	2013	2011	2012	2012
Region	USA	Canada	Germany	New Zealand	Japan	Japan	Japan
Number of case	128	125	354	133	174	250	203
Mean age at diagnosis of AIH (year)	44.6	43.5	39	50.0	56.7	55.6	55.0
Female ratio	80%	NA	75%	74%	91%	89%	75%
Mean follow-up (year)	3.3	7.9	10	9	8.0	6.8	10.9
Cirrhosis at presentation (%)	28.9% (37/128)	33.6% (42/125)	25% (76/309)	34% (45/133)	12.1% (21/174)	17.2% (43/250)	12.8% (26/203)
Development of cirrhosis during follow-up (%)	39.6% (36/91)	NA	NA	NA	9.2% (14/153)	3.9% (8/217)	NA
5-year survival for patients with cirrhosis	97%	76.3%	NA	NA	NA	91.8%	NA
10-year survival for patients with cirrhosis	89%	61.9%	NA	NA	NA	71.2%	NA
Cumulative survival for patients with or without cirrhosis at baseline (logrank test)	$P = 0.85$	$P = 0.003$	$P = 0.003$	P = NS	NA	$P < 0.001$	$P = 0.952$ (F1-2 vs F3-4)

NA not available, *NS* not significant

10-year survival (93%) was similar to that of an age- and sex-matched cohort from the population at large (94%) [5]. In another study, including 126 AIH patients, Feld reported that 33% of patients had histological evidence of cirrhosis at diagnosis. Except for platelet count, which was lower in patients with cirrhosis, laboratory parameters, patient demographics, and AIH scores did not differ between cirrhotic and non-cirrhotic patients. A similar frequency of patients from each group was symptomatic at diagnosis, and an equivalent proportion had good response to treatment [10]. On the other hand, liver cirrhosis at presentation was found in 12.1–17.2% of Japanese AIH cases [11–13]. The incidence of cirrhosis at presentation of AIH in Japan is lower than that reported in previous studies conducted in the USA and European countries (25–34%) [5, 10, 14, 15]. In addition, cirrhosis developed during the follow-up period in 3.9% and 9.2% of the Japanese AIH patients with non-cirrhosis at presentation. One possible reason for this is that revised and simplified scoring criteria for AIH have been clearly established, and more early stage cases of AIH have been diagnosed. Moreover, human leukocyte antigen DR status is considered to affect the clinical features of patients with AIH. In Japanese patients, DR4 is dominantly associated with the disease. Patients with DR4 are typically older and respond better to corticosteroid treatment than do those with DR3.

Among AIH patients, the presence of cirrhosis at the time of AIH diagnosis, advanced age, lack of treatment, and the appearance of symptoms have been reported to be negative factors for survival [10]. Another study showed that cirrhosis at presentation is associated with poorer prognoses [10, 12, 14]. In contrast, other studies have reported similar outcomes in patients with and without cirrhosis at presentation [5, 13, 15]. The significantly lower levels of platelet count in the group that developed cirrhosis during treatment are important predictors of the development of cirrhosis, although there were no differences in liver histology findings. However, since the relapse rate and the rate of immunosuppressant use were significantly higher in the patients who developed cirrhosis during the follow-up period than in non-cirrhosis patients, it seems that many patients who exhibited relapse from steroid resistance developed cirrhosis. There are indications that cirrhosis is more common among AIH type-1 patients compared to patients with type-2 AIH. In a pediatric study, 69% of ANA/SMA positive patients had evidence of definite cirrhosis on initial biopsy, whereas only 38% of patients positive for anti-LKM-1 were cirrhotic.

2.2.2 Liver Cirrhosis in PBC

PBC is a disease that occurs frequently in middle-aged women; in PBC, the intrahepatic lobule bile duct is destroyed by an autoimmune mechanism and exhibits chronic cholestasis. Destruction of the intrahepatic bile ducts causes the loss of bile ducts, cirrhosis, and liver failure. The concept of disease of “primary biliary cirrhosis” has been proposed for 50 years. Although previous cases were diagnosed after progression to cirrhosis, due to the development of examination methods such

as the measurement of anti-mitochondrial antibodies, many cases are diagnosed before progression to liver cirrhosis. In addition, ursodeoxycholic acid (UDCA) came into use, which made it possible for patients to be diagnosed before progression to cirrhosis. Recently, the name of the disease has been changed to "primary biliary cholangitis." PBC progresses through several stages such as asymptomatic, symptomatic, and liver failure. Biochemical abnormalities eventually appear after a median time of 5.6 years [16], but this phase is not yet associated with the presence of symptoms. When symptoms eventually develop, they are most commonly fatigue and pruritus and, later, varices, edema, or ascites. Liver failure is characterized by the accelerated development of jaundice and is associated with poor prognosis [17]. The clinical course of PBC is classified into three types: slow progressive type, portal hypertension type, and liver failure type. The anti-gp210 antibody positive hepatic failure type has poor prognosis. The anti-centromere antibody positive portal hypertension type progresses slowly [18].

Previous study showed that observed survival of non-cirrhotic patients was not different from that of the control population. In contrast, survival of cirrhotic patients was significantly lower than that of the control population (Fig. 2.1). Prognostic factors of survival were defined. The multivariate analysis identified two independent prognostic factors: the presence of cirrhosis and a high serum bilirubin level [19].

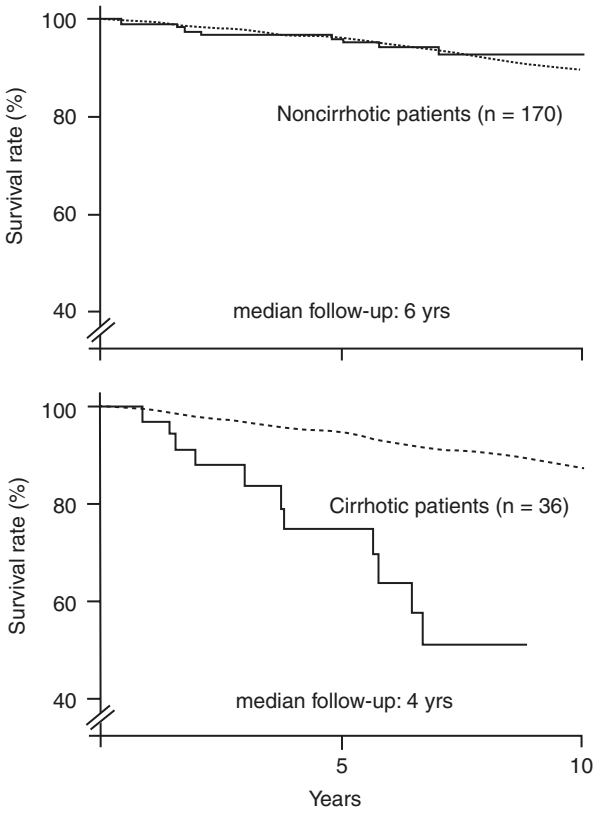

Fig. 2.1 Survival of the UDCA-treated PBC patients according to the absence or presence of cirrhosis at the beginning of treatment [19]

The other study suggested that mean survival for patients with a bilirubin of 2.0 mg/dL is 4 years, while for those with bilirubin of 6.0 mg/dL it is only 2 years [17]. Histological stages can predict survival of PBC patients [20]. In untreated PBC patients, the median time to the development of extensive fibrosis is 2 years. The probability of remaining in early stages after 4 years is 29%, whereas the development of cirrhosis occurs in 50% of patients originally demonstrating histological evidence of interface hepatitis without fibrosis [21].

2.3 Diagnosis of Liver Cirrhosis in AILDs

2.3.1 Serum Indirect Markers

The gold standard for the evaluation of hepatic fibrosis is a liver biopsy, but the procedure is invasive and has problems such as sampling errors. A variety of indirect markers have been reported in AILDs. Table 2.2 shows the main indirect markers that are reported according to AUROC for their fibrotic diagnostic ability as indicators in AIH and PBC [22–27]. In the report of Nyblom, the AST/ALT ratio was measured for 160 patients with PBC, and the cirrhosis cases were significantly higher than the non-cirrhotic cases [28]. Hino reported that the AST/ALT ratio was a risk factor for liver-related death along with steroid reactivity in patients with AIH [29]. Even in AILDs, it has been studied with various serum liver fibrosis markers, and its diagnostic ability is also increasing.

2.3.2 M2BPGi

The new sugar chain marker M2BPGi (Mac-2 binding protein glycosylation isomer) is a liver fibrosis marker developed in Japan and is useful not only to diagnose hepatic fibrosis in patients with chronic hepatitis C but also in relation to liver carcinogenesis. In addition, the usefulness of liver fibrosis diagnosis in nonalcoholic fatty liver disease has been reported. The report of diagnosis of liver fibrosis in AILDs is

Table 2.2 Serum liver fibrosis markers in AILDs: AUROC

Serum liver fibrosis marker	Formula	AIH			PBC		
		F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4	F0-1 vs F2-4	F0-2 vs F3-4	F0-3 vs F4
AST/ALT ratio	AST/ALT	0.60	0.66–0.68	0.77–0.80	0.61	0.59	0.66–0.82
APRI	AST/ULN/platelet (10^9/L) × 100	0.60	0.53–0.70	0.66–0.77	0.65–0.75	0.75–0.84	0.41–0.82
FIB-4	Age + AST/platelet (10^3/mL) × $ALT^{1/2}$	0.66	0.61–0.79	0.77–0.84	0.68–0.72	0.71–0.79	0.83–0.92

Table 2.3 Diagnosis of hepatic fibrosis of AILDs by M2BPGi

Author/year	*n*	AILDs	Cut-off (COI)	AUROC	Sensitivity	Specificity	PPV	NPV
Nishikawa [22]/2015	84	AIH	F3: 3.7 F4: 3.9	0.75 0.85	64 94	83 76	NA	NA
Umemura [23]/2015	137	PBC	F1: 0.7 F2: 1.0 F3: 1.4 F4: 2.0	0.88 0.98 0.83 0.97	70 93 83 39	100 93 90 100	100 90 69 100	21 95 95 70
Nishikawa [24]/2016	57	PBC	F3: 3.4 F4: 3.7	0.73 0.97	50 100	92 98	NA	NA

NA not available

shown in Table 2.3. Nishikawa reported the relationship between M2BPGi and liver histological findings before treatment in patients with AIH [22]. The cut-off value by M2BPGi (COI) was F1, 1.5; F2, 2.1; F3, 3.3; F4, 9.8, and the extraction of liver cirrhosis was more effective than the serum liver fibrosis marker, such as FIB-4 or APRI, when the cut-off value was 3.9 COI, AUROC 0.853. In addition, the cut-off value by liver inflammation was A1, 1.6; A2, 2.5; A3, 5.4, and M2BPGi showed a positive correlation with high sensitivity CRP. In AIH, this showed that M2BPGi strongly reflects not only hepatic fibrosis but also the effect of liver inflammation.

On the other hand, Umemura reported an association between pretreatment M2BPGi and liver histological findings in patients with PBC [23]. The cut-off value by M2BPGi was F1, 0.7; F2, 1.0; F3, 1.4; F4, 2.0, which is low compared with that of AIH but increased with increasing liver fibrosis stage. In extraction of the liver fibrosis stage, AUROC was 0.965 with a cut-off value of 2.0 COI. In addition, it was found from the results of Cox regression analysis that M2BPGi 2.0 COI ≤ is an independent risk factor for liver-related death and liver transplantation in PBC. In addition, Nishikawa and colleagues reported that M2BPGi is useful for hepatic fibrosis stage and liver inflammation grade prediction and shows a positive correlation with IP-10 [24]. M2BPGi is closely related to the pathology of AILDs and is useful for predicting liver histology and prognosis.

2.3.3 Ultrasonic Elastography and MR Elastography

FibroScan developed as a noninvasive examination device for liver fibrosis more than 10 years ago. Transient elastography (TE) has been reported as a means for diagnosis of hepatic fibrosis of AILDs. The cut-off value of liver stiffness in an existing diagnosis of liver cirrhosis is 12.5–19.0 kPa in AIH and PBC. It has been reported that AUROC is 0.84–0.95 for AIH and 0.96–0.99 for PBC (Table 2.4) [25–27, 30–32]. In addition to fibrosis, inflammation such as ALT, fatty liver, congestion, cholestasis, diet, and deep breathing are affected factors. In AIH, atypical cases

Table 2.4 Diagnosis of hepatic fibrosis of AILDs by transient elastography

Author/year	*n*	AILDs	Cut-off (kPa)	AUROC	Sensitivity	Specificity	PPV	NPV
Hartl [30]/2016	94	AIH	F2: 5.6 F3: 10.4 F4: 16.0	0.87 0.93 0.95	90 83 88	72 98 100	83 92 100	84 91 98
Xu [25]/2016	100	AIH	F2: 6.45 F3: 8.75 F4: 12.50	0.88 0.88 0.91	82 80 87	88 84 90	97 84 71	49 81 96
E Anastasiou [26]/2016	53	AIH	F2: 10.05 F3: 12.1 F4: 19	0.78 0.74 0.84	61 59 82	89 83 93	96 81 76	32 62 95
Corpechot [31]/2006	73	PBC	F2: 7.10 F3: 11.10 F4: 17.30	0.88 0.91 0.96	76 71 93	93 96 95	96 94 78	61 76 99
Gomez-Dominguez [32]/2008	80	PBC	F3: 14.7 F4: 15.6	0.86 0.96	56 88	100 98	100 88	83 98
Corpechot [27]/2012	103	PBC	F1: 7.1 F2: 9.8 F3: 10.7 F4: 16.9	0.80 0.91 0.95 0.99	64 67 90 93	100 100 93 99	100 100 84 93	25 75 96 99

of acute hepatitis-like onset have been increasing in recent years. Hartl reported that liver stiffness correlated with the inflammation grade of the liver and did not correlate with the hepatic fibrosis stage within 3 months from the start of immunosuppressive treatment. Moreover, the correlation between liver stiffness and liver fibrosis stage was better 6 months after treatment [30]. It seems that 6 months after the initiation of immunosuppressive treatment is preferable for the assessment of hepatic fibrosis stage in AIH.

On the other hand, Corpechot conducted liver biopsy and TE in 103 patients with PBC and reported that liver fibrosis stage evaluation was superior in liver stiffness compared with other serum liver fibrosis markers. In addition, in 150 patients with PBC repeatedly observed with TE for 5 years, the group with hepatic stiffness of $\leq$2.1 kPa/year experienced increased decompensation, liver transplantation, and liver-related death. Ultrasonic elastography such as TE in AIH and PBC is associated with liver histology and prognosis [27].

Along with the progress of MRI technology in recent years, it has become possible to diagnose fibrosis from elastic modulus measurement by MR elastography (MRE). Wang reported that MRE was more useful than serum liver fibrosis marker (APRI, FIB-4) in the diagnosis of hepatic fibrosis in AIH. In MRE, AUROC in the diagnosis of cirrhosis is 0.98 (APRI: 0.78, FIB-4: 0.80) with a cut-off value of 4.5 kPa, a sensitivity is 92%, and a specificity is 96% [33].

2.4 Treatment for Liver Cirrhosis in AILDs

2.4.1 Treatment for Liver Cirrhosis in AIH

The aim of treatment in AIH is to obtain complete remission of the disease and to prevent further progression of liver disease. This requires mostly permanent maintenance therapy or induction of a sustained remission following treatment withdrawal. In symptomatic patients and patients with advanced fibrosis or cirrhosis, treatment should always be initiated, as these findings represent a negative prognostic predictor.

In addition, even in advanced fibrosis and cirrhosis, substantial regression of scarring after successful treatment has been reported. In view of the progressive nature of AIH and the effectiveness of immunosuppressive therapy, the consensus group recommends that all patients with active disease should receive treatment [34]. Prednisone as initial therapy followed by the addition of azathioprine after two weeks is the first-line treatment for AIH. The initial dose of prednisone should be between 0.5 and 1 mg/kg/day.

Individuals with cirrhosis at presentation have a higher frequency of drug-related complications than do those without cirrhosis (25% versus 8%) [35]. They also have high frequency of cytopenia that may compromise their tolerance for azathioprine. Patients with cirrhosis must be closely monitored during therapy, and those individuals with cytopenia should be assessed for thioprine methyltransferase activity prior to the administration of azathioprine [36].

Liver transplantation is indicated for AIH patients presenting with advanced cirrhosis. The immunosuppressive strategy most commonly adopted consists of the combination of prednisolone and a calcineurin inhibitor [37], leading to excellent outcome with 5- and 10-year patient survivals of 90% and 75%, respectively [3].

2.4.2 Treatment for Liver Cirrhosis in PBC

The introduction of UDCA as the first-line treatment for PBC patients has changed the natural history of the disease [2]. UDCA slows fibrosis progression and delays cirrhosis development [38]. In clinical trials, UDCA treatment of PBC patients decreased the development of esophageal varices and prolonged survival [39–41]. Several papers have also assessed the impact of UDCA therapy on the progression rate of cirrhosis in PBC patients. Corpechot et al. [42] examined progression to cirrhosis in 183 UDCA-treated PBC patients. In this study, 21% of patients developed cirrhosis during follow-up. The incidence of cirrhosis in patients followed-up from stages 1, 2, and 3 was 4%, 12%, and 59%, respectively, and the median length of time to cirrhosis development was 25, 20, and 4 years, respectively. Albumin and bilirubin levels and the histological severity of interface hepatitis were independently associated with progression to cirrhosis. Cirrhosis does, however, still

develop in UDCA-treated PBC patients [42]. Indeed, the development of cirrhosis under UDCA treatment is an independent predictor of negative outcome [42]. Indeed, the number of PBC patients requiring LT decreased by 20% between 1996 and 2006 [43].

2.5 HCC in AILD

2.5.1 HCC in AIH

Liver cirrhosis has been reported as a risk factor for hepatocellular carcinoma (HCC) in AIH [44–48]. In Japan, a recent study reported that a primary survey of the 496-member institutions of the Liver Cancer Study Group of Japan was carried out, and a secondary survey was carried out for 250 HCC patients from 4869 AIH patients (5.1%) identified in the primary survey [49]. One hundred twenty-seven patients were enrolled throughout Japan. Mean age at diagnosis of HCC was 69 years, and the male-to-female ratio was 1: 5.7, and 77.9% had liver cirrhosis. Another study showed that the presence of cirrhosis at presentation was a risk factor for HCC, according to a Cox proportional hazard model [50]. On the other hand, Yoshizawa et al. showed that on multivariate analysis, the prognosis of two or more relapses was identified as the only risk factor for the development of hepatic malignancy [13].

In the EU and US, and even in Japan, AIH patients showing steroid resistance need to use other immunosuppressants (e.g., azathioprine), and attendant carcinoma has also been reported in these cases [51, 52]. While steroids are used as the first-line therapy for AIH, it has been shown that approximately 10% of AIH patients in Japan are resistant to steroid therapy. For steroid-resistant patients, azathioprine, which is not covered by national health insurance, is considered first-line therapy. When the maintenance dose of steroid was higher, the incidence of HCC was significantly higher. However, neither steroid nor azathioprine therapies were significant factors for the development of HCC. There was no significant difference between the two therapies [53].

2.5.2 HCC in PBC

With respect to HCC, its incidence in patients with PBC varies from 0.76% to 5.9%, depending on reports [54–57]. The number of PBC patients associated with HCC has been increasing recently, which may be due to the improvement of therapeutic effects and prognosis. In Japan, surveys involved 8509 patients registered in the 1st–15th surveys performed between 1980 and 2012. According to the 15th National Survey performed in 2012, the incidence of malignancy at the time of PBC

diagnosis was 2.4%. Liver cancer was the most common (24%) [58]. Moreover, most female patients with PBC and with HCC develop the advanced stage at the time of HCC diagnosis, supporting several reports stating that cirrhosis is a risk factor for HCC [54, 55]. In males, HCC cases arising from an early PBC stage are not rare. Hence, male patients with PBC should be carefully followed from an early stage to identify HCC.

In contrast, a previous study reported on the possibility that UDCA may protect against HCC [59]. In UDCA-treated patients with PBC, the risk of HCC was relatively low, but the main risk factor for HCC was the absence of a biochemical response to UDCA and the development of cirrhosis. Another study showed that, of 4565 patients with PBC, 123 developed HCC, yielding an incidence rate of 3.4 cases/1000 patient-years. HCC was significantly more common in men, and on univariate analysis, factors at PBC diagnosis associated with future HCC development were male sex, elevated serum aspartate transaminase, advanced disease, thrombocytopenia, and hepatic decompensation. A 12-month biochemical non-response is associated with increased future risk of developing HCC in PBC [60].

2.6 Conclusion

Among AIH patients, the presence of cirrhosis at the time of AIH diagnosis has been reported to be a negative factor for survival. In contrast, other studies have reported similar outcomes in patients with and without cirrhosis at presentation. Furthermore, the prognosis of two or more relapses was identified as a risk factor for the development of cirrhosis and HCC in patients with AIH. On the other hand, in UDCA-treated patients with PBC, the risk of HCC was relatively low, but the main risk factor for HCC was the absence of a biochemical response to UDCA and the development of cirrhosis. AILD patients with liver cirrhosis must be carefully monitored during treatment.

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Chapter 3
Ultrasound Imaging for the Diagnosis of Liver Cirrhosis

Hiroko Iijima

Abstract Ultrasound evaluation of liver fibrosis has been performed by using B-mode and Doppler ultrasound (US). In recent years, ultrasound elastography, being noninvasive, is replacing liver biopsy. Several elastographies that are integrated into a conventional ultrasound system are available to evaluate liver fibrosis and are approved for health insurance coverage.

Keywords Ultrasound elastography · Noninvasive liver fibrosis assessment Transient elastography · Point shear wave elastography · 2D shear wave elastography

3.1 Introduction

In ultrasound diagnosis of liver fibrosis, it is important to evaluate complications in addition to morphological changes. In patients with chronic liver disease, especially viral hepatitis, fibrosis septa are developed and extend to form bridges in the portal area with progression of the disease that results in lobular reconstruction and pseudo-lobule formation. With the progression of fibrosis, regenerative nodules develop. Fibrosis appears as speckle noise on ultrasound images due to differences in acoustic impedance. Fibrosis process can be observed in the changes of the speckle pattern. Morphological changes on the surface and in marginal area of the liver also gradually occur.

Among complications of liver fibrosis, portal hypertension is associated with increased risk of liver cancer. It may lead to ascites and esophagogastric varices, and can be a factor to determine prognosis of the disease. There are different imaging modalities for assessing liver fibrosis including ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and scintigraphy, but this article reviews noninvasive ultrasound elastography.

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3.2 B-mode Diagnosis

Evaluating morphological changes in the liver in B-mode imaging is important as these changes correlate with background liver lesions. Nodules in patients with hepatitis B (HBV) are larger than the ones in those with hepatitis C (HCV). B-mode imaging generally reflects it. As fibrosis progresses, blunt edges and an irregular surface of the liver occur. Coarse and speckled echotexture in the liver parenchyma are the most specific features in B-mode imaging. Left lobe hypertrophy and right lobe atrophy, and enlarged spleen due to portal hypertension are commonly displayed in cirrhotic patients. However, these changes are subjective and less objective for determining progression of liver fibrosis, portal hypertension, and the risk of developing liver cancer [1]. Though it is invasive and has the potential for sampling error, currently liver biopsy is the gold standard for diagnosing liver fibrosis. In recent years, ultrasound elastography has been widely used. Its usefulness, especially in the diagnosis of chronic hepatitis and liver cirrhosis, is recognized as seen in the clinical practice guideline by European Association for the Study of the Liver [2].

The following figures show cirrhotic liver on B-mode imaging. In patients with chronic hepatitis, liver surface irregularity and edge bluntness increase with fibrosis progression. Coarsened echotexture of the liver parenchyma, irregular running of portal veins and hepatic veins also occur. Atrophy of the right lobe and hypertrophy of the caudate and left lobe follow with further progression of fibrosis. Irregular surface, rough parenchyma, and narrowed hepatic vein vessels, and changes in diameter of vessels are displayed (Fig. 3.1a, b). In response to portal hypertension, enlarged spleen and collateral vessels are developed (Fig. 3.1c, d). Enlarged spleen is evaluated by calculating a splenic index (SI) using ultrasound. SI is obtained by multiplying the distance (line) between the hilar indentation and anterior cranial end (A cm) by length of the line perpendicular to it (B cm) (A × B). SI greater than 20 or ≥10 cm when simplified SI (the major axis from the spleen dome to the tip) is used is considered suspected splenomegaly, and greater than 30 is considered splenomegaly [3] (Fig. 3.2). Ascites, gallbladder wall thickening, collateral vessels occur (Fig. 3.3). As cirrhosis progresses, portal vein flow velocity decreases (Fig. 3.4). However, blood flow volume does not change because the cross-sectional area of the blood vessel increases. In patients with HCV, portal lymphadenopathy is frequently found. Different speckled patterns appear in the liver surface or the parenchyma depending on the causes of cirrhosis. In alcoholic cirrhosis, the liver, being steatotic, appears blighter. Small, micronodules are also a common manifestation. In HBV hepatitis, a number of small nodules, 2–3 mm in size, may grossly appear with fibrosis progression, and are displayed as a “mesh pattern” on ultrasound images. These small nodules need to be evaluated to distinguish from atypical nodules or borderline lesions, and early hepatocellular carcinoma (HCC). When distinction of these nodules is difficult by using only B-mode ultrasound, contrast-enhanced ultrasonography or ethoxybenzyl-diethylenetriamine Primovist-

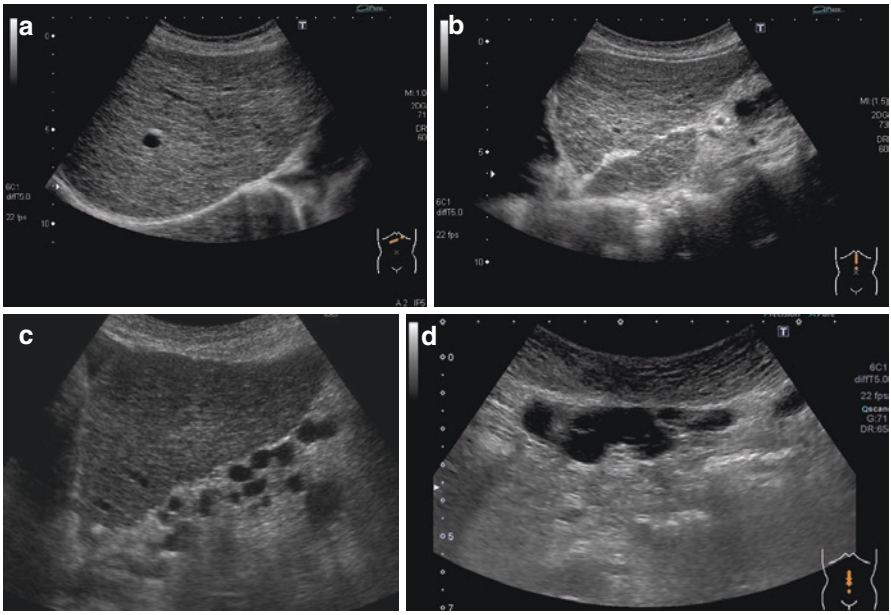

Fig. 3.1 Ultrasound images of liver cirrhosis. (**a**) Uneven left hepatic lobe, enlarged caudate lobe, rough parenchyma are displayed. (**b**) Narrowing and irregular hepatic vein vessels are seen. (**c**) Dilation of left gastric veins is seen. (**d**) Dilation of paraumbilical veins to superior epigastric veins is seen

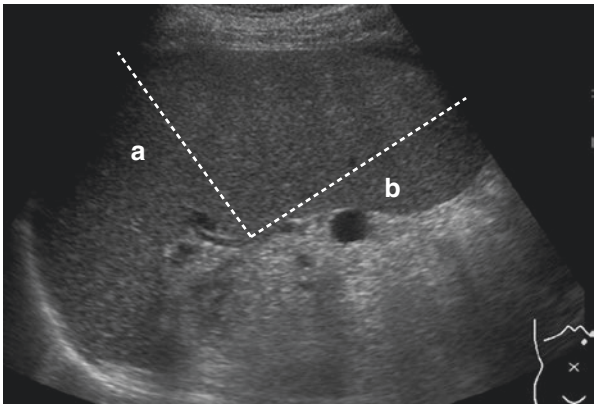

Fig. 3.2 The spleen in liver cirrhosis. The spleen is considered enlarged when spleen index (SI), $a \times b$, is >20

enhanced MRI (EOB Primovist-enhanced MRI) may be useful. In HCV hepatitis, because nodules are smaller than the ones of HBV hepatitis, nodules on the surface gradually progress to cirrhosis and do not display changes drastically on images. Nevertheless, portal hepatic lymphadenopathy develops more in HCV hepatitis than in HBV hepatitis.

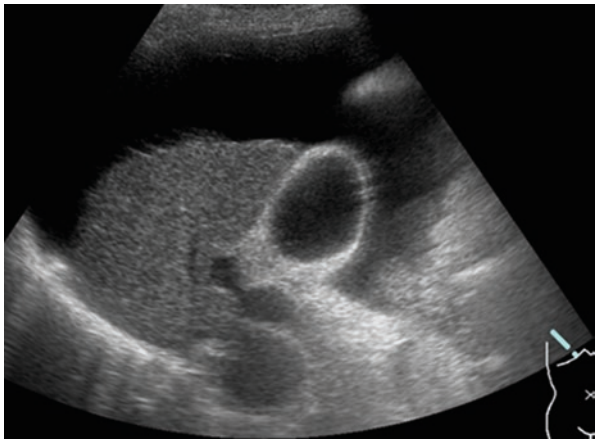

Fig. 3.3 HCV-related liver cirrhosis. Ascites and gallbladder wall thickening is observed

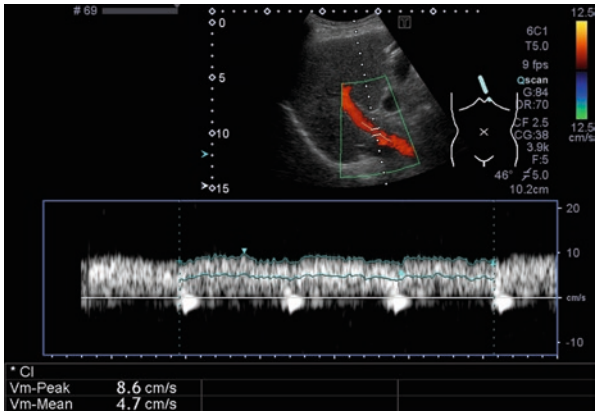

Fig. 3.4 Portal vein flow in HCV-related cirrhosis. Portal vein flow measurement in a patient with cirrhosis. In this patient, portal vein flow velocity showed a decrease of 4.7 cm/s (17–18 cm/s in normal liver)

3.3 Principles of Ultrasound Elastography

Elastography is a technology that creates displacement in the tissue, and then visualizes and quantifies the response of the deformed tissue as "stiffness" of the tissue. Strain imaging is a technique that measures liver tissue displacement (strain) where a certain amount of force is exerted on, and provides the measurement as stiffness. Shear wave imaging is a technique that measures the shear wave speed that propagates through liver tissue. Shear wave travels faster through hard tissues and slower through soft tissues. Shear wave elastography has become mainstream. It uses either one of the following two excitation methods: mechanical vibrating method that is used in transient elastography (TE) and acoustic radiation force impulse method (ARFI). ARFI is a physical phenomenon in which acoustic pushing pulse is generated and pushes liver tissues downwards. In this method, shear waves are generated in response to ARFI. The detection pulse is sent after shear waves to measure the

Table 3.1 Classification of elastography

	Strain imaging	Shear wave imaging
Manual compression	Strain elastography	
	RTE (Hitachi) Elastography (GE, Philips, Canon)	
ARFI	ARFI imaging	Point shear wave elastography
	VTI (Siemens)	VTQ (ACUSON S2000, 3000) ElastPQ (Affiniti, EPIQ) SWM (ARIETTA 850LE, 850, 850SE, S70, E70, 70)
		2D Shear wave elastography
		2DSWE (ACUSON Sequoia) SWE (Aixplorer) SWE (Aplio300, 400, 500, i700, i800, i900) SWE (LOGIQS8) ElastQ (EPIQ)
Mechanical impulse		Transient elastography
		FibroScan (Echosens)

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propagation velocity of shear waves. This method that uses ARFI is categorized into two technical approaches: point shear wave elastography (pSWE) and 2D shear wave elastography (2D-SWE) (Table 3.1).

3.4 Diagnostic Capability of Each Elastography for Liver Fibrosis

3.4.1 Strain Imaging

3.4.1.1 Strain Elastography

Real-time tissue elastography (RTE, Hitachi Medical Systems, Tokyo, Japan) is imaging modality for the diagnosis of liver fibrosis in which liver displacement caused by heartbeat is displayed. RTE was put into clinical use for the first time in Japan in 2003. With RTE, differences in stiffness in the region of interest (ROI) are color-coded with a 256 stepwise grading and displayed on B-mode images in a superimposed fashion, such as blue areas indicate relatively hard tissues, green areas indicate relatively average stiffness, and red areas indicate relatively soft tissues. Yada et al. performed RTE imaging of 245 patients with chronic viral hepatitis and proposed liver fibrosis index (LFI) using nine RTE parameters. In the study, they observed significant differences between all fibrosis stages except between F2

and F3, and reported the usefulness of RTE in staging liver fibrosis [4]. It is said that RTE is less affected by inflammation, ascites, and jaundice compared to shear wave imaging; however, its methods and procedure are rather complicated.

3.4.2 Shear Wave Imaging

3.4.2.1 Transient Elastography (TE)

FibroScan (Echosens, Paris, France) induces shear waves by mechanical vibration, and measures liver stiffness which is expressed in kilopascal (kPa). The liver stiffness measurement (LSM) is the median value of minimum 10 measurements. When the rate of successful measurements out of all measurements is <60% or when an interquartile range (IQR)/median (med) is >30%, LSM is considered unreliable [5]. FibroScan is the most extensively evaluated elastography for liver stiffness assessment. We studied 881 patients with chronic hepatitis and cirrhosis. 199 of them had HBV, 350 had HCV, 4 had HBV+HCV, and 328 had nonBnonC, and their fibrosis stages were F0-1/F2/F3/F4; 403/188/192/98, respectively. LSMs (kPa) were F0-1: 5.72 ± 3.01, F2: 7.99 ± 4.39, F3: 13.0 ± 10.6, F4: 24.47 ± 13.55, respectively, and they increased significantly with the increase of fibrosis stage ($p < 0.001$). The areas under the receiver operating characteristic curve (AUROCs) for differentiating F2≤, F3≤, F4 (cirrhosis) were 0.821, 0.862, 0.936, respectively, with cutoff values 7.20, 7.80, 10.8 kPa, respectively, and showed a good diagnostic capability. Our results showed a comparable diagnostic capability of FibroScan to the ones in many studies including by Castèra et al. [5].

3.4.2.2 Point Shear Wave Elastography (pSWE)

Shear wave velocity, expressed as Vs (m/s), increases with the progression of liver fibrosis. Measurement area can be positioned at any desired location. Measurement is repeated 5–10 times to obtain the mean or median Vs value. Many reported that sensitivity of VTQ (virtual touch quantification; Siemens Medical Systems, Mountain View, USA) was equivalent to TE [6–8]. We studied 1482 patients with chronic hepatitis and cirrhosis. 294 of them had HBV, 680 had HCV, 10 had HBV+HCV, and 498 had nonB, nonC, and their fibrosis stages were F0-1/F2/F3/F4; 660/299/316/207, respectively. The mean Vs values were 1.16 ± 0.26 for F0-1, 1.35 ± 0.38 for F2, 1.61 ± 0.57 for F3, 2.27 ± 0.62 for F4, respectively, and they increased with the increase of fibrosis stage ($p < 0.001$). The AUROCs for differentiating F2≤, F3≤, F4 were 0.800, 0.833, 0.916, respectively, with cutoff values 1.29, 1.38, 1.63 m/s, respectively, and showed a good diagnostic capability. Friedrich-Rust et al. reported the diagnostic accuracy for F2≤, F3≤, F4 were 0.82, 0.91, 0.91, respectively, with cutoff values 1.37, 1.45, 1.75 m/s, respectively [6]. ElastPQ

(Philips Healthcare, Bothell, USA) is also one of the pSWE. The diagnostic performance of ElastPQ was evaluated using TE as the reference method in the study included 228 patients with viral hepatitis. AUROC for F2≤ was 0.94, F3≤ was 0.97, and F4 was 0.97 and showed a good accuracy for staging liver fibrosis [9]. Shear wave measurement (SWM, Hitachi Ltd., Tokyo, Japan) also uses pSWE technique, and its usefulness in evaluating liver fibrosis in patients with chronic hepatitis C is reported [10].

3.4.2.3 Two Dimensional (2D) Shear Wave Elastography (2D-SWE)

With 2D shear wave elastography (2D-SWE), liver stiffness is measured by placing a ROI of where the stiffness needs to be measured on top of a ROI of where the area is observed. The mean value of 6–10 measurements taken at two different locations on an image is used as a liver stiffness value. The observational ROI of this technique is larger than the one of pSWE, and the difference of stiffness can be color-coded on a display (Fig. 3.5).

We studied 521 patients with chronic hepatitis and cirrhosis with a 2D-SWE device (Canon Medical Systems, Otawara, Japan). 105 of them had HBV, 204 had HCV, 1 had HBV+HCV, and 211 had nonBnonC, and their fibrosis stages were F0-1/F2/F3/F4; 239/114/117/51, respectively. Vs values were 1.42 ± 0.20 for F0-1,

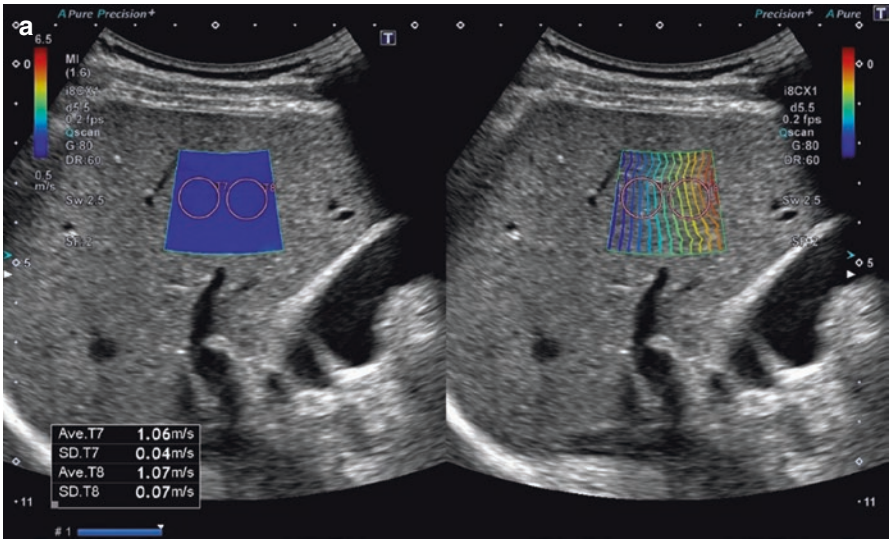

Fig. 3.5 2D-SWE. (**a**) Normal liver. Vs value is 1.06, and the intervals between contour lines are narrow and even. (**b**) HCV-related cirrhosis. Vs value is 2.0 and shear wave speed is fast, and the intervals between contour lines are wide

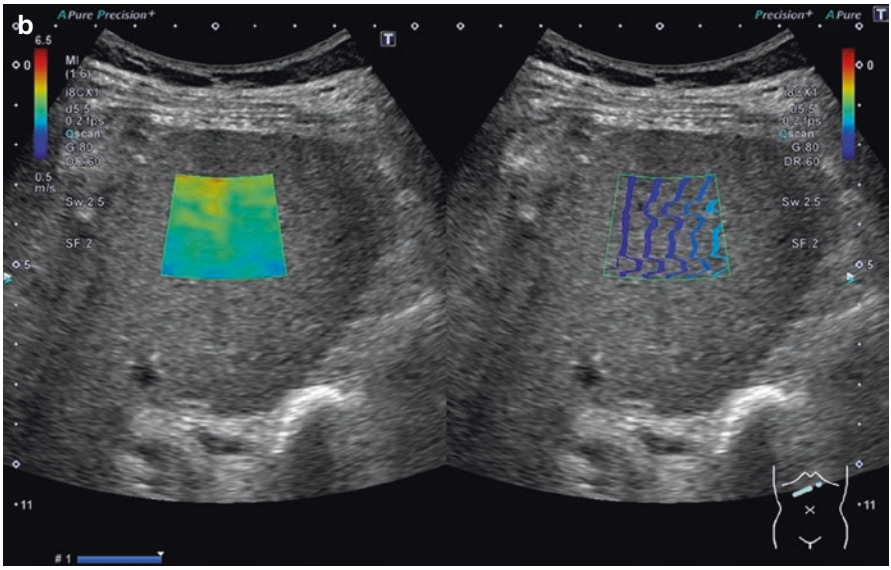

Fig. 3.5 (continued)

1.62 ± 0.29 for F2, 1.95 ± 0.54 for F3, 2.55 ± 0.61 for F4, and they increased with the increase of fibrosis stage ($p < 0.001$). The AUROCs for differentiating F2≤, F3≤, F4 were 0.840, 0.870, 0.933, respectively, with cutoff values 1.56, 1.64, 1.87 m/s, respectively, and showed an excellent diagnostic capability.

In the study to evaluate the diagnostic capability of 2D-SWE by SuperSonic Imagine (SSI, Aix-en-Provence, France) for differentiating liver cirrhosis, 349 patients with chronic liver disease are studied. The AUROCs for differentiating F1≤, F2≤, F3≤, F4 were 0.89, 0.88, 0.93, 0.93, respectively, with cutoff values 7.8 kPa, 8 kPa, 8.9 kPa, 10.7 kPa, respectively [11]. Another 2-D SWE, ElastPQ developed by Philips, provides swift stiffness measurement on a color-coded display by using observational ROI.

Diagnostic ability of a SWE by GE (GE Healthcare, Milwaukee, USA) for evaluating liver fibrosis showed a good correlation with the one of TE, and cutoff values for differentiating F2≤, F3≤, F4 were 6.7, 8.2, and 9.3 kPa, respectively [12].

3.5 Recommendation for Examination Procedure Using Shear Wave Imaging

Measurement of liver stiffness by shear wave imaging including TE should be performed through the right intercostal space while holding breath slightly on an empty stomach to avoid the influence of food intake.

Measurement of TE requires confirmation of positioning as the ROI positioning in TE cannot be monitored with B-mode imaging. In the presence of ascites and

severe hepatic atrophy, measurement may be unsuccessful. In highly obese patients, obtaining LSM was difficult; however, usage of the XL probe made it possible to perform TE in patients with BMI ≥30 kg/m^2 or skin to capsular distance (SCD) ≥25 mm [13]. In patients with acute liver damage, obstructive jaundice, congestion, and excessive alcohol intake, LSM may be found to be higher; therefore, these factors need to be taken into account when interpreting the results. PSWE is also influenced by food intake, necrosis-inflammation, and acute liver damage.

The study by Chen et al. reported that both LSM and the collagen proportionate were significantly higher in the HCV group than in the HCB group within all fibrosis stages [14]. Moreover, antiviral therapies for chronic viral hepatitis is also a factor that affects LSM [15–17]; close attention must be paid to these factors including background liver disease and treatment history.

3.6 Conclusion

Each ultrasound elastography technique and the diagnostic capability of each elastography for assessing liver fibrosis were described. The diagnostic capability of all elastography technique for assessing liver fibrosis is good. They are also useful in assessing pathology and prognosis of portal hypertension and hepatocarcinogenesis. Moreover, all ultrasound device manufacturers sell elastography-available device, and insurance reimbursement is expected to expand. Ultrasound elastography is an essential tool in the diagnosis of chronic liver disease.

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Chapter 4
Liver Cirrhosis with Inherited Liver Disease: Hemochromatosis

Keisuke Hino and Sohji Nishina

Abstract Although liver cirrhosis is most commonly caused by hepatitis B and C viruses, alcohol, and nonalcoholic fatty liver disease, hereditary hemochromatosis also causes cirrhosis as one of the hereditary liver diseases. Hereditary hemochromatosis is characterized by iron deposition not only in the liver but also in heart and endocrine organs. Therefore, hereditary hemochromatosis potentially progresses to liver cirrhosis, diabetes mellitus, heart failure, and/or hypogonadism without early diagnosis and prompt initiation of treatment. On the other hand, the identification of important iron metabolic molecules and genes such as hepcidin, ferroportin, and *HFE* has made it possible to understand the molecular mechanisms underlying hereditary hemochromatosis and to introduce proper treatment at the early stage of disease. This chapter will review and discuss the iron metabolic regulation and the molecular and clinical characteristics of hereditary hemochromatosis.

Keywords Hepcidin · Hemojuvelin · Ferroportin · Human antimicrobial peptide (HAMP) · Juvenile hemochromatosis

4.1 Introduction

Essential trace elements such as iron, copper, and zinc are biologically indispensable for mitochondrial electron transport, signal transduction, redox reaction, oxygen transport, and/or physiological catalytic reaction such as hydrolysis. Because these elements are transition metal, they also function as active region of various enzymes, cytokines, and hormones. Thus, metabolic disturbance of these elements results in critical disorder of biological functions, leading to the development of various diseases.

On the other hand, liver is a crucial organ for metabolism of iron. Therefore, primary metabolic disorders of iron give rise to liver diseases such as liver cirrhosis

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and hepatocellular carcinoma (HCC) in addition to iron deposition in vital organs such as heart and endocrine organs. This chapter will review and discuss (1) iron absorption, (2) hepatocellular iron transport, (3) regulation of iron homeostasis, and (4) hereditary hemochromatosis as primary iron metabolic disorder.

4.2 Iron Absorption

Iron can be absorbed from the diet in two forms in the proximal intestine: as inorganic (nonheme) iron predominantly released from foods such as vegetables or cereals, or as heme iron from the breakdown of hemoglobin and myoglobin contained in red meat [1]. Heme (ferrous protoporphyrin IX) is more efficiently absorbed than inorganic iron from the diet. The mechanism responsible for heme uptake is not yet well understood, but it is known to occur by receptor-mediated endocytosis. The hem-carrier protein 1 (HCP1) has been identified as the most probable receptor involved in this process [2]. However, it has low-affinity to heme and is more involved in folate absorption [3]. Once in the enterocyte, heme is broken by heme oxygenase 1 (HO1) and iron is released in its ferric state.

Nonheme-iron exists primarily in the bio-unavailable, oxidized form (Fe^{3+}), which must first be reduced to Fe^{2+} for transport across the intestinal epithelium. At the apical membrane, there is a cytochrome b-like ferrireductase (Dcytb) [4]. Fe^{2+} then enters the cell through divalent metal transporter 1 (Dmt1), an iron transporter. Dmt1 is responsible for the absorption of the ionic forms of iron, cobalt, zinc, cadmium, and others, and takes advantage of the proton gradient existing between the gut lumen and the enterocyte cytoplasm to perform the transport of Fe^{2+} coupled with H^+ [5].

In the cytoplasm iron is transferred to the basolateral membrane of the enterocyte or stored in ferritin, a multi-subunit protein shell that can accommodate up to 4500 atoms of iron. The export of iron from the enterocyte to the circulation is a critical step for the entrance of iron in the body. The mammalian iron transporter, ferroportin-1, exists on the basolateral membrane of the enterocytes [6]. Ferroportin-1 transports Fe^{2+} to the extracellular side of the basolateral membrane, where Fe^{2+} is oxidized by the ferroxidase, hephaestin, and ceruloplasmin in order to be associated with the circulatory transferrin [7, 8].

4.3 Hepatocellular Iron Transport

Hepatocytes take up iron through at least two distinct pathways. They have a functional transferrin cycle and a transport system to take up non-transferrin-bound iron. The cellular uptake of transferrin-bound iron is mainly mediated by the transferrin receptor 1 (TfR1). The molecules important for non-transferrin-bound iron transport have not yet been identified. Hepatocytes store iron in ferritin. When iron is

needed elsewhere in the body, they can release it to transferrin through autophagy-dependent mechanism (ferritinophagy) [9, 10]. This mechanism is described in detail later. The mechanism of hepatocyte export is not well known, but it may involve ferroportin-1. Ceruloplasmin seems to aid in iron export from hepatocytes, but its precise function has not yet been defined.

4.4 Regulation of Iron Homeostasis

Systemic iron homeostasis, the control of iron balance throughout the body, requires controlled absorption, recycling, and storage, because there is no efficient pathway for iron excretion in the human body. All the stages required for keeping iron homeostasis are strictly regulated at both systemic and cellular levels.

4.4.1 Hepcidin

The major systemic regulator of iron homeostasis is hepcidin, which is a 25 amino-acid peptide hormone exclusively synthesized in the liver and a soluble regulator that acts to attenuate both intestinal iron absorption and iron release from reticuloendothelial macrophages [11, 12]. Hepcidin acts by triggering internalization of ferroportin-1 and consequent degradation, and traps iron in absorptive enterocytes, macrophages, and hepatocytes [13]. Thus, coupling the internalization of ferroportin-1 to hepcidin levels generates a homeostatic loop regulating the iron plasma level and the tissue distribution of iron. Hepcidin is expressed from the *human antimicrobial peptide* (*HAMP*) gene located at the long arm of chromosome 19. The increase of iron levels and inflammation upregulate the transcription of *HAMP* gene, while reactive oxygen species (ROS), hypoxia, and anemia/erythropoiesis repress its expression [14–18].

Hemojuvelin (Hjv), HFE, TfR1, and TfR2 that are located at the surface of hepatocytes are considered to be "iron sensors." The Hjv-hepcidin axis is the most important mechanism for the upregulation of *HAMP* expression during iron overload. Bone morphogenic protein (BMP) binding to the Hjv and BMP receptor complexes induces the phosphorylation of cytosolic sons of mothers against decapentaplegic (SMADs) 1, 5, and 8 [14, 19]. The phosphorylated SMADs form complexes with SMAD4, which are translocated to the nucleus where they bind to the BMP responsive elements present at *HAMP* promoter, inducing its transcription [20]. Proinflammatory cytokine interleukin 6 (IL-6) activates *HAMP* gene transcription through a pathway that involves Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling and a binding site for the transcription factor STAT3 [17, 21]. The transcription factor CCAAT/enhancer-binding protein α (C/EBPα) is also clearly involved in regulating hepcidin transcription [22].

4.4.2 Iron Regulatory Protein (IRP)/Iron Responsive Element (IRE) System

Besides the systemic regulation of iron homeostasis by hepcidin, the IRP/IRE system controls both mRNA stability and translation of transcripts coding proteins involved in iron uptake (Dmt1 and TfR1), storage (ferritin), and export (ferroportin-1) [23, 24]. The IPP1 and IRP2 proteins are the main regulators of cellular iron in humans, but the IPR2 protein is assumed to play a central role in IRP/IRE system [25]. Under iron deficient conditions, IRP binds to the IRE present at the 5′ or 3′-untranslated regions (UTRs) of mRNAs that code for iron regulatory proteins. Under iron depleted condition in cells, IRPs bind to the IREs present at 5′-UTRs of *FTH*, *FHL* (genes coding heavy chain and light chain of ferritin), and *SLC40A1* (gene coding ferroportin-1), preventing ribosome assembly and further translation [26, 27], while they bind to the IREs present at 3′-UTR of TfR1 and *SLC11A2* (gene coding Dmt1), increasing the transcripts stability and subsequently their translation [28, 29]. Thus, cellular iron depletion downregulates iron storage and export, and upregulates iron uptake. Alternatively, cellular iron increase makes IRPs unable to bind to the IREs, resulting in suppression of iron acquisition. Cellular regulation other than IRP/IRE system for iron homeostasis will be discussed elsewhere.

4.4.3 Ferritinophagy

In mammalian cells, iron homeostasis is maintained by compensatory regulation of iron uptake and storage depending on the availability of iron. Ferritin is the major iron storage protein in mammals. Ferritin forms a three-dimensional protein shell consisting of 24 protein subunits that can store up to 4500 atoms of iron [30]. Two isoforms of ferritin, ferritin heavy chain (H chain) and light chain (L chain), cooperate in storing iron in the ferritin shell. The production of H and L chains is regulated by iron availability at the posttranscriptional level through IRP/IRE system as mentioned above. While the mechanism of iron-mediated regulation of ferritin expression has been well defined, comparatively little is known so far regarding the fate of the iron that is stored by ferritin. Ferritin is degraded via lysosomal in response to iron deficiency [31]. This process is mediated with autophagy [9]. Recently, nuclear receptor coactivator 4 (NCOA4) has been identified as the cargo receptor mediating autophagic turnover of ferritin (ferritinophagy) [10]. These results suggest that the targeting of ferritin to autophagosomes by NCOA4 is a general cellular mechanism for regulating bioavailable iron.

4.5 Inherited Iron Metabolic Disorder

Iron overload, especially excess divalent iron can be highly toxic, mainly via the Fenton reaction producing hydroxyl radicals [32]. This is particularly relevant for hereditary iron-overloaded liver diseases such as hemochromatosis, in which

oxidative stress has been proposed as a major mechanism of liver injury. Oxidative stress and increased iron levels strongly favor DNA damage, genetic instability, and tumorgenesis. Indeed, a significant correlation between 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidatively generated DNA damage [33], and hepatic iron excess has been shown in iron-overloaded liver diseases.

4.5.1 Hereditary Hemochromatosis

Hereditary hemochromatosis is a heterogeneous group of inherited iron-overload conditions that is characterized by increased intestinal absorption and deposition in vital organs, including the liver, heart, and endocrine organs. The hemochromatosis group shows common features with respect to increased transferrin saturation and parenchymal iron deposition in organs, resulting in the development of liver cirrhosis, HCC, heart failure, diabetes mellitus (DM), and hypogonadism, even though the severity of the different forms of hemochromatosis varies. Hereditary hemochromatosis has been clinically classified into two phenotypes. The classical form induces mainly cirrhosis, DM, and/or skin pigmentation in middle-aged patients, while the other form, juvenile hemochromatosis, results in cardiac failure and hypogonadism before patients reach the age of 30 [34]. On the other hand, four types (type 1, 2, 3, and 4) of hemochromatosis have been genetically classified on the basis of mutation in five genes (*HFE*, *HAMP*, *Hiv*, *TFR2*, and *SLC40A1*). Responsible genes and dysregulated iron metabolism in hereditary hemochromatosis are summarized in Table 4.1. The molecular mechanism common to all types but type 4 hereditary hemochromatosis fails to regulate hepcidin expression in response to cellular iron levels [35]. Figure 4.1 depicts the molecular mechanisms underlying various types of hereditary hemochromatosis.

4.5.1.1 Type 1 Hereditary Hemochromatosis (Classical Form)

Type 1 hereditary hemochromatosis, known as classic hemochromatosis, is affected by *HFE* mutations and accounts for approximately 90% of all cases of hereditary hemochromatosis. It is characterized by mild disease progression with

Table 4.1 Responsible genes and dysregulated iron metabolism in hereditary hemochromatosis

Type	Subtype	Responsible gene	Inheritance	Transferrin saturation	Serum hepcidin
1		*HFE*	Autosomal recessive	High	Low
2	2A	*HJV*	Autosomal recessive	High	Low
	2B	*HAMP*		High	Absent
3		*TFR2*	Autosomal recessive	High	Low
4	4A	*SLC40A1*	Autosomal dominant	Normal	High
	4B	*SLC40A1*		High	High

HJV hemojuvelin, *HAMP* human antimicrobial peptide, *TFR2* transferrin receptor 2

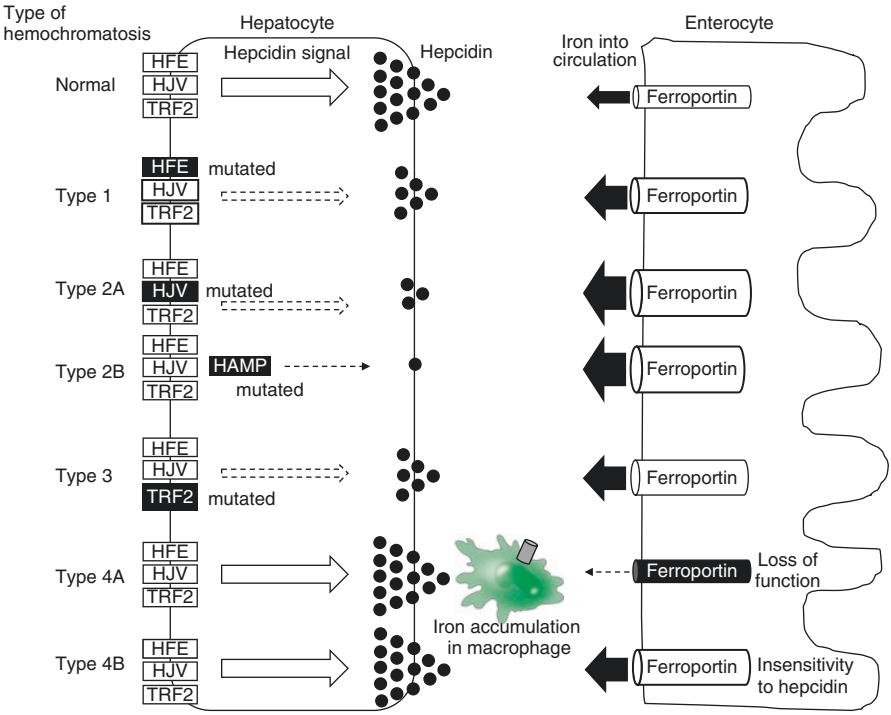

Fig. 4.1 The impaired hepcidin–ferroportin system in genetic hereditary hemochromatosis. Hepcidin secreted by the liver regulates iron release from macrophages and duodenal enterocytes by interacting with the ferroportin expressed on their surface. Ferroportin transports Fe^{2+} to the extracellular side of the basolateral membrane. HFE, transferrin receptor 2 (TfR2), and hemojuvelin (HJV) are all required to adjust hepcidin expression to current iron needs. In hemochromatosis except for type 4, loss of any one of these hepcidin regulators diminishes intracellular hepcidin signal transduction and hepcidin secretion, leading to unrestricted flow of iron into the plasma iron pool. In type 4A hemochromatosis, loss of function mutants of ferroportin is unable to export iron from cells, resulting in iron accumulation predominantly in reticuloendothelial cells. In type 4B, ferroportin mutations are responsible for a gain of function with full iron export capability but insensitivity to downregulation by hepcidin, leading to iron accumulation in parenchymal cells and a phenotype similar to other hepcidin deficiency-based types of hemochromatosis

a gradual iron deposition in organs [36, 37]. *HFE* encodes an atypical histocompatibility class I protein that heterodimerizes with β-2 microglobulin [37]. Most affected patients are homozygous for a missense mutation (C282Y) that partially disrupts HFE function [37]. The C282Y mutation is known to be widespread in populations of Northern European descent [38], but its prevalence is extremely low in Asians [39]. Mutated HFE protein cannot bind to β-2 microglobulin and be transferred to the cell surface, which presumably results in failure to regulate hepcidin transcription, since HFE protein is considered to be one of "iron sensors" at the surface of hepatocytes.

4.5.1.2 Type 2 Hereditary Hemochromatosis (Juvenile Hemochromatosis)

Type 2 hereditary hemochromatosis, known as juvenile hemochromatosis, is an autosomal recessive disease and affected by *Hjv* or *HAMP* mutations. This type of hemochromatosis is a rare but more progressive disease which includes hypogonadism, diabetes, and cardiomyopathy. Severe iron overload and organ damages usually occur before patients reach the age of 30. Juvenile hemochromatosis is further classified into two types: (A) Hjv-associated hemochromatosis [40], and (B) HAMP-associated hemochromatosis [41]. Hjv protein is also one of the "iron sensors" at the surface of hepatocytes.

4.5.1.3 Type 3 Hereditary Hemochromatosis

Camaschella et al. reported six patients who met the diagnostic criteria for hereditary hemochromatosis but were not linked to *HFE* from two families of Sicilian origin, and identified homozygous Y250X mutation in *TRF2* in these patients [42]. This type of hemochromatosis affects middle-aged adults but also adolescents and young adults and resembles type 1 hemochromatosis. The Y245X mutation of this gene in mice, equivalent to Y250X in humans, causes downregulation of hepcidin expression and iron accumulation in the liver [43]. In type 1, 2, and 3 hereditary hemochromatosis, serum hepcidin level is inappropriately low despite iron overload, and the diseases are inherited in the autosomal recessive pattern.

4.5.1.4 Type 4 Hereditary Hemochromatosis

Type 4 hemochromatosis, which is affected by *SLC40A1* mutations, is also known as ferroportin disease, and is less rare than type II or III [36]. The inheritance pattern is autosomal dominant. This disease is phenotypically heterogeneous with two forms (A and B). In form A, the loss of function mutants of ferroportin are unable to export iron from cells, resulting in iron accumulation predominantly in reticuloendothelial cells and decreased availability of iron for transferrin [36]. In form B, ferroportin mutations are responsible for a gain of function with full iron export capability but insensitivity to downregulation by hepcidin, leading to iron accumulation in parenchymal cells and a phenotype similar to other hepcidin deficiency-based types of hemochromatosis [36, 44]. Thus, ferroportin disease form B shows elevated transferrin saturation-associated tissue iron accumulation, preferentially within hepatocytes.

4.5.1.5 Other Type of Hereditary Hemochromatosis

Mutations in *BMP6* gene have recently been reported in several families [45, 46]. It has been reported that serum hepcidin levels of patients with heterozygous mutations of this gene were markedly low or inappropriately low for the iron overload.

Also, a heterozygous mutation in the IRE in the 5′-UTR of *FTH* gene has been demonstrated in patients with systemic iron overload [47]. The proband had iron deposition in hepatocytes and Kupffer cells/macrophages in the liver and spleen.

4.5.2 Management of Hereditary Hemochromatosis

Early diagnosis and prompt initiation of iron depletion therapy are essential for preventing irreversible organ damage. Although the penetrance of HFE C282Y is low among the general population except for northern European population, it is important to consider biochemical screening for hemochromatosis (followed by genetic testing when indicated) when we see the patients with iron overload. Phlebotomy is the standard treatment for hereditary hemochromatosis, but there are no evidence-based guidelines on the use of therapeutic phlebotomy. It should be repeated at appropriate intervals for at least 1 week [48]. Treatment is conventionally initiated when serum ferritin levels exceed the normal range [36]. The standard volume of phlebotomy is 400–500 mL which contains approximately 200–250 mg of iron, and it should be modified according to the patient's age, body weight, hemoglobin level, and comorbidities [48]. Maintenance therapy is performed to keep serum ferritin levels 50 to 100 ng/mL, but iron deficiency with lower serum ferritin levels should be avoided. In patients with both iron overload and anemia, phlebotomy is inappropriate. In such cases, iron chelation therapy using desferrioxamine, deferasirox, or deferiprone may be considered [48].

Although glucose tolerance, cardiac function, and gonadal function should be monitored in patients with hereditary hemochromatosis, we also should bear in mind that HCC is at least twice as frequent among patients with hereditary hemochromatosis compared with those who have other types of liver diseases because hepatic iron overload strongly favors DNA damage, genetic instability, and tumorgenesis through enhanced oxidative stress.

4.6 Conclusion

Because of the identification of important iron metabolic molecules and genes such as hepcidin, ferroportin, and *HFE*, our understanding of systemic iron regulation and the mechanisms of iron overload-related diseases has largely progressed in the past two decades. As liver is a crucial organ for iron metabolism, liver is likely to be involved in iron overload-related disorder. Hereditary hemochromatosis is one of the inherited liver diseases and occasionally diagnosed after the development of liver cirrhosis and/or HCC. Liver cirrhosis significantly reduces survival in concert with disorders in vital organs due to the parenchymal iron deposition. Therefore, early diagnosis and prompt initiation of iron depletion therapy are essential for improving the prognosis of patients with hereditary hemochromatosis. Recognition

of this disease is critical for hepatologists, hematologists, cardiologists, endocrinologists, and family physicians because inadequate management is fatal for patients with hereditary hemochromatosis.

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Chapter 5
Liver Cirrhosis with Inherited Liver Diseases: Wilson Disease

Masaru Harada

Abstract Wilson disease is a genetic disorder of copper metabolism. Wilson disease is treatable by several pharmacological agents. If untreated, this disease results in severe disability and death. Therefore, early diagnosis and adequate treatments are important for this disease.

Keywords ATP7B · Copper · Late endosome · Oxidative stress

5.1 Introduction

Copper is an essential trace element and plays many important biological processes. The processes include mitochondrial energy generation (cytochrome c oxidase), iron metabolism regulation (ceruloplasmin), melanin formation (tyrosinase), oxygen-radical scavenging (superoxide dismutase), and collage cross-linking (lysyl oxidase). However, excess copper is toxic, because it induces oxidative stress [1]. Therefore, both shortage and excess of copper can induce serious problems as illustrated by Menkes disease and Wilson disease [2–4]. Therefore, accurate regulations of copper absorption, mobilization, and excretion are necessary for our healthy life [5].

5.2 Copper Absorption

In mammals, copper absorption mainly occurs in the small intestine. Copper transporter 1 (Ctr1) transports copper across the apical membrane of enterocytes [6, 7]. The copper in the cytoplasm of enterocytes is delivered to Atox1, a copper chaperone,

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which delivers copper to ATP7A [8]. In copper-depleted conditions, ATP7A localizes at the trans-Golgi network (TGN). ATP7A localizes in the peripheral vesicles in copper-rich conditions [9]. Copper containing vesicles release copper from the basolateral membrane to interstitial space of the small intestine by exocytosis.

5.3 Copper Transport to the Liver

Copper absorbed from the enterocytes enters the portal circulation [2–4, 10]. The liver is the central organ of copper metabolism, and the liver takes up copper from the portal circulation [10, 11]. Ctr1 transports copper across the sinusoidal plasma membrane [12].

5.4 Copper Metabolism in Hepatocytes

Wilson disease is an autosomal recessive inherited disorder of copper metabolism. It is characterized by the accumulation of copper in the body because of decreased biliary copper excretion from hepatocytes into bile. Wilson disease gene, *ATP7B*, has been cloned and it encodes a cation-transporting P-type ATPase (ATP7B) [13–15]. It is clear that ATP7B functions in both incorporation of copper into apo-ceruloplasmin to form stable (mature) holo-ceruloplasmin and biliary copper excretion [16, 17]. Long-Evans Cinnamon (LEC) rat is an animal model of Wilson disease and it has mutated gene of *Atp7b,* the rat gene homologous to *ATP7B* [18]. Introduction of ATP7B protein to hepatocytes of LEC rats restored the normal ceruloplasmin secretion and biliary copper excretion [16, 17]. First, the gene product, ATP7B (Wilson disease protein), was detected in the TGN [19, 20]; however, the localization is still controversial [21–29]. We examined the intracellular localization of green fluorescent protein-ATP7B (GFP-ATP7B), ATP7B-DsRed, transfected-ATP7B, and endogenous ATP7B in primary isolated rat hepatocytes, OUMS29 (a human hepatocytes cell line), Huh7 and Hep3B (human hepatoma cell lines), HEK293, and MDCK cells [21–27]. Our examinations demonstrated that ATP7B colocalized with lysosomes associated protein (lamp) 1 and 2 (late endosome and lysosomes localized protein), Rab7 (late endosome localized protein), Niemann-Pick C1 protein (NPC1, late endosome localized protein), incubated rhodamine-dextran (all endocytic structures), but not with galactosyltransferase (TGN localized protein), γ-adaptin (TGN localized protein), 58-kd Golgi protein (TGN localized protein), cathepsin D (lysosome localized protein), or lysosomal glycoprotein 85 (lysosome localized protein) [21–27]. Therefore, we consider that ATP7B localizes in the late endosomes. ATP7B translocates copper from the cytoplasm into the late endosomes. Then, copper in the late endosomes is transported to the lysosomes and copper is excreted into bile. About the copper incorporation into ceruloplasmin, we

demonstrated the importance of NPC1 by the experiments of NPC1 knockdown and introduction using cultured cells [26, 27]. Some other studies reported the importance of NPC1 in copper metabolism in hepatocytes [30, 31].

5.5 Systemic Regulation of Copper Metabolism

Systemic regulation of copper has been little understood. Cardiac copper deficiency produced by cardiac specific knockout of Ctr1 induced increase of copper in serum and decreased hepatic copper contents. Furthermore, the expression of ATP7A increased in the intestine and liver. These results indicated the existence of systemic regulation of copper metabolism [5].

5.6 History

Wilson disease is an autosomal recessive disorder characterized by the accumulation of copper in the body [2–4]. First, Wilson SAK described strange familial cases with nervous system and liver cirrhosis as progressive lenticular degeneration in 1912 [32]. Copper accumulation in the liver and central nervous system is associated with this disease [33]. Serum ceruloplasmin concentration had been found to be low in patients with Wilson disease [34]. The prevalence of Wilson disease is estimated one in about 40,000 individuals [2–4].

5.7 Clinical Manifestations

Clinical manifestations of patients with Wilson disease vary and the onset of the age is variable [35]. Symptoms include hepatic manifestations, neuropsychiatric manifestations, Kayser–Fleischer ring, and hemolysis in association with acute liver failure. Various manifestations of the other organs, such as kidney, heart, bone, muscle, and endocrine organs, are possible.

Hepatic manifestations include asymptomatic mild liver dysfunction to cirrhosis. Some patients may present as acute liver failure. Hepatocellular carcinoma (HCC) may be possible, although the prevalence of HCC is not so frequent [4].

Some patients manifest broad spectrum of neurological, behavioral, and psychiatric manifestations. Most, probably all, patients with neurological manifestations have liver abnormalities. Many of them have already progressed to cirrhosis.

Kayser–Fleischer rings represent deposition of copper in Descemet's membrane of the cornea. Sunflower cataract represents copper deposition in the lens. These findings of the eyes represent copper deposition in the extrahepatic tissues including central nervous system [2–4].

Patients with successful treatment can become pregnant [36, 37]. Treatment must be continued throughout the duration of pregnancy. D-penicillamine, trientine, and zinc have been demonstrated safe for pregnant patients and fetus [2–4, 38, 39]. Interruption of the treatment during pregnancy may induce acute liver failure [2–4].

5.8 Diagnosis

There is no single test to diagnose Wilson disease. Therefore, combined evaluation of clinical and biochemical findings is important. The Wilson disease scoring system is useful for the diagnosis (Table 5.1) [3, 40]. If the cumulative score from this scoring system is 4 or above, the diagnosis of Wilson disease is likely.

Table 5.1 Scoring system for the diagnosis of Wilson disease

Symptoms and tests	Points
Kayser–Fleischer ring	2
Neurologic symptoms or MRI findings	1
Coombs negative hemolytic anemia	1
Urinary copper	1
1–2 × ULN	
>2×	2
Normal, but >5× after D-penicillamine	2
Liver copper content	
1–5 × ULN	1
>5×	2
Normal	−1
Rhodanine staining	
Positive[a]	1
Serum ceruloplasmin (mg/dL)	
10–20	1
<10	2
Mutation analysis	
Disease causing mutations on both chromosomes	4
Disease causing mutations on one chromosome	1

Total score: 4 or more: diagnosis of Wilson disease highly likely; 2–3: diagnosis of Wilson disease probable, do more investigation; 0–1: diagnosis of Wilson disease unlikely

ULN upper limit of normal, urinary copper <40 μg/day, liver copper content <50 μg/g, serum ceruloplasmin <20 mg/dL

[a]If measurement of liver copper content is not available

5.9 Treatments

Wilson disease was a fatal disease until the treatment was introduced. Now, Wilson disease is a genetic metabolic disorder that can be treated with some drugs. The treatments are based on the use of copper chelators to remove accumulated copper from the body and zinc to reduce absorption of copper from the small intestine [2–4, 41]. Since chocolate, nuts, mushrooms, shellfish, and organ meats contain high concentration of copper, patients should avoid these foods.

D-penicillamine promotes urinary excretion of copper from the body. Side effects appear in approximately 30% of patients treated with this drug. The side effects include fever, eruptions, neutropenia, thrombocytopenia, proteinuria, nephrotoxicity, lupus-like symptoms, myopathy, and hepatotoxicity [2–4]. Usually, the initial dose is 200–400 mg/day. Maintenance dose is usually 800–1000 mg/day. D-penicillamine should be taken 1 h before or 2 h after the meals, because food in the gastrointestinal tract inhibits absorption of the drug. Long-term prognosis of patients treated with D-penicillamine is favorable, although some patients needed the change of the treatment due to adverse events [42].

Trientine is another copper chelator [43]. When patients suffer from severe adverse effects by the use of D-penicillamine, trientine should be administrated. Typical dosages are 750–1500 mg/day. A recent long-term observation demonstrated that trientine produced comparable outcomes with D-penicillamine and less adverse events [42].

Bis-choline tetrathiomolybdate is now tried as a new chelation therapy [44].

Zinc induces the expression of metallothionein, a metal binding protein, in various cells including enterocytes [45, 46]. Metallothionein has greater affinity with copper than zinc. Copper binds with metallothionein in the cytoplasm of enterocytes. Therefore, copper is not absorbed but is lost into the intestinal lumen with enterocytes. Zinc should be administrated 1 h before or 2 h after the meals. Zinc has very few side effects [46]. Zinc has direct cell protection effects against copper toxicity [1]. Zinc is used for presymptomatic patients or for maintenance of those who have already received chelation therapy [47].

Combination of D-penicillamine or trientine and zinc can be used for treatment for patients with Wilson disease [48, 49].

Patients with acute liver failure or decompensated liver failure may require liver transplantation [2–4]. Living donor liver transplantation from a family member who has heterozygote mutation is also successful [50]. Liver transplantation does not always improve the neurological manifestations [2–4]. For consideration of the indication of liver transplantation for patients with Wilson disease, the revised King's college score is very useful [51]. Sometimes intensive therapy with copper chelators, plasma exchange, and artificial liver support can avoid liver transplantation in patients with acute liver failure [52].

5.10 Prognosis of Wilson Disease

Patients with Wilson disease who receive adequate care have usually favorable prognosis. However, prognosis is poor in patients diagnosed after the development to cirrhosis or advanced neurological symptoms [53, 54]. Discontinuance of the treatment induces intractable hepatic failure [55–57]. Suicide is one of the problems of patients with Wilson disease [56, 57]. Education of patients is important to prevent these serious problems.

5.11 Conclusions

The liver is the central organ for the metabolism of copper. Abnormal metabolism of copper in the liver is detected in patients with Wilson disease. Wilson disease is a rare genetic metabolic disorder that can be treated by pharmacological treatments. Therefore, recognition and adequate treatment are essential, because inadequate management is fatal for these patients.

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Chapter 6
Nutrition in Liver Cirrhosis

Masahito Shimizu, Makoto Shiraki, and Yohei Shirakami

Abstract Nutritional/metabolic disorders such as protein–energy malnutrition are frequently observed with liver cirrhosis. Nutritional therapy prevents complications of liver cirrhosis and improves prognoses as well as quality of life. Branched chain amino acids are key drugs of nutritional therapy for liver cirrhosis, improve hypoalbuminemia, and are useful as a late evening snack for energy malnutrition. Appropriate nutritional therapy must be conducted for liver cirrhosis patients associated with sarcopenia or obesity.

Keywords BCAA · Liver cirrhosis · Nutritional therapy · PEM · Sarcopenia

6.1 Pathology and Nutrition of Liver Cirrhosis

The liver plays a central role in nutritional/energy metabolism control and liver cirrhosis patients with decreased hepatic functional reserve are associated with various nutritional/metabolic disorders. Particularly because protein–energy malnutrition (PEM), which is common in patients with liver cirrhosis, is deeply involved in the prognosis and deterioration of quality of life (QOL) in the same patients, appropriate diagnosis (nutritional assessment) along with early intervention (nutritional therapy) is important [1, 2].

Although the resting energy expenditure of liver cirrhosis patients is elevated, the uptake of glucose into the liver and the ability to synthesize/store glycogen in the liver are decreased as liver parenchymal cells decrease. In particular, as liver cirrhosis progresses, liver cirrhosis patients are frequently associated with abnormal glucose metabolism such as diabetes and postprandial hyperglycemia/hyperinsulinemia because the utilization efficiency of carbohydrates decreases, while the

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utilization efficiency of fat as a physiological energy substrate increases. Patients with cirrhosis show a compromised ability to store glycogen and blunted gluconeogenesis [3, 4].

With liver cirrhosis, a decrease in branched chain amino acids (BCAAs) and an increase in aromatic amino acids along with a decrease in the Fischer ratio, which is a molar ratio of these (amino acid imbalance), are observed. Among BCAAs, leucine in particular promotes protein synthesis through the activation of mTOR signaling. BCAA administration for protein malnutrition raises the serum albumin levels and improves the QOL and survival of patients with liver cirrhosis. BCAAs play an important role in maintaining and increasing skeletal muscle mass and the decline in BCAA in liver cirrhosis patients is deeply involved in the development of hypoalbuminemia and sarcopenia [5–7].

Sarcopenia is a syndrome characterized by reduced skeletal muscle mass and muscle strength. With liver cirrhosis, because BCAAs are more energy efficient than glucose and the substrate burned as an energy source in skeletal muscle is mainly BCAAs, progression of PEM, decline in BCAAs, and the development of sarcopenia are observed as a series of pathological conditions. Moreover, with liver cirrhosis, because ammonia that cannot be treated due to a decline in hepatic detoxification function is metabolized in skeletal muscle in a compensatory manner using BCAAs as a substrate, the BCAA concentration further decreases [8, 9]. The loss of hepatic functional reserve and skeletal muscle mass is also involved in glucose intolerance (Fig. 6.1).

In addition to malnutrition, hypernutrition also exacerbates the prognosis of liver cirrhosis patients. Obesity and diabetes in particular have been reported to increase the risk of hepatocellular carcinoma (HCC), so attention is required. Currently, one-third of liver cirrhosis patients are obese [10]. Moreover, liver cirrhosis with backgrounds of nonalcoholic steatohepatitis related to obesity and lifestyle diseases is also increasing. Based on the fact that the nutritional status of liver cirrhosis patients is shifting from PEM/malnutrition to obesity/hypernutrition, improvements of nutritional therapy, exercise therapy, and lifestyle habits should be promoted.

6.2 Basics of Nutritional Therapy

When starting nutritional therapy of liver cirrhosis, it is important to accurately evaluate the nutritional status of patients, especially PEM. PEM is strongly associated with the severity of hepatic decompensation in the setting of cirrhosis and the Child–Pugh classification is a commonly used tool for measuring the severity of chronic liver failure. Cirrhotic patients with Child–Pugh classes B and C have been shown to be most likely to develop PEM [11]. The subjective global assessment (SGA), an attractive test due to its accuracy, is also used as a standard nutritional evaluation in hospitals. The SGA is simple to execute because it is a questionnaire with two main components, history and physical examination [12]. A biochemical

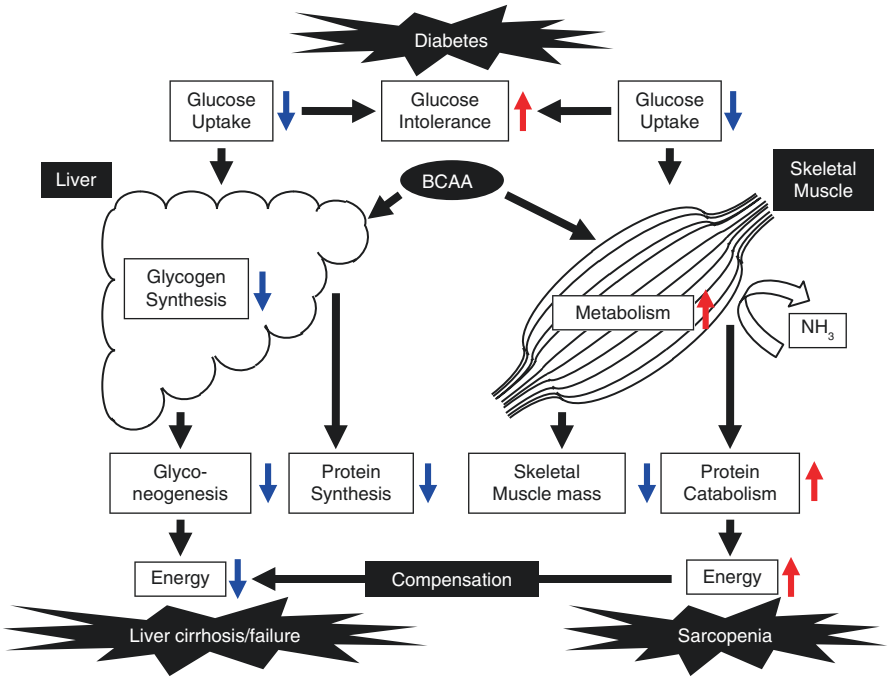

Fig. 6.1 Pathophysiological mechanisms linking metabolic abnormalities, sarcopenia, and glucose intolerance in patients with liver cirrhosis. *BCAA* branched chain amino acids

assessment is commonly performed to evaluate malnutrition and serum albumin is a common tool to measure nutritional status.

In 2015, the Japanese Society of Gastroenterology revised the evidence-based clinical practice guidelines for liver cirrhosis, which is useful to undergo nutritional therapy for such disease [13]. In the guidelines, the protein malnutrition status of liver cirrhosis patients is evaluated using their serum albumin level. A serum albumin level of less than 3.5 g/dL significantly decreases survival rate. In liver cirrhosis patients, serum albumin levels are correlated with BCAA concentrations and are the basis for demonstrating the utility of BCAA replacement therapy for the same patients. Energy malnutrition is evaluated using the nonprotein respiratory quotient, arm muscle circumference length/arm circumference length, and serum free fatty acid levels. For hypoalbuminemia, amino acid imbalance, and energy malnutrition, it is necessary to proactively conduct nutritional therapy [3, 14] (Fig. 6.2).

Diet plays a substantial role in cirrhosis. For liver cirrhosis, a nutritional care plan is prepared by paying attention to complications such as ascites/edema, impaired glucose tolerance, and hepatic encephalopathy/protein intolerance. Physical measurements along with a subjective comprehensive evaluation are conducted and a nutritional assessment is conducted over time according to changes in the pathological conditions. Although the energy requirement is calculated based on the

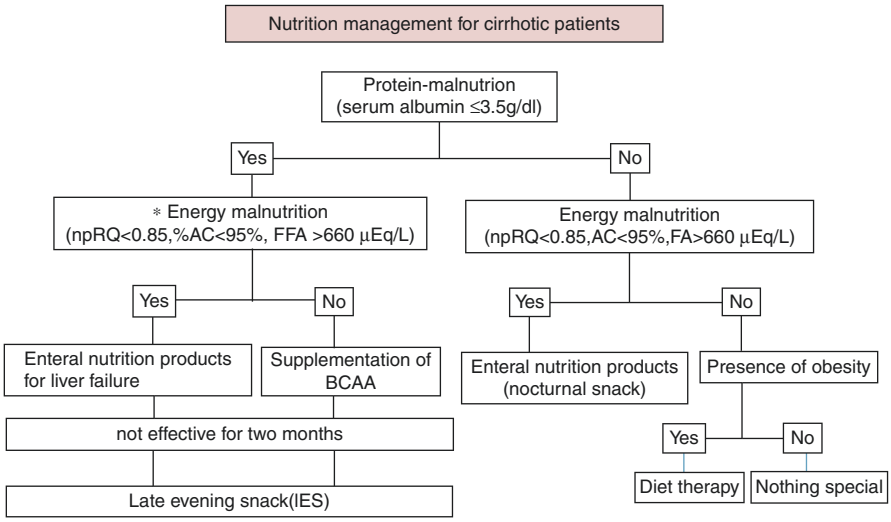

Fig. 6.2 Algorithm for nutritional therapy in patients with liver cirrhosis. *npRQ* nonprotein respiratory quotient, *%AC* percent arm circumference, *FFA* free fatty acid. This figure is referred from [13]

intensity of daily activity, particularly in the event of impaired glucose tolerance, attention must be paid to avoid excessive caloric intake (25–35 kcal/kg ideal body weight/day as a guide). The recommendation for carbohydrates is 50–70% of daily calories; however, simple sugar, especially fructose, should be avoided as much as possible [15]. A low salt diet is effective against ascites/edema; however, excessive sodium restrictions require attention because they reduce appetite and deteriorate nutritional status.

Protein restriction is no more a recommended strategy unless contraindicated by clinical complications, such as hepatic encephalopathy. Because protein deficiency is a significant problem in malnutrition, the required protein intake in cirrhotic patients is 1.0–1.5 g/kg/day if there is no protein intolerance [16]. Although protein intake is useful as a countermeasure to sarcopenia, because excessive protein load may induce hepatic encephalopathy, particularly in the event of protein intolerance, low protein diet (0.5–0.7 g/kg/day) or enteral nutrients for liver failure including BCAAs is used. Fat requirements are set to 20–25% in terms of energy ratio. It is also important to supplement zinc and take appropriate amounts of vitamins and dietary fiber (measures for constipation). There should be an increased emphasis on BCAA and fiber with decreased ammonia when the patients suffer from hepatic encephalopathy (Table 6.1).

It should be emphasized that total nutritional management, including both diet and nutritional supplements, is important in order to prevent the progression of chronic liver disease and onset of HCC. In 2012, the Japanese Nutritional Study Group for Liver Cirrhosis published the guidelines on nutritional management in

Table 6.1 Recommendation for nutritional management of liver cirrhosis

1. Daily calories
25–35 kcal/kg ideal body weight/day
If any abnormalities are seen in glucose tolerance, intake should be 25 kcal/kg ideal body weight/day
2. Proteins
If there is no protein intolerance:
1.0–1.5 g/kg/day (including BCAA granules)
If there is protein intolerance:
0.5–0.7 g/kg/day (low protein diet) + BCAA-enriched enteral nutrient mixture
3. Carbohydrates
50–70% of daily calories with decreased simple sugar, especially fructose
4. Lipids
20–25% of daily calories
5. Sodium chloride
If there is ascites and/or edema: 5–7 g/day
6. Divided meal (4–6 times/day) and/or LES (amounts to 200 kcal)

cirrhotic patients from the perspective of preventing HCC [17]. This guideline is useful for the actual nutritional management of patients with liver cirrhosis.

6.3 Nutritional Therapy Using BCAA

To improve hypoalbuminemia and amino acid imbalance, oral BCAA preparations are useful. Although oral BCAA preparations include BCAA granules and enteral nutrients for liver failure, they need to be properly used depending on the energy malnutrition state or the presence of hepatic encephalopathy. While supplemental administration of BCAA granular preparation maintains/increases the serum albumin concentration in decompensated liver cirrhosis patients, it prevents adverse events of liver cirrhosis and improves vital prognosis as well as QOL. A multicenter, randomized, and nutrient intake-controlled trial has revealed that long-term oral BCAA granules supplementation (12 g/day) improves event-free survival (death by any cause, development of HCC, rupture of esophageal varices, or progress of hepatic failure), increases serum albumin levels, and improves QOL in patients with decompensated liver cirrhosis with hypoalbuminemia [5]. The mean annual changes in the model for end-stage liver disease score and Child–Pugh score were smaller and the incidence of overall major cirrhotic complications, such as ascites, was lower in cirrhotic patients taking BCAA granules, which suggests that early interventional oral BCAA administration may prolong the liver transplant waiting period by preserving hepatic reserve in cirrhosis [18]. BCAA supplementation relieves minimal hepatic encephalopathy and increases muscle mass [19]. More

importantly, BCAA supplementation is also involved in reduced incidence of HCC in patients with cirrhosis [20–22].

For energy malnutrition, divided meals and late evening snacks (LES), such as rice ball, liquid nutrients, and BCAA-enriched supplementation, are recommended. Approximately 200 kcal is divided from the target total daily calories and taken as a snack/energy before going to bed to improve nighttime starvation. LES improves nutritional status, increases body protein content, and diminishes fat and protein oxidation in patients with liver cirrhosis [23, 24]. LES is associated with suppression of serum free fatty acid levels, recovery of energy metabolism, and improvement of health-related QOL [25, 26]. In patients with cirrhosis, divided meals with LES fortified with BCAA prevented hypoglycemia and led to increased nutrition due to reduced catabolism overnight [27]. As divided meals/LES need to be continued, one which is easy to prepare and ingest is preferred. Specifically, 1 pack (approximately 200–300 kcal) of enteral nutrition for liver failure containing mostly BCAA is used. BCAAs are a key drug in nutritional therapy of liver cirrhosis patients (Table 6.1 and Fig. 6.3).

Low level of serum BCAA predicts sarcopenia in patients with liver cirrhosis [28]. In a retrospective study of liver cirrhosis patients with sarcopenia, the oral administration group of a BCAA preparation has been reported as having a significantly better prognosis compared to the non-oral administration group [8]. A leucine-enriched BCAA diet is able to reduce the elevated whole-body protein breakdown in patients with cirrhosis [29]. A recent clinical trial has revealed that combination of BCAA supplementation and walking exercise is effective for improving muscle volume and strength in liver cirrhosis patients [30]. As preven-

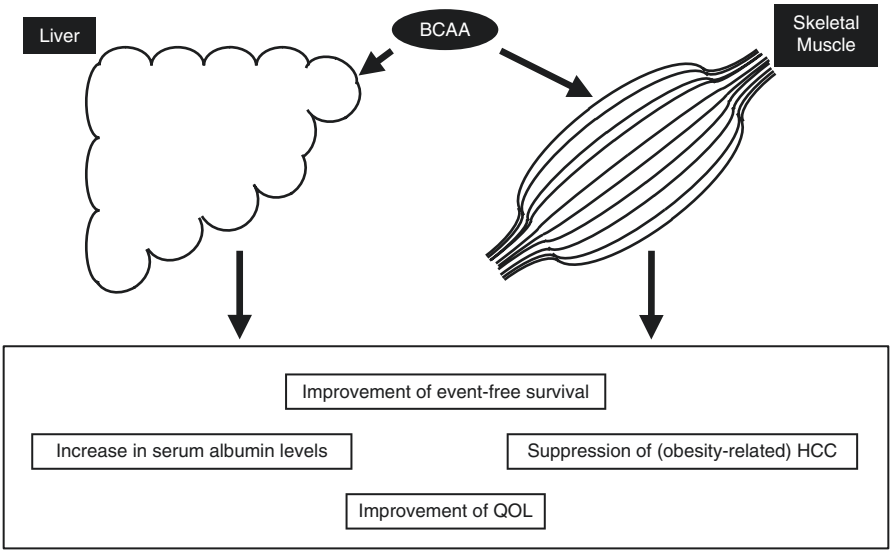

Fig. 6.3 Beneficial impacts of BCAA in patients with liver cirrhosis

tion/treatment of sarcopenia in liver cirrhosis patients, the usefulness of nutritional therapy mainly including BCAAs as well as exercise therapy is anticipated.

6.4 Liver Cirrhosis and Obesity

It has recently been revealed that the nutritional status of liver cirrhosis patients is shifting from PEM/malnutrition to obesity/hypernutrition. Currently, one-third of liver cirrhosis patients exhibit a BMI of 25 or more and liver cirrhosis with a background of obesity and nonalcoholic steatohepatitis is increasing [10]. Obesity exacerbates the prognosis of liver cirrhosis patients and increases the risk of HCC; however, replacement therapy of oral BCAA preparations has been reported to suppress liver carcinogenesis in patients with hepatitis C and cirrhosis who are obese [21]. The beneficial effects of BCAA supplementation on the regulation of glucose metabolism have been reported in recent clinical and experimental studies, which suggest that BCAA may suppress liver carcinogenesis in obese patients with liver cirrhosis, at least in part, by improving insulin resistance [7, 31]. It is necessary to practice nutritional therapy aimed at improvement of the long-term prognosis of liver cirrhosis patients associated with obesity as well as suppression of liver failure and HCC.

6.5 Conclusion

PEM is a serious problem, especially in cirrhotic patients. Appropriately evaluating nutritional/metabolic disorders in liver cirrhosis patients and proactively conducting nutritional therapy lead to the prevention of complications and improved prognoses/QOL. Nutritional therapy for liver cirrhosis should make sure the patients reach the recommended daily calories and nutrients by increasing oral intake or by using other measures, such as oral supplementation, divided meal, and LES. It is also necessary to conduct nutritional therapy including measures for sarcopenia and obesity in coordination with registered dietitians.

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Chapter 7
Microbiome in Liver Cirrhosis

Akira Sakamaki, Masaaki Takamura, and Shuji Terai

Abstract Humans have a codependent relationship with gut microbiota. Changes in microbiota are proposed to be associated with various pathological conditions. New analyses facilitate the assessment and exhaustive search of these nonculturable bacteria. These new technologies also provide evidence of a relationship between gut microbiota and liver cirrhosis (LC).

Gut microbiota is closely involved in maintenance of the relationship between gut and liver in which commensal gut microbiota inhibits harmful bacteria, produces short-chain fatty acids to protect from mucosal infection, and metabolize bile acids to monitor the intestinal environment. Gut microbiota in patients with LC was different from that in healthy individuals due to dysbiosis regardless of background hepatitis status. In patients with LC, dysbiosis, small intestinal bacterial overgrowth, leaky gut syndrome, and immune paralysis of the gut-associated lymphoid tissue occurred, and the interaction between these induces a disruption of the gut–liver barrier. Dysfunction of the immune system induces translocation of harmful bacteria and endotoxin into the liver. Bacterial translocation worsens LC and contributes to complications such as hepatic encephalopathy, hepatocellular carcinoma, hepatorenal syndrome, and spontaneous bacterial peritonitis. Poorly absorbable antibiotics, probiotics, prebiotics, synbiotics, and fecal microbiota transplantations were reported as potential treatment interventions for dysbiosis in patients with LC.

Keywords Gut microbiota · Liver cirrhosis · Metagenomics · Gut–Liver axis Dysbiosis · Bacterial translocation · Hepatic encephalopathy · Poorly absorbable antibiotics · Fecal microbiota transplantation

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7.1 Introduction

In humans, the intestinal tract contains up to 100 trillion gut bacteria, a number that is up to ten times more than that of somatic and germ cells [1]. Genes in gut bacteria that synthesize amino acids and vitamins are more extensive than those in humans, and their products are commonly utilized by humans [2], who are in a codependent relationship with the gut bacteria that act as a superorganism [3]. The cooperative relationship with gut bacteria in healthy humans reverts to a damaging one in pathological conditions. Several reports indicated that changes in microbiota were associated with various pathological conditions including liver diseases [4–6], obesity [7], diabetes [8], cardiac diseases [9], inflammatory bowel diseases [10], irritable bowel syndrome [11], and autism [12]. The development of new analytical tools such as rRNA gene sequencing and metagenomic methods [13] facilitated the assessment and exhaustive search of these nonculturable bacteria that could not be found and accessed using earlier techniques that utilized cultivation [14].

The most commonly used identification method without cultivation is sequencing of 16S ribosomal RNA (rRNA) gene, which consists of a super variable region with species-specific base arrangement and a conserved region that is nearly identical across bacterial species. Therefore, universal primers that bind the conserved region can amplify almost all species in gut bacteria [15]. Moreover, metagenomics, which analyzes extracted undiluted DNA or RNA sequences, provides extensive genetic information with high-performance gene sequencers and computing systems or tools. Especially, functional analysis of individual genes can provide important information through metagenomics [16].

In this chapter, we discuss recent studies on gut bacteria that utilized new technologies, with a focus on liver cirrhosis (LC).

7.2 Gut–Liver Axis and Gut Microbiota

Liver and the intestinal environment are closely related through portal vein and bile duct. Foreign agents, including nutrients, drugs, and a small amount of pathogens that pass across intestinal epithelial cells are delivered to liver through the portal vein, are processed, decomposed, and reserved in liver. Intestinal epithelial cells allow for only detoxified products can pass through the liver to be dispersed to the whole body. The digestive tract and liver play an important role in the biological defense against foreign agents in a coordinated manner by forming the gut–liver axis [17] (Fig. 7.1a).

Over 90% of human gut microbiota consist of four main divisions: *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria. Firmicutes* and *Bacteroidetes* are more dominant, and relative percentages and species differ among individuals [2, 18] and countries [19]. Gut microbiota is closely involved in maintenance of the relationship between gut and liver. Specifically, commensal gut microbiota inhibits harmful bacteria and produces short-chain fatty acids (SCFAs) to protect mucosa from bacterial infection (Fig. 7.1b).

Intestinal epithelial cells have important, life-supporting functions including absorption as well as barrier formation against invasion of harmful bacteria, that is, pathobionts. These cells have participated in an aggressive host defense by formation of a thick mucus layer and tight junctions [20, 21]. Mucus layer on the surface of epithelial cells is composed predominantly of mucin that is secreted by these cells, which physically prevents the invasion of foreign matter. Furthermore, secretory immunoglobulin (Ig) subtype A (IgA) produced by plasma cells in lamina propria covers intestinal epithelial cells [22].

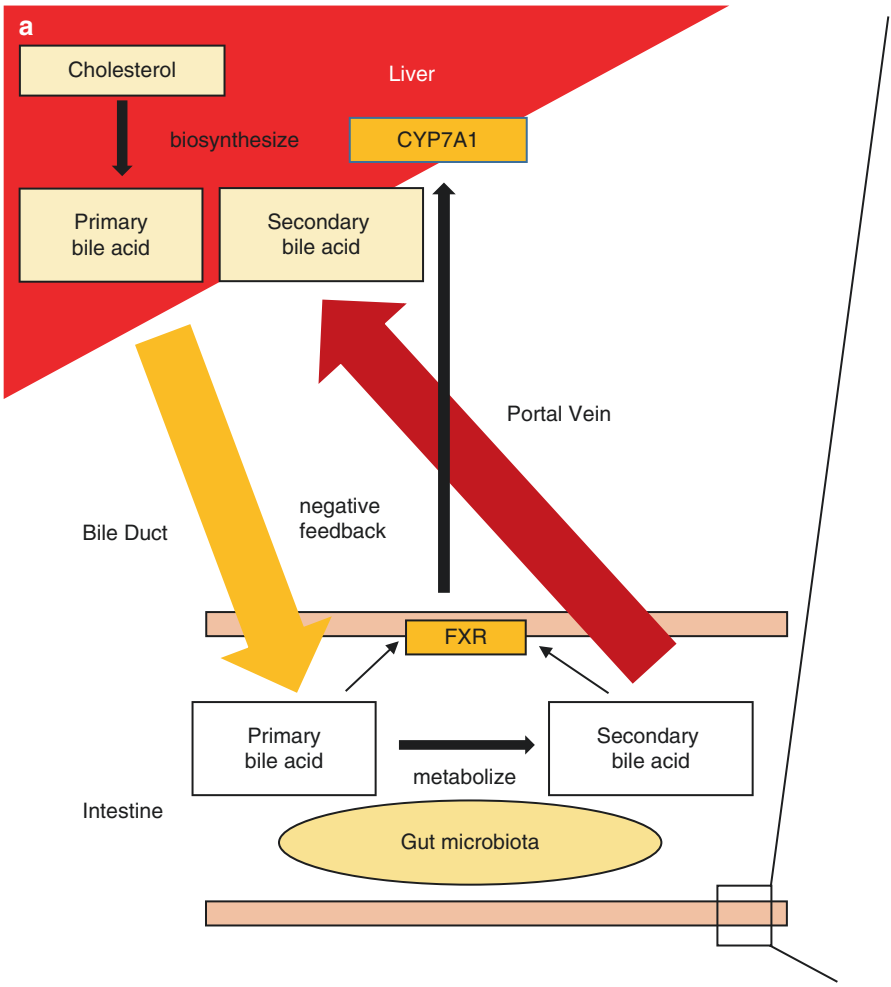

Fig. 7.1 The relationship between gut and liver mediated by gut microbiota. Enterohepatic circulation has a role to monitor and control the intestinal environment (**a**), and host immunological defense mechanisms maintain the intestinal environment and protect against the invasion of harmful foreign substances (**b**) in coordination with gut microbiota. *CYP7A1* cholesterol 7α-hydroxylase, *FXR* farnesoid X receptor, *MAMP* microbe-associated molecular pattern, *SCFA* short-chain fatty acid, *TLR* toll-like receptor, *Treg cell* regulatory T cell, *IgA* immunoglobulin subtype A

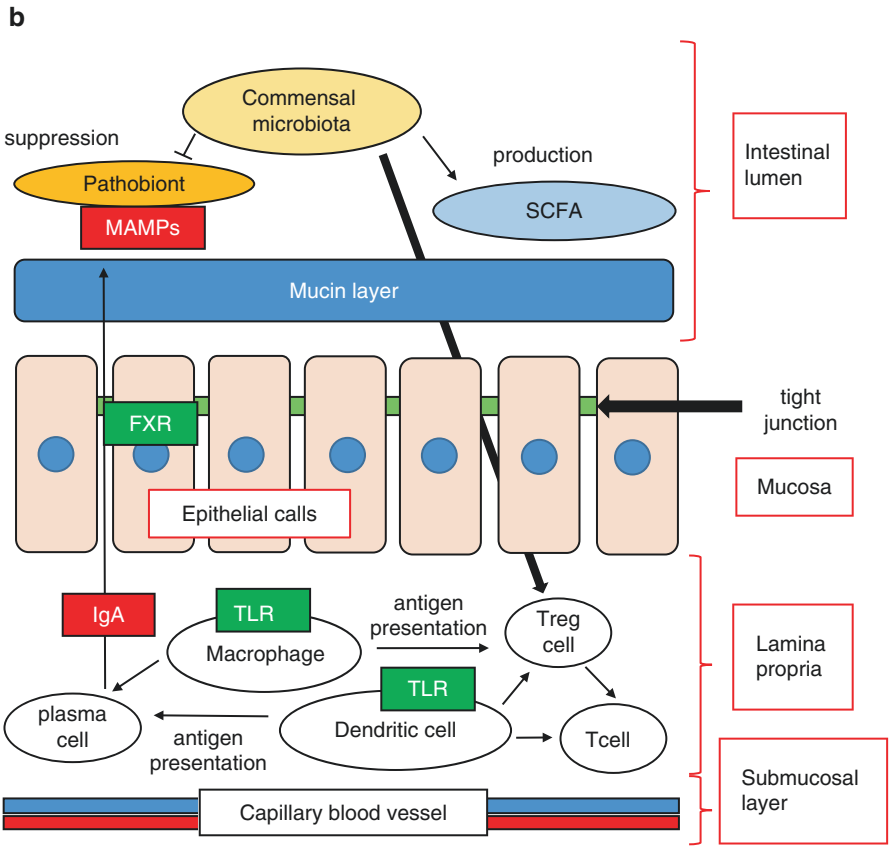

Fig. 7.1 (continued)

Secretory IgA plays a key role in innate immunity, as part of the initial immunological response to pathogens. Innate immunity starts with recognition of common and characteristic molecular structures by a limited number of pattern-recognition receptors (PRRs) expressed on natural killer cells and antigen-presenting cells such as macrophages and dendritic cells [23]. Secretory IgA has wide-ranging functions such as inhibition of bacterial adhesion to epithelial cells and neutralization of bacterial and viral toxins, and establishment and maintenance of cohabitation with gut bacteria via recognition of microbe-associated molecular patterns by several PRRs such as toll-like receptors (TLRs) [24]. Furthermore, particular indigenous species such as *Clostridium* induce regulatory T cells that are critical for the regulation of immunological response in gut [25, 26].

Moreover, gut bacteria ferment and disaggregate indigestible components such as dietary fiber and produce SCFAs such as acetic, propionic, and butyric acid by interaction among different bacteria, mainly *Firmicutes* and *Bacteroidetes* divisions [27]. These SFCAs act as nutrients for epithelial cells [28], induce mucin production [29], and regulate energy homeostasis [30, 31]. These immunological defense mechanisms, in coordination with commensal microbiota, maintain the intestinal environment and protect against the invasion of harmful foreign substances (Fig. 7.1b).

Conversely, bile acids secreted to the gut are reabsorbed and returned to the liver via the portal vein, which is named as enterohepatic circulation [32]. Bile acids are biosynthesized from cholesterol and secreted to duodenum through the bile duct as the main component of bile. Cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of bile metabolism, receives negative feedback from the intestinal epithelial cell nuclear receptor, farnesoid X receptor (FXR), to regulate bile acid levels [33–35]. Primary bile acids are metabolized to secondary bile acids through deconjugation and dehydrogenation by gut bacteria in small intestine. Thereafter, about 95% of secondary bile acids as well as primary bile acids are reabsorbed and returned to liver via portal vein to be resecreted in bile. CYP7A1 and FXRs monitor and control the intestinal environment by bile acids (Fig. 7.1a).

7.3 Microbiome in Patients with Liver Cirrhosis

In patients with LC, a disruption in the gut–liver barrier was found by the combination of dysbiosis, small intestinal bacterial overgrowth (SIBO), leaky gut syndrome (LGS), and immune paralysis of gut-associated lymphoid tissue (GALT) [36].

Several metagenomic analysis indicated that gut microbiota in patients with LC was different from that in healthy individuals because of dysbiosis independent of background hepatitis status [4–6]. Dysbiosis indicates failure of the normal intestinal environment and is classified into four states: decrease in commensal bacteria; pathological increase in pathobionts; decrease in the diversity of gut flora; and change of the function of gut flora [37, 38]. In summary, an increase in *Enterobacteriaceae* and *Streptococcaceae* families and a decrease in *Bifidobacteriaceae* and *Lachnospiraceae* families are found in patients with LC. Furthermore, changes in gut microbiota of patients with decompensated or complicated LC are different from that in compensated patients [6, 39], and liver transplantation improves dysbiosis in patients with good post-transplantation congestion [40]. A study showed that the source of SIBO such as *Veillonella* and *Streptococcus* in patients with LC was the oral cavity and that proton pump inhibitors might worsen the cascade [41].

The rate of SIBO in patients with LC ranges from 48% to 73% [36, 42]. SIBO is defined as the presence of $>10^5$ colony-forming units/ml in proximal jejunum and a significant change in gut flora, with the emergence of bacteria not normally found in the intestines [43, 44]. Accumulating evidence suggests that SIBO might be associated with a reduction in intestinal movement, impaired bile secretion, and decreased IgA secretion.

Intestinal epithelial cells form tight junctions that allow selective passage of nutrients, whereas physiological permeability is achieved by nutrients, pathological increase in permeability occurs via immune cells, cytokines, and pathogens [45], which is termed LGS. In patients with LGS, because of an increase in not only pathobionts and its components, such as lipopolysaccharide (LPS), which is an endotoxin produced by gram-negative bacteria, but also high molecular-weight chemical agents and food allergens in the intestinal mucosa, LGS leads to the inhibition of physiological absorption and was shown to be associated with allergic

and autoimmune diseases [46]. Studies also reported that LGS was worsened by ingestion of particular food items, drugs, alcohol [47], fructose [48, 49], and non-steroidal anti-inflammatory drugs [50, 51].

GALT, the largest immune organ in the human body that is located in the lamina propria, is the first defense mechanism against invasion of gut bacteria [36]. In healthy individuals, a small amount of bacteria pass the intestinal mucosa, whereas this amount increases in parallel with the progression of liver fibrosis; these bacteria produce inflammatory cytokines such as tumor necrosis factor (TNF)-α. Release of reactive oxygen species and nitric oxide in GALT precipitates epithelial cell dysfunction. In patients with decompensated LC, more pathogens pass the mucosa and lead to the paralysis of GALT.

Dysfunction of the immune system within the gut–liver axis induces pathobionts and the release of LPS into the epithelial mucosa, peripheral veins, portal vein, and liver in a process called bacterial translocation. Pathological processes that take place in patients with LC are summarized in Fig. 7.2.

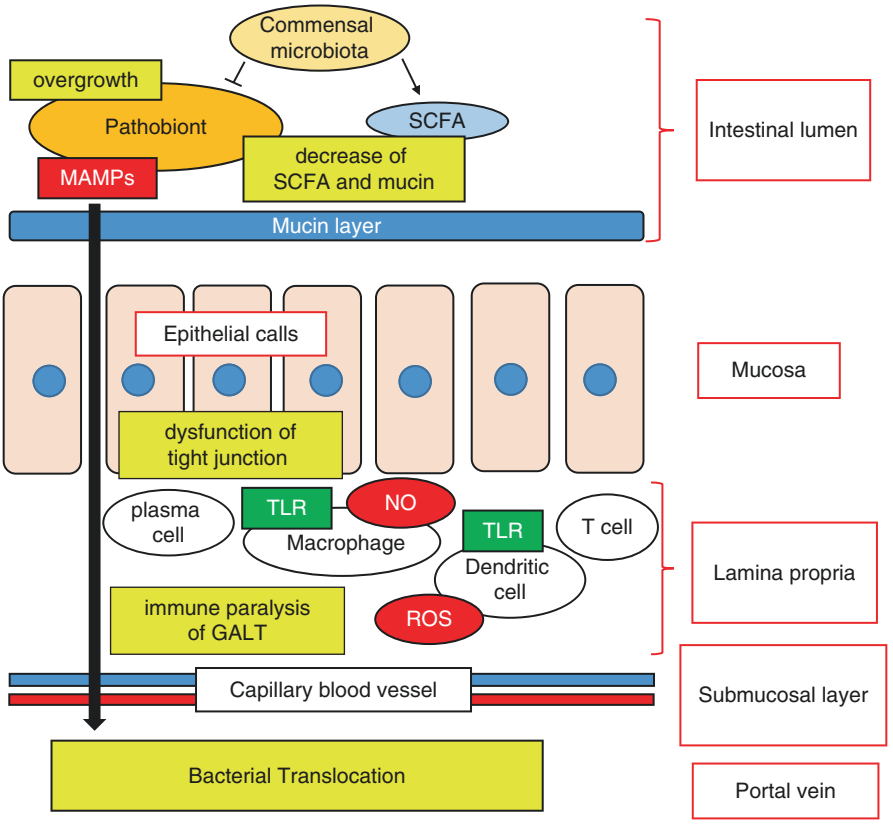

Fig. 7.2 The mechanism of bacterial translocation in patients with liver cirrhosis. *MAMP* microbe-associated molecular pattern, *SCFA* short-chain fatty acid, *TLR* toll-like receptor, *ROS* reactive oxygen species, *NO* nitric oxide, *GALT* gut-associated lymphoid tissue

7.4 Influence of Bacterial Translocation in LC

Bacterial translocation from gut to liver worsens not only in patients with chronic liver inflammation and fibrosis, especially those with alcoholic LC and nonalcoholic steatohepatitis (NASH) [52], but also in those with complications that are directly linked to worse LC prognosis, such as hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP) [20].

Chronic intake of alcohol induces LGS, which leads to an increase in serum levels of LPS that activates Kupffer cells, culminating in liver injury [47]. Furthermore, Kupffer cells secrete TNF-α, interleukin (IL)-6, and IL-8, leading to the infiltration of neutrophils into liver tissue [53, 54]. Conversely, obese patients and those with type 2 diabetes exhibit increased serum LPS levels, suggesting that endotoxins might have an important role in the progression of NASH and nonalcoholic fatty liver [55]. A high-fat diet was reported to be associated with an increase in serum endotoxin levels in mice and human [56, 57]. LPS stimulates TLR-4 in adipose and Kupffer cells and leads to an increase in secretion of the inflammatory cytokines, which induce inflammation, fat deposition, and fibrotic changes in liver.

These mechanisms may furthermore contribute to the development and progression of hepatocellular carcinoma [58, 59]. Several studies reported that intestinal gram-positive bacteria were increased in obese individuals by metagenomic analysis [7, 60] and that deoxycolic acid, one of their metabolized products that is also a secondary bile acid, was significantly increased in blood. Deoxycolic acid induces DNA damage by reactive oxygen species [61]; in mice, hepatocellular carcinoma rate was significantly decreased by reduction in blood deoxycolic acid levels [62].

HE is characterized by altered consciousness accompanied by psychological and neurological symptoms in patients with acute or chronic liver dysfunction. HE is triggered by the production of encephalopathy-inducing factors in response to nitrogen compounds originating in the gut [63]. *Proteus, Klebsiella, Pseudomonas*, and *Bacteroides* are important as urease-producing bacteria [64, 65]. As a consequence of SIBO or LGS in patients with LC, these factors gain easy access to the portal vein; their blood levels increase due to the disruption in the clearance capability of liver and formation of the portal-venous shunt that bypasses the liver [66]. Studies reported that gut microbiota in patients with LC and HE was also different from that in healthy individuals [67].

Peritonitis-like symptoms such as fever and abdominal pain with unknown etiology were reported in patients with decompensated LC and clinically evident ascites. Previous studies reported high mortality rates of 16–23% in these cases and suggested that peritonitis might also arise from bacterial translocation due to a breakdown in the defense mechanism of gut in these patients [68, 69].

Hepatorenal syndrome is characterized by progressive renal failure complicated by decompensated LC. SBP in patients with LC with ascites is associated with high risk for hepatorenal syndrome. Poorly absorbable antibiotics, such as rifaximin, were reported to reduce acute kidney injury and hepatorenal syndrome [70]. The relationship between bacterial translocation and general complications is illustrated in Fig. 7.3.

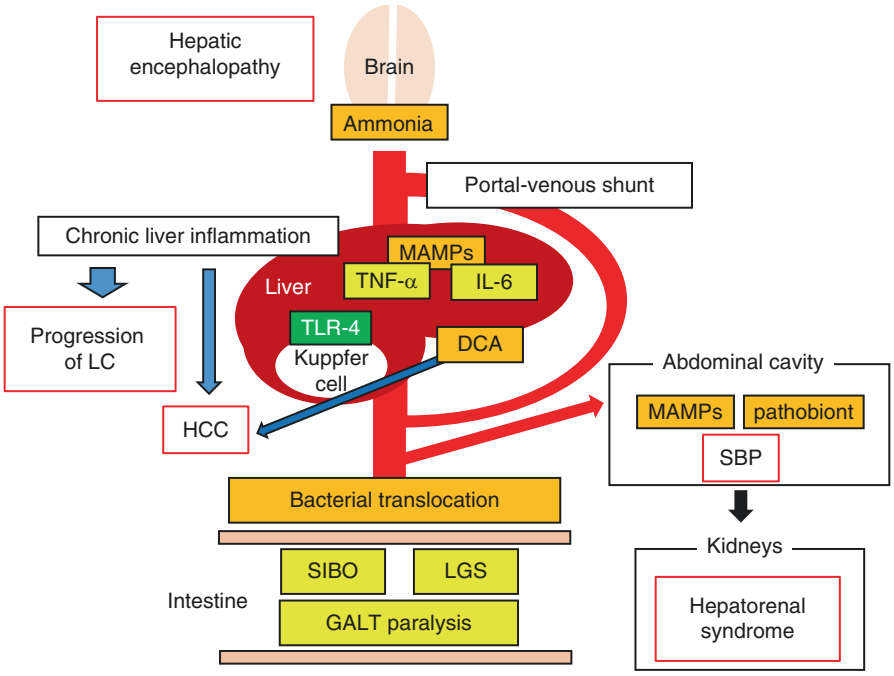

Fig. 7.3 Bacterial translocation and general complications. *LC* liver cirrhosis, *HCC* hepatocellular carcinoma, *MAMP* microbe-associated molecular pattern, *TNF* tumor necrosis factor, *IL* interleukin, *TLR* toll-like receptor, *DCA* deoxycolic acid, *SBP* spontaneous bacterial peritonitis, *SIBO* small intestine bacterial overgrowth, *LGS* leaky gut syndrome, *GALT* gut-associated lymphoid tissue

7.5 Treatment Interventions for Dysbiosis

Poorly absorbable antibiotics, probiotics, prebiotics, synbiotics, and fecal microbiota transplantation (FMT) were evaluated as treatment interventions for dysbiosis in patients with LC.

Poorly absorbable oral antibiotics aim to inhibit pathobionts that produce endogenous ammonia. Neomycin, kanamycin, vancomycin, metronidazole, and rifaximin are used; rifaximin has been increasingly preferred due to fewer side effects owing to the low absorbance ratio of <0.4% in gut [71]. Efficacy of rifaximin was reported not only in HE [72] but also in SBP and hepatorenal syndrome [73, 74]. Rifaximin reduced *Veillonella* and *Streptococcus* without significantly affecting the gut microbiome diversity [75]. Although *Clostridium difficile* infection has been reported during rifaximin administration, the incidence rate of the infection was not increased in patients with LC who were not administered rifaximin [76].

Probiotics are live bacteria that are utilized for correcting the composition of gut flora or for their beneficial effects. Most commonly used probiotics are *Lactobacillus* and *Bifidobacterium*. Specifically, VSL#3, composed of eight strains that has been the most studied probiotic, was found to reduce HE and to improve liver function in randomized control trials [77, 78]. Moreover, prebiotics such as oligosaccharides, poorly digestible starch, and dietary fiber, which are poorly digestible dietary constituents that encourage the propagation of beneficial bacteria and the suppression of harmful bacteria, were previously used for the treatment of HE. Lactulose, the representative synthetic disaccharide, was shown to improve HE by reducing blood ammonia levels [73, 79]. Furthermore, synbiotics that are a combination of prebiotics and probiotics were shown to inhibit the recurrence of overt HE by reducing blood ammonia levels and increasing *Lactobacillus* in gut flora [80].

FMT is aimed at rectifying abnormal gut microbiota by introduction of fecal material from healthy donors to the gut of the patient. Candidates for FMT donors are relatives, partners, friends, and healthy volunteers. Although the risk of unexpected infections is low by FMT from relatives or partners because of similar lifestyle and environment, the efficacy of FMT is also low due to the closely related gut flora. Indeed, FMT from healthy volunteers has been reported to be more efficient than that from relatives, and fecal material from healthy volunteers with strict examination for the exclusion of pathogenic bacteria and viruses is recommended [81]. FMT was found to improve dysbiosis and reduce recurrent HE in a randomized control trial [82].

Finally, although not utilized for direct treatment of gut bacteria, obeticholic acid, an FXR agonist, was found to improve liver fibrosis in patients with NASH [83]. Therapeutic alternatives for dysbiosis are summarized in Table 7.1.

Table 7.1 Summary of treatment intervention for dysbiosis

Treatment	Functional mechanism or active ingredient	Therapeutic targets	Major side effects
Poorly absorbable antibiotics	Inhibit urease-producing bacteria	HE, SBP, hepatorenal syndrome	Pseudomembranous enterocolitis
Probiotics	Living beneficial bacteria	HE, Child–Pugh score	Not significant
Prebiotics	A poorly digestible dietary constituent	HE	Diarrhea, distaste
Synbiotics	A combination of prebiotics and probiotics	HE, Child–Pugh score	Not significant
Fecal microbiota transplantation	Microbiota transplantation from healthy control	HE	Unexpected infections
Obeticholic acid	FXR agonist	Liver fibrosis	Pruritus

HE hepatic encephalopathy, *SBP* spontaneous bacterial peritonitis, *FXR* farnesoid X receptor

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Chapter 8
Hepatic Encephalopathy in Liver Cirrhosis

Yasuhiro Takikawa, Takuro Sato, and Keisuke Kakisaka

Abstract Hepatic encephalopathy (HE) is the most severe and often fatal complication of liver cirrhosis. Intestine-derived neurotoxic substances, such as ammonia, are believed to be the cause of HE. As the results from hepatocellular dysfunction or portosystemic shunt associated with liver cirrhosis, those toxins, which should be detoxified in the liver, flow into the systemic circulation and perturb the brain function. The symptoms of HE include consciousness disorders that range from mild disorders, such as minimal hepatic encephalopathy, to severe disorders that result in deep coma. HE associated with liver cirrhosis develops when some additional triggers overlie liver failure or portosystemic shunt, such as constipation, a high-protein diet, and gastrointestinal bleeding, which are the therapeutic targets of medical treatment. Synthetic disaccharides and rifaximin are used to suppress intestinal ammonia production, and BCAA and zinc are used to support ammonia detoxification in the liver or muscle.

Keywords Ammonia · Portosystemic shunt · Minimal hepatic encephalopathy (MHE) · Quantitative neuropsychological tests · Branched-chain amino acids (BCAAs) · Synthetic disaccharides · Rifaximin

8.1 The Concept and Classification of Hepatic Encephalopathy (HE)

Hepatic encephalopathy (HE) is among the most severe complications of liver cirrhosis, which also include jaundice and ascites. The diagnosis of HE is sufficient for making diagnosis of liver failure.

HE is a neuropsychiatric symptoms caused by brain toxins, which escaped from the hepatic detoxification system and increased in systemic circulation due to

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Table 8.1 Clinical classification of hepatic encephalopathy

Type	Grade		Tie course	Spontaneous or precipitated
A (Acute) type B (Bypass) type C (Cirrhosis) type	MHE	Covert	Episodic Recurrent Persistent	Spontaneous[a] Precipitated (specify)
	1			
	2	Overt		
	3			
	4			

[a]Without recognized precipitating factors

hepatic failure, portal-systemic shunt, or congenital insufficiency of the urea cycle. The symptoms include consciousness disorders that range from mild disorders to severe disorders that result in deep coma. HE is largely classified into three types: type A or HE associated with acute liver failure (ALF) with coma; type B or HE associated with portal-systemic shunts without intrinsic hepatocellular disease; and type C or HE associated with cirrhosis and portal hypertension/or portal-systemic shunts. HE is further classified based on the severity of clinical manifestations (described below), time course, and whether or not a precipitating factor as shown in Table 8.1 [1]. In the grade of HE, minimal HE (MHE) is defined as a state with subtle abnormalities that are only detected by the use of specific neuropsychometric and/or neurophysiological tools [2] in cirrhosis patients with otherwise normal neurological examination results. MHE is clinically indicated by a lack of awareness and cognitive impairment, and has been reported to be observed as a complication in approximately 30% of cirrhosis patients [3]. MHE can be recognized as a stage prior to overt encephalopathy based on a report that overt HE corresponding to grade II or higher occurred in 23% of cases within 6 months of the initial diagnosis of MHE [4], although it has not yet been determined. The diagnostic significance of MHE is that it is associated with a decline in quality of life (QOL) [5], impairment of driving skills [6], and the poor prognosis in cirrhosis patients [7].

8.2 The Pathogenic Mechanism of HE in Liver Cirrhosis

As mentioned above, toxic substances derived from the intestinal tract are believed to be the greatest pathogenic factor. With hepatic failure- or portosystemic shunt-associated liver cirrhosis, it is believed that toxic substances that are derived from intestinal tract and which should be detoxified in the liver flow into the systemic circulation and pass the blood–brain barrier (BBB), and thereby perturb the brain function (Fig. 8.1).

Ammonia is a neurotoxic substance that is derived from the intestinal tract. In addition to direct neurotoxicity, ammonia is considered to cause a range of conditions including cerebral edema, pseudo-neurotransmitter, and amino acid imbalance, and abnormalities in GABA and benzodiazepine (BZ) receptor complex, which work in combination, leading to the onset of encephalopathy.

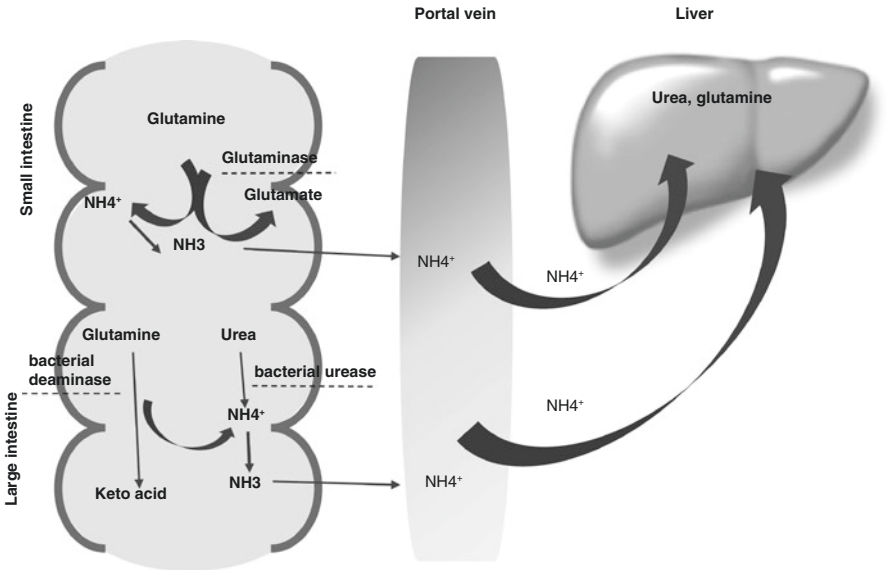

Fig. 8.1 Ammonia metabolism in the intestinal tract with the portal vein and liver. Ammonia is constantly generated in the intestinal tract through the hydrolysis of glutamine or urea by human and bacterial glutaminase and by bacterial urease, respectively. The generated ammonia is absorbed in the portal vein and transferred to the liver

Besides the simple diffusion of ammonia through the BBB, increased vascular permeability caused by inflammatory cytokines is considered to be implicated in the increase in ammonia inside brain tissue [8].

8.2.1 The Neurotoxicity of Ammonia

8.2.1.1 Decline in Glutamate, an Excitatory Neurotransmitter

Ammonia inside the brain is metabolized by the conversion of glutamate into glutamine by glutamine synthase in astrocytes followed by a decline in glutamate (Fig. 8.2). Because the glutamate is an excitatory synaptic transmitter, the decline in glutamate associated with ammonia processing is considered to be the primary causes of encephalopathy.

On the other hand, the glutamine level increases inside the brain particularly in astrocytes as a result of ammonia detoxification in the brain, which has been clinically confirmed in patients with minimal HE by a magnetic resonance spectroscopy [9]. Because the glutamine has a strong osmotic pressure effect, the increase in the glutamine level leads to a brain edema and astrocyte swelling and then irreversible morphological degeneration of astrocytes called Alzheimer-type II degeneration [10].

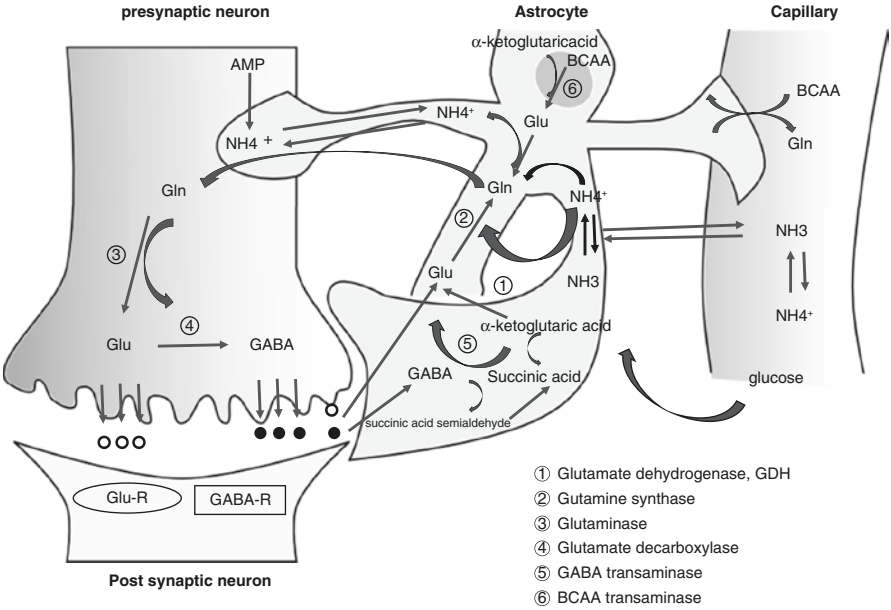

Fig. 8.2 Ammonia metabolism in the brain. Glutamate (Glu) is derived from glutamine (Gln) by glutaminase in the presynaptic neurocytes and acts as an important excitatory neurotransmitter. With excess ammonia (NH_3), Glu is depleted via an ammonia-detoxification mechanism by glutamine synthetase in astrocytes

8.2.1.2 Disturbance of the Energy Metabolism of Brain

It has been proven by both experimental and clinical studies that hyperammonemia inhibits energy metabolism and glucose metabolism inside the brain [11, 12]. This pathological mechanism tends to be especially evident in the reticular activating system and cerebral cortex, and is believed to be involved in consciousness disorders and higher cortical dysfunction in HE.

Regarding the ammonia-detoxification system in brain, a mechanism has been considered in which α-ketoglutarate, which is the matrix of the tricarboxylic acid (TCA) cycle, becomes amidated to process excessive ammonia, which is converted to glutamate and glutamine, thereby decreasing the substrates of the TCA cycle and decreasing the production of ATP (Fig. 8.3) [13, 14].

8.2.1.3 Amino Acid and Neurotransmitter Imbalance

During chronic hyperammonemia, branched-chain amino acids (BCAAs) are consumed by the muscles via the metabolism of ammonia; as a result, an imbalance of amino acids in the blood, in particular, decreased levels of BCAAs and increased levels of aromatic amino acids (AAAs), is observed [15]. Because

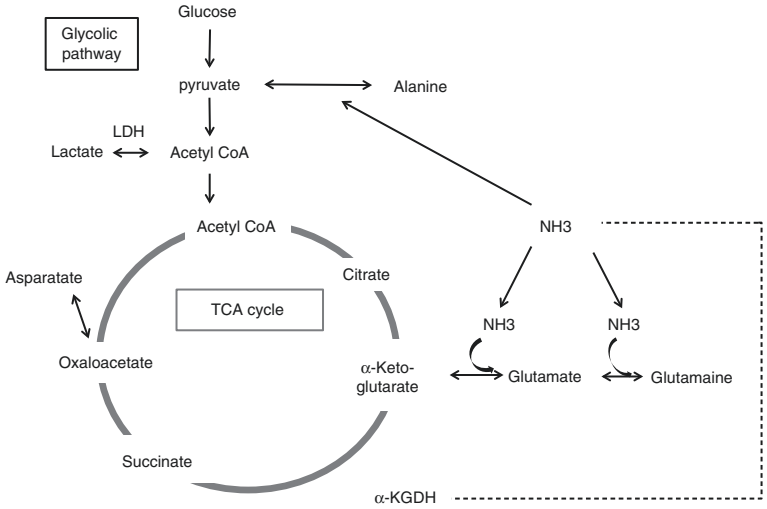

Fig. 8.3 The potential effect of ammonia on glucose metabolism. A schematic representation of the potential effects of ammonia on glucose metabolism. Ammonia stimulates amidation by glutamine synthetase and possibly α-ketoglutarate (solid arrows) leading to the increased synthesis of glutamine. Ammonia has also been shown to inhibit the enzyme α-ketoglutarate dehydrogenase α-KGDH (dashed line) and to enhance the activity of glycolytic enzymes

the migration of AAAs in the brain via the BBB competes the migration of BCAAs, it is hypothesized that excessive AAAs migrates inside the brain during chronic hyperammonemia. As a result, the presence of excessive AAAs inside the brain inhibits the generation of norepinephrine, dopamine, and other catecholamines (which are neurotransmitters), and instead promotes the production of β-hydroxide sympathomimetic amines (pseudo-neurotransmitters), such as octopamine, β-phenylethanolamine, and serotonin. This process is believed to be one of the causes of HE.

Several agonists of the GABA-benzodiazepine (BZ) receptor including GABA, BZ themselves, a similar substance, and neurosteroids are known to increase under hyperammonemia [16]. The GABA-BZ receptor is linked to Cl^- channel and its agonists open the channel, which generates an inhibitory postsynaptic potential. Thus the increase in the receptor agonists is considered to be one of the causes of HE: however, the precise through which this occurs has not been clarified.

8.3 Diagnosis and Grade

In Japan, mental state diagnoses are made based on the Inuyama classification system. In contrast, Western countries base their diagnoses on the West Haven criteria (Table 8.2). In the clinical setting, however, it is difficult to diagnose and differentiate

Table 8.2 The West Haven criteria for the semi-quantitative grading of the mental state of hepatic encephalopathy

Grade 0	No abnormality detected
Grade I	Trivial lack of awareness
	Euphoria or anxiety
	Shortened attention span
	Impaired performance of addition and subtraction
Grade II	Lethargy or apathy
	Minimal disorientation for time or place
	Subtle personality change
	Inappropriate behavior
Grade III	Somnolence to semi-stupor, but responsive to verbal stimuli
	Confusion
	Gross disorientation
Grade IV	Coma (unresponsive to verbal or noxious stimuli)

Table 8.3 Suggested modifications of the West Haven criteria for the grading of the mental state of patients with cirrhosis

Grade	Proposed operative definition
I	• Not able to complete TMT-A *a* in 120 s (individuals with ≥5 years of education), or naming ≤7 animals in 120 s
	• Orientated in time and space
II	• Disorientated in time: (≥3 items incorrect)
	– Day of the week
	– Day of the month
	– The month
	– The year
	• Orientated in place
III	• Disorientated in place: (≥2 items incorrect), and
	– State/country
	– Region/county
	– City
	– Place
	– Floor/ward
	• Disorientated in time, and reduction of Glasgow score (8–14)
IV	• Coma, unable to test mental state
	• Unresponsive to pain stimuli (Glasgow score <8)

grade 0–I. Amodio et al. suggested a method using the West Haven criteria with a quantitative neuropsychological tests to diagnose grades I–IV [17, 18] (Table 8.3).

The following criteria are said to be required for an accurate diagnosis of MHE: (1) an evaluation of the QOL, including changes in the behavioral patterns of daily life, such as appetite, sleep, and physical activity; (2) an evaluation of the mental

Table 8.4 Clinical examinations for the diagnosis of minimal hepatic encephalopathy

• Quantitative neuropsychological tests
Wechsler adult intelligence scale (WAIS)
Number connection test A (NCT-A), number connection test B (NCT-B)
Digit symbol test (DST), block design test (BDT), etc.
• Electrophysiology tests
Electroencephalogram (EEG)
Evoked potentials (EP), P300 wave
Flicker frequency test (CFF test)
• Non-invasive brain functional imaging and spectroscopy
Magnetic resonance imaging (MRI, functional MRI)
Magnetic resonance spectroscopy (MRS)
Positron-emission tomography (PET)

state such as memory, concentration, cognition, and consciousness; (3) quantitative neuropsychological testing; and (4) the presence of speech disorders, such as lisp, along with an evaluation of cognitive activity disorders, such as increased reaction time, and disordered spatial recognition [17]. However, it is difficult to perform such a comprehensive diagnosis in everyday clinical practice: thus, the early objective diagnosis of slight abnormalities in neurological function is generally attempted with a combination of quantitative neuropsychological tests (Table 8.4). Moreover, attempts have been made to use 3.0 T magnetic resonance imaging (MRI) and MRS for the minimally invasive detection of metabolic disturbances in the brain based on an increase in glutamine and a decline in myo-inositol in the brain [9].

8.4 Treatment

The fundamental causes of HE including acute or chronic hepatocyte dysfunction and portosystemic shunts are not easily resolved by medical therapy alone. Chronic HE develops when some additional triggers overlie these fundamental causes, such as constipation, a high-protein diet, and gastrointestinal bleeding, which are the therapeutic targets of medical treatment.

Moreover, while there is room for discussion regarding the choice of active therapeutic intervention with respect to MHE, when cases are believed to be in the stage prior to overt encephalopathy, it is important that any improvements are carefully considered if hyperammonemia is observed. The principal therapies for hyperammonemia in liver cirrhosis are listed in Table 8.5 according to their mechanism of action.

Table 8.5 Principal treatments for hyperammonemia and hepatic encephalopathy in patients with liver cirrhosis

Suppression of synthesis and absorption of ammonia in intestine 1. Suppression of the protein intake and BCAA supplementation 2. Osmotic diarrhea and a reduced pH in the intestine: Synthetic disaccharides (lactulose and lactitol) 3. Suppression of urease-positive intestinal bacteria: Minimally absorbed antibiotics (rifaximin), probiotics
Assist in ammonia detoxification 1. Suppression of the increase in pseudo-neurotransmitters inside brain: BCAA supplementation 2. Assist in ammonia detoxification in muscle: Zinc preparations, BCAA preparations 3. Activation of ammonia detoxification in liver: Carnitine preparations, zinc preparations

BCAA branched-chain amino acid

8.4.1 Synthetic Disaccharides (Lactulose and Lactitol)

Synthetic disaccharides are administered with the objective of inhibiting the production and absorption of ammonia in the intestinal tract [19]. Synthetic disaccharides are led to the large intestine without being absorbed by the small intestine. Due to β-galactosidase, which is produced by enteric bacteria, lactulose is hydrolyzed to galactose and fructose, while lactitol is hydrolyzed to galactose and sorbitol, ultimately becoming organic acids (e.g., lactic acid, acetic acid). The following factors are believed to play a role in the decline of ammonia: (1) the inhibited growth of urease-producing bacteria accompanying the decrease in pH in the large intestine that occurs due to the production of organic acids; (2) the ionization of ammonia (NH_3) to NH_4^+ due to the decreased pH in the large intestine, which results in decreased absorption from the intestinal tract membrane; and (3) the promotion of intestinal movement due to the production of organic acids and the increase in water content, and the shortening of the transit time of the intestinal tract content due to osmotic diarrhea.

8.4.2 Minimally Absorbed Antibiotics

Minimally absorbed antibiotics are administered with the objective of inhibiting the growth of gram-negative bacillus, which is an ammonia-producing bacterium that is found inside the intestinal tract, along with inhibiting the breakdown of urea and glutamine in the intestine.

Rifaximin, a rifamycin antimicrobial agent, is used worldwide for the treatment of HE. It has been shown to achieve a greater [20] or equal [21] improvement of HE and hyperammonemia in comparison to lactitol. Besides the effects on the decrease of the circulating ammonia level and the HE grade, rifaximin showed

beneficial effects in patients with liver cirrhosis, improved overall survival in liver cirrhosis patients with a Child–Pugh score of ≥7 [22], and prevented the recurrence of HE [23].

Moreover, when rifaximin is administered, <0.4% is absorbed from the gastrointestinal tract into the blood, and it is associated with few side effects [24]. Based on these findings, rifaximin was officially approved in Japan in 2016 with an indication for the improvement of hyperammonemia in patients with hepatic encephalopathy.

8.4.3 BCAA

Ammonia is metabolized in the process of generating glutamine from α-ketoglutarate and glutamate in the brain and skeletal muscles. This results in BCAA becoming conjugatively oxidized. Thus, in patients with cirrhosis and hyperammonemia, a vicious cycle occurs in which the serum BCAA concentration declines due to the promotion of BCAA consumption, thereby encouraging hyperammonemia. Furthermore, a decline in serum BCAA promotes the transition of AAA inside the brain, where it causes an imbalance of amino acids, leading to an increase in the levels of pseudo-neurotransmitters. For these reasons, HE is treated with the oral and intravenous administration of a BCAA preparation.

The clinical effect of oral BCAA supplementation on HE prevention has been confirmed by a meta-analysis [25]. Besides the treatment of HE, BCAA supplementation also provides nutritional support for patients who also require protein restriction. Indeed, it is reported that oral BCAA supplementation improved the event-free survival of liver cirrhosis patients [26].

8.4.4 Zinc

Ornithine transcarbamylase (OTC) is a zinc enzyme in the urea cycle in the liver. Zinc is also involved in maintaining ammonia metabolism, through its promotion of the activation of glutamine synthase in the skeletal muscles. Most of the ammonia that migrate inside the liver is processed via the urea cycle, with residual ammonia in the blood processed by glutamine synthase. It is known that zinc is depleted during cirrhosis and that ammonia metabolism declines due to the reduced activity of the two enzymes. There are reports [27] that suggest that encephalopathy is improved by the oral administration of zinc acetate or zinc sulfate preparations, along with reports [28] that indicate that the concomitant administration of synthetic disaccharides (as mentioned above) is effective in combination with BCAA granules.

8.4.5 Probiotics

The usefulness of probiotic preparations on MHE has been reported [29], with improvements in the neuropsychiatric function and ammonia concentration in the blood observed in patients who underwent treatment with a probiotic preparation alone and patients who used probiotics in combination with lactulose. Probiotics affect the intestinal flora inside the intestinal tract, causing a decline in the pathogenesis of urease-producing bacteria, and thereby decreasing the production of toxic substances that are derived from the intestinal tract, in particular, ammonia.

8.4.6 Carnitine

Carnitine (CA) is a vitamin-like substance that plays an essential role in supplying fatty acids (long chain) inside the mitochondria for beta-oxidation. Cirrhosis is considered to be a state of CA deficiency because the absolute intake is insufficient due to a protein-restricted diet, reduced biosynthesis due to hepatocellular dysfunction, and reduced internal storage due to a reduced skeletal muscle mass. The metabolic disturbance of CA is related to disorders in the urea cycle and TCA cycle in the mitochondria; such a decline may therefore ultimately lead to hyperammonemia. Malaguamera et al. [30] performed a randomized, controlled, double-blind trial on HE patients and observed a significant improvement in the blood ammonia concentration along with improvement in the cognitive function of 88% of patients who received CA.

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Chapter 9
Hepatic Ascites in Liver Cirrhosis

Hideto Kawaratani and Hitoshi Yoshiji

Abstract Common complications of decompensated liver cirrhosis include esophageal varices, hepatic encephalopathy, and ascites. They are associated with a poor prognosis and quality of life. The 5-year mortality rate of patients with ascites is 44%. A decrease in ascites improves the quality of life and survival. A newer diuretic tolvaptan (a vasopressin V2 receptor antagonist) has been found to be effective in treating hepatic ascites, but there is as yet little evidence of its effect on prognosis. Other treatments for ascites include large-volume paracentesis, cell-free and concentrated ascites reinfusion therapy, and transjugular intrahepatic portosystemic or peritoneovenous shunts. Although these measure may improve quality of life, liver transplantation remains the only curative form of treatment. This paper discusses the therapeutic management of cirrhotic ascites according to Japanese guidelines.

Keywords Ascites · Portal hypertension · Vasopressin receptor antagonists Paracentesis · Peritoneovenous shunt · Transjugular intrahepatic portosystemic shunt

9.1 Introduction

Ascites is the pathologic accumulation of fluid in the peritoneal cavity. It is the most common complication of liver cirrhosis. Over 50% of patients develop ascites within 10 years of a diagnosis of liver cirrhosis [1], with 15% having ascites within 1 year and 44% by 5 years [2]. Additional conditions often associated with ascites, such as hyponatremia, spontaneous bacterial peritonitis (SBP), and hepatorenal syndrome (HRS), further worsen the prognosis. Therefore, successful treatment of ascites is needed. In this paper, we provide an overview of the management of hepatic ascites and its various complications according to Japanese guidelines.

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9.2 Diagnosis of Ascites

Ascites is diagnosed in the presence of more than 25 mL of fluid in the peritoneal cavity. Symptoms may include increased abdominal circumference, weight gain, ankle edema, abdominal discomfort, and shortness of breath. The most common cause of ascites is liver cirrhosis, although it can be caused by cancer, heart failure, tuberculosis, pancreatitis, or hepatic vein obstruction. Diagnostic abdominal paracentesis is performed to determine the underlying cause. Paracentesis is considered even in patients with a low platelet count and prolonged prothrombin time. The overall complication rate of the procedure does not exceed 1% [3], with severe complications (e.g., bowel perforation or hemorrhage) occurring in <0.1% [4]. Therefore, prophylactic administration of platelets or fresh frozen plasma before paracentesis is not recommended [5].

9.3 Evaluation of Ascitic Fluid

Evaluation of the fluid helps to determine the underlying cause of the ascites. The fluid may appear clear, pale-yellow, cloudy, bloody, or chylous. Clear or pale-yellow ascites usually indicates a transudate; cloudy fluid suggests infection; bloody fluid may be associated with malignancy; and chylous fluid suggests trauma, postoperative effects, or malignancy. The initial laboratory investigation of ascites should include a cell count and both serum and ascitic fluid levels of total protein, albumin, and lactate dehydrogenase (LDH). Ratios of ascites-to-serum total protein >0.5 and LDH >0.6 are suggestive of an exudate. The serum-ascites albumin gradient (SAAG), calculated by subtracting the ascites albumin level from the serum albumin level, is more useful than other values for correctly diagnosing the cause of ascites. A SAAG ≥1.1 g/dL indicates portal hypertension with an accuracy of approximately 97% [6]. Patients who have portal hypertension but also a second cause of ascites still generally have a SAAG ≥1.1 g/dL. This measure retains its diagnostic accuracy even in the face of fluid infusions and use of diuretics [7]. On the other hand, a SAAG <1.1 g/dL suggests the ascites arises from a cause other than portal hypertension [8].

9.4 Treatment of Ascites

Ascites is categorized in three grades. Grade 1 is mild, with fluid visible only on ultrasound or computed tomography; grade 2 indicates fluid apparent on physical examination with flank bulging and shifting dullness; and grade 3 includes a visibly distended abdomen with a positive fluid wave. According to American

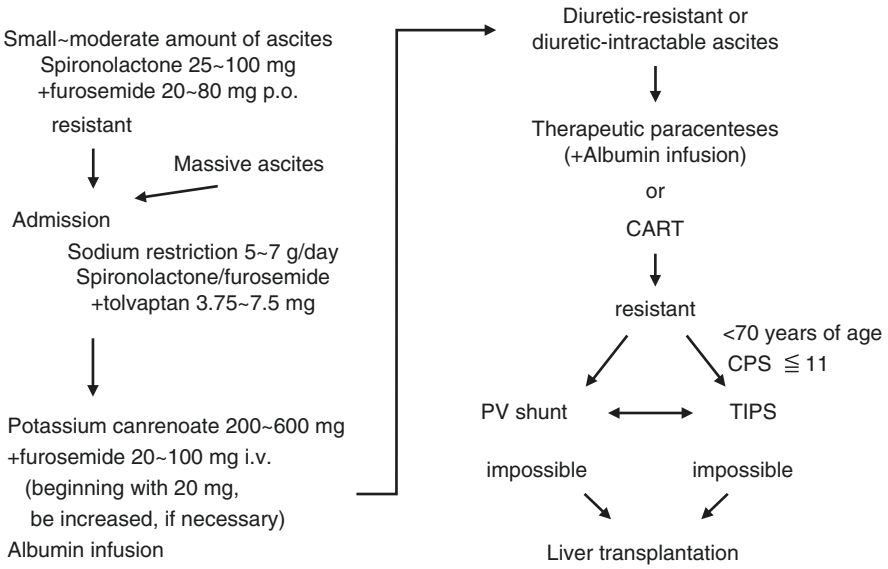

Fig. 9.1 Treatment algorithm of patients with ascites. *CART* cell-free and concentrated ascites reinfusion therapy, *CPS* Child–Pugh score, *PV shunt* peritoneovenous shunt, *TIPS* transjugular intrahepatic portosystemic shunt. Modified from reference [11]

guidelines, no treatment is recommended for grade 1 ascites. Grade 2 is managed with sodium restriction and diuretics. For grade 3 ascites, large-volume paracentesis (LVP) is performed, followed by sodium restriction and diuretics (except for refractory ascites) [9]. The Japanese guidelines, however, advise administering diuretics even for grade 1 ascites. Refractory ascites is defined as "ascites that cannot be mobilized or the early recurrence of which (i.e. after paracentesis) cannot be satisfactorily prevented by sodium restriction and diuretic treatment [10]." Figure 9.1 shows the treatment algorithm for ascites modified from the Japanese guidelines [11].

9.4.1 Sodium Restriction

Previously, strict sodium restriction (less than 5 g/day of NaCl) was recommended. However, recently, the Japanese guidelines have recommended milder restriction (5–7 g/day of NaCl). The stricter limit may worsen malnutrition in patients with cirrhosis. Water restriction was also recommended in the past, but there is now controversy about this measure in the treatment of ascites. Fluid intake can rarely be restricted to <1 L/day, an amount insufficient to reduce the ascites [12]. The effect of water restriction may also depend on the level of hyponatremia [13].

9.4.2 Diuretics, Including Vaptans

In patients with cirrhosis, the renin–angiotensin–aldosterone system (RAAS) is activated, resulting in hyperaldosteronism. Therefore, the first-line diuretics to treat ascites are aldosterone antagonist, such as spironolactone. The American guidelines recommend an initial spironolactone dose of 50–100 mg, gradually increasing to 400 mg until the ascites is resolved. However, Japanese patients are usually intolerant to high doses of diuretics because they result in dehydration or hyponatremia. In such cases, the patient may have diuretic-intractable ascites. Therefore, Japanese clinical guidelines recommend an initial dose of spironolactone of 25–50 mg, increasing to a maximum of 100 mg. If the response to spironolactone is insufficient by 2 weeks after initiation, a loop diuretic such as furosemide (20–40 mg/day) is added, with the dose increased as needed to 80 mg. Monotherapy with furosemide is less effective than spironolactone and is not recommended [14].

Tolvaptan is a newly introduced vasopressin V2-receptor antagonist, a class of drugs called vaptans. The use of tolvaptan has drastically changed the approach to ascites treatment. Japanese guidelines recommend that, in cases of ascites persisting despite conventional diuretics (25–100 mg/day of spironolactone and 20–80 mg/day of furosemide), 3.75–7.5 mg/day of tolvaptan should be administered. More recently, tolvaptan has been used even earlier, without waiting until maximal doses of spironolactone and furosemide are reached (START study) [15]. Tolvaptan was not found to be superior to placebo in terms of long-term survival in patients with heart failure (EVEREST study) [16]. Some studies have demonstrated the safety and efficacy of intermediate- and long-term tolvaptan treatment for decompensated cirrhosis [17, 18]. However, there is little evidence concerning long-term safety and management of hyponatremia in patients with refractory ascites or diuretic intolerance. Tolvaptan is known to have a protective effect on renal function compared with conventional diuretics. Early introduction of tolvaptan to treat ascites may be effective before renal function deteriorates.

9.4.3 Albumin Supplementation

Serum albumin is a nonglycosylated negatively charged plasma protein that helps to maintain colloid osmotic pressure. It also has ligand-binding, antioxidant, free-radical scavenging, and anti-inflammatory properties. It maintains vascular integrity and modulates neutrophil function. Albumin increases the response to diuretics and prevents paracentesis-induced circulatory disturbance [19]. Japanese guidelines suggest that patients with ascites resistant to tolvaptan be treated with intravenous potassium canrenoate (200–600 mg) and furosemide (20–100 mg) along with albumin. However, the high cost of albumin is a concern. Compared with saline or other plasma expanders, albumin has been shown to reduce mortality and morbidity in patients with massive ascites undergoing LVP [20]. Moreover, albumin reduces the incidence of type 1 HRS in patients with SBP who are receiving antibiotics [9].

9.4.4 Other Drugs

Non-selective β-blockers (NSBBs) are known to reduce portal vein pressure in patients with liver cirrhosis. Lebrec et al. reported that NSBBs effectively prevented recurrent bleeding from esophageal varices in patients with cirrhosis [21]. However, in 2010, Serste et al. reported that NSBBs may be associated with poor survival in patients with refractory ascites [22]. Thus, the use of NSBBs is controversial, requiring further studies to clarify the issue. Angiotensin receptor blockers also reduce portal vein pressure in patients with cirrhosis, but they have not been directly compared with NSBBs.

Midodrine, a potent peripherally acting α1-adrenergic receptor agonist, increases effective arterial blood volume by causing splanchnic vasoconstriction. Oral midodrine has been shown to increase urine volume, urine sodium, arterial pressure, and survival in patients with refractory ascites [23]. Midodrine can be added to diuretics to increase blood pressure and restore sensitivity to diuretics [24].

Clonidine, an α2-adrenergic receptor agonist, has sympathoinhibitory effects and suppresses RAAS in patients with liver cirrhosis [25]. Clonidine augments the effect of spironolactone, facilitating an earlier diuretic response with lower diuretic dose requirements and fewer complications [26].

Terlipressin is a synthetic vasopressin analog that improves renal sodium excretion by enhancing renal perfusion. This agent increases V1A receptor affinity and decreases V2 receptor affinity. The V1A receptor causes vasoconstriction in the splanchnic vessels, redistributing systemic blood flow, maintaining blood pressure, and lowering portal pressure. Terlipressin increases sodium excretion and decreases plasma renin activity in patients with cirrhosis. The combination of terlipressin and albumin led to better control of refractory ascites than diuretics plus albumin [27]. However, it has not yet been approved in Japan or the USA. Recently, vasopressin 1a receptor partial agonist (FE204038) has been developed. It increases sodium excretion, and reduces portal hypertension and ascites in a cirrhotic rodent model [28]. It would be a useful drug for managing decompensated patients with cirrhosis.

9.4.5 Paracentesis

Therapeutic paracentesis is a fast, safe, and effective therapy for hepatic ascites. It reduces intra-abdominal, intrathoracic, pulmonary, and portal pressures without causing renal or hepatic dysfunction. Side effects seen with diuretics, such as hyponatremia, acute kidney injury, and hepatic encephalopathy, are significantly less frequent with paracentesis. LVP is performed in patients with massive ascites to minimize the number of paracenteses needed. LVP significantly alters the systemic circulation, with an acute increase in cardiac output and a reduction in systemic vascular resistance and arterial blood pressure [29, 30]. During LVP, an albumin

infusion of 6–8 g per liter of fluid removed is recommended. A meta-analysis demonstrated reduced mortality when albumin was used [20]. However, some investigators have suggested that 4 g of albumin per liter of fluid removed may be adequate to maintain circulating plasma volume [31]. Albumin is superior to other plasma expanders during paracentesis. However, paracentesis does not improve the prognosis in cirrhosis [32].

9.4.6 Cell-Free and Concentrated Ascites Reinfusion Therapy

Cell-free and concentrated ascites reinfusion therapy (CART) has been developed in Japan for patients with massive ascites due to liver cirrhosis [33]. It has been proven to be as safe and effective as LVP with albumin infusion [34]. The aim of this therapy is to maintain serum albumin levels by filtering and concentrating ascitic fluid that has been removed and then reinfusing the protein-rich fluid intravenously [33]. CART has been approved as a bi-weekly therapy by the Japanese medical insurance system. The advantages of CART are a reduction in the need for albumin transfusions and a lower risk of infection or allergic reaction. However, CART is difficult to perform in patients with SBP because the filtering and concentrating process may increase the level of endotoxins in the fluid to be reinfused, potentially resulting in fever or shock. A filtration membrane cleaning function was recently added to conventional CART, resulting in better filtering of many cell components. CART is thus also effective in patients with malignant ascites [35]. The risk of worsening renal failure with CART is unlikely. Moreover, a trend toward the stabilization of the sodium concentration during CART has been observed [36]. Studies comparing CART with LVP plus albumin infusion should be conducted.

9.4.7 Peritoneal-Urinary Drainage

The Alfapump® is a battery-operated automated low-flow ascites pump. It is implanted subcutaneously for the treatment of refractory ascites. The Alfapump automatically pumps ascitic fluid from the peritoneal cavity into the urinary bladder [37], thus enabling continuous low-volume paracentesis [37]. It is used for patients who have contraindications to transjugular intrahepatic portosystemic shunt (TIPS) placement or liver transplantation. The amount of ascitic fluid to be removed daily is controlled by a wireless programming system [38]. It is activated every 10–15 min and moves 3–30 mL of ascitic fluid into the bladder in each cycle. The pump is inactivated at night while the patient is asleep [38]. A recent randomized controlled trial demonstrated that, compared with standard LVP treatment, the Alfapump was effective in reducing the need for paracentesis in >50% of patients over 6 months and improving health-related quality of life, especially in the first 3 months [39]. Compared with LVP, the system was associated with improvement in patients'

nutritional status as assessed by body mass index, hand grip strength, triceps skinfold thickness, and midarm muscle circumference. The authors speculated that this nutritional benefit may involve attenuation of an increased resting energy expenditure [40, 41]. On the other hand, it may be associated with enhanced endogenous vasoconstrictor systems and impaired renal function. Continuous ascitic fluid drainage by the Alfapump may impair effective arterial blood volume, an effect mimicking LVP-induced circulatory dysfunction. There are a number of adverse events related to the procedure and the device, such as wound dehiscence, wound infection, abdominal wall hematoma, kinking of the bladder catheter, and pump pocket infection, any of which may require surgery [38, 42]. Due to frequent and serious comorbidities, careful patient selection and postoperative monitoring are required [42].

9.4.8 Peritoneovenous Shunt

The peritoneovenous shunt (PVS) was designed to palliate ascites by reinfusing ascitic fluid into the systemic circulation. It was intended for patients with refractory ascites who were not candidates for transplantation or TIPS. The Denver shunt was developed in the 1970s as a physiologic treatment of ascites [43, 44]. PVS reportedly improved the glomerular filtration rate [45] and the quality of life. This procedure is covered by Japanese medical insurance. Japanese guidelines suggest that contraindications for TIPS include a total bilirubin ≥10 mg/dL, respiratory failure, disseminated intravascular coagulation, SBP, gastrointestinal bleeding, peritoneal adhesions, or untreated risky varices. PVS reportedly achieved earlier control of ascites than TIPS, but long-term efficacy favored TIPS [46]. PVS prolonged the time to recurrence of ascites compared with diuretic treatment [44] and LVP with albumin infusion. However, PVS is seldom used because of poor long-term patency, excessive complications, and the lack of survival advantage over medical therapy [44, 47]. PVS is still reasonable to consider in patients requiring serial paracenteses or in whom paracentesis is difficult because of multiple abdominal scars, as well as not having a physician available who is willing and capable of performing paracenteses. Interventional radiologists have reported that PVS can be performed without the participation of a surgeon [48].

9.4.9 Transjugular Intrahepatic Portosystemic Shunt

TIPS can be used to treat complications of portal hypertension, including ruptured esophageal varices, refractory ascites, hepatic hydrothorax, portal thrombus, and HRS. TIPS is recommended in the Japanese guidelines for patients under 70 years of age with a Child–Pugh score of <12 and ascites refractory to LVP or CART. TIPS reduces the portosystemic pressure gradient by shunting the blood from the portal vein to the hepatic vein in over 90% of cases. It is usually inserted by an

interventional radiologist using local anesthesia [49, 50]. In studies comparing TIPS with LVP, TIPS was associated with better control of ascites but a higher incidence of hepatic encephalopathy and reduced quality of life [51, 52]. Another study reported that TIPS prevented HRS but at a higher financial cost [53]. TIPS was shown to yield good survival with similar hospitalization rates but also with more severe encephalopathy [54]. TIPS with covered stents has been reported to have a better prognosis than LVP [41]. A meta-analysis found significantly better transplant-free survival with TIPS and similar cumulative occurrence of developing hepatic encephalopathy [55].

However, the beneficial effect of TIPS on survival is diminished beyond 1 year [56], possibly because it induces long-lasting cardiac overload [57, 58]. Thus, TIPS should be considered as bridging therapy in patients with refractory ascites who are awaiting liver transplantation [57].

9.4.10 Liver Transplantation

While liver transplantation is the only curative option for refractory ascites [59], many patients are not eligible for the procedure. For this reason, there is continued interest in developing alternative approaches for managing refractory ascites in individuals who cannot undergo successful transplantation.

9.5 Complications

9.5.1 Spontaneous Bacterial Peritonitis (SBP)

SBP was first defined by Kerr et al. by describing the infection of ascitic fluid in the absence of previous antibiotic therapy and an intra-abdominal source of infection [60]. The pathogenesis of SBP is a disruption of the intestinal membrane, resulting in bacterial translocation. For the diagnosis of SBP, culture of ascites and polymorphonuclear leukocyte (PMN) count of ascites are necessary. Positive cultures, ascitic fluid white blood cell count $\geq$500 cells/mm^3, or PMN count $\geq$250 cells/mm^3 are diagnostic of SBP. To detect ascitic bacteria, ascitic fluid should be cultured in aerobic and anaerobic blood culture bottles. Ascitic granulocyte elastase (GE) has been reported of the usefulness in SBP patients [61, 62]. In situ hybridization (ISH) of bacterial DNA in leukocytes of the ascites in SBP patients has high sensitivity and specificity [63]. The mortality rate is higher in patients with culture-positive SBP than in patients with culture-negative SBP [64]. For the treatment of SBP, a third-generation cephalosporin, cefotaxime, is administered until the results of the specific pathogenic bacteria are determined. As the mortality rate increases, empirical therapy should be started as soon as diagnosed. The most common bacteria of SBP are gram-negative bacteria, including *Escherichia coli* and *Klebsiella*

pneumoniae. A third-generation cephalosporin covers over 90% of these bacteria. And oral ofloxacin is considered a substitute for cefotaxime in patients without recent administration of quinolones [65]. Nosocomial infection, long-term norfloxacin prophylaxis, or recent use of β-lactam antibiotics will lead to resistance in bacteria [66]. Infections with resistant bacteria are associated with a higher mortality rate. When the patients of SBP have serum Cr ≥ 1.0 mg/dL, BUN ≥30 mg/dL, or T. Bil ≥ 4.0 mg/dL, 1.5 g per kg body weight of albumin should be administered. Albumin administration reduces the risk of renal dysfunction by improving the effective circulating blood volume and improves the mortality. Primary prophylactic antibiotics (norfloxacin 400 mg/day) decreased the risk of SBP [67], as well as mortality [68]. And rifaximin also reduces the occurrence of SBP [69]. SBP's usual recurrence has been reported to be 69% in 1 year [70]. Once SBP has occurred, patients should be prescribed long-term prophylactic antibiotics, and be considered for liver transplantation [71].

9.5.2 Hepatorenal Syndrome (HRS)

HRS is a type of renal dysfunction that is reversible and occurs as a result of advanced liver disease. The pathophysiology of HRS is a reduction of circulating blood volume due to the increased resistance to blood flow in the cirrhotic liver. The diagnostic criteria of HRS are shown in Table 9.1. HRS is categorized into two types. Type 1 HRS is associated with a doubling of the initial serum creatinine to a level of more than 2.5 mg/dL, or reflecting a 50% reduction in creatinine clearance in less than 2 weeks [72]. Type 2 HRS is defined as renal impairment that is less severe than that observed with type 1 HRS. Type 1 HRS usually occurs following a precipitating factor, such as hyponatremia, gastrointestinal bleeding, bacterial infections, LVP without albumin infusion, or SBP [73]. The most characteristic cause of type 2 HRS is renal vasoconstriction. LVP with plasma expander decreases the incidence of HRS. Albumin infusion decreases the incidence of type 1 HRS better than plasma expanders [74]. Synthetic vasopressin analogs have improved the prognosis during treatment for HRS. Terlipressin is a vasoconstrictive agent that is effective in mesenteric circulation compared to renal and other vascular systems. Combination

Table 9.1 Definition of hepatorenal syndrome (HRS)

1. Cirrhosis with ascites
2. Serum creatinine levels >1.5 mg/dL
3. Absence of hypovolemia as defined by no sustained improvement of renal function following at least 2 days of diuretic withdrawal and treated with albumin at 1 g/kg/day (up to a maximum of 100 g/day)
4. Absence of shock
5. No current or recent treatment with nephrotoxic drugs
6. Absence of parenchymal renal disease such as proteinuria >0.5 g/dL, microhematuria (>50 red cells/high powered field), and abnormal renal ultrasonography

therapy with terlipressin and albumin infusion is the most effective for type 1 HRS [75]. TIPS also improves renal function in patients with type 1 HRS [76] and in type 2 HRS [77], and improves the survival rate. The goal of HRS treatment is to achieve a serum creatinine level below 1.5 mg/dL. And the principle of HRS treatment should be an early diagnosis and quick treatment. Despite all the possible treatments available, mortality rates remain high, especially in type 1 HRS. To reverse HRS, treatment may be extensive and it demonstrated a recurrence in 50% of patients. In cases of recurrence, the same treatment regimen is usually found to be successful. The only definitive treatment of HRS is a liver transplantation. However, HRS is an important risk factor after liver transplantation [78, 79]. The 3-year survival rate after liver transplantation in patients with HRS is 60%; in patients without HRS is 70–80% [80].

9.6 Conclusion

Although invasive treatments (LVP, CART, TIPS, PVS, and liver transplantation) are somewhat useful for decreasing the mortality rate of refractory ascites, the condition is still associated with substantial morbidity and mortality in patients with liver cirrhosis. The selection of the most appropriate therapy for each patient is necessary. While some treatments may improve the prognosis of ascites, once complications occur, the prognosis worsens. The earlier the treatment begins, the better the outcome to be expected.

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Chapter 10
Sarcopenia in Liver Disease

Hiroki Nishikawa and Shuhei Nishiguchi

Abstract Because skeletal muscle is the largest store of proteins in the body, protein homeostasis is essential for the maintenance of skeletal muscle mass. Aging disrupts the balance between protein synthesis and breakdown in skeletal muscle, resulting in muscle strength decline, walking disorders, falls, and other problems. The decreased muscle mass and muscle strength that accompanies aging is defined as primary sarcopenia, while the decreased muscle mass and muscle strength that accompanies an underlying disease is defined as secondary sarcopenia. Several potential biomarkers associated with skeletal muscle mass loss have been reported. The most conceivable mechanism which can cause sarcopenia in patients with liver disease is protein energy malnutrition. Skeletal muscle mass is not only a good indicator of nutrition in patients with liver disease, but also has recently been shown to be closely related to survival in patients with liver disease. In 2016, the Japan Society of Hepatology established its own assessment criteria for sarcopenia in liver disease as the number of liver disease patients with sarcopenia is expected to increase and there is compelling evidence to indicate patients with sarcopenia have unfavorable clinical outcomes, and in subsequent several studies, its usefulness was validated. On the other hand, exercise and branched-chain amino acid supplementation may be recommended in sarcopenic patients with liver disease. Here, in this article, we will summarize the current knowledge of sarcopenia in liver disease.

Keywords Sarcopenia · Liver · Japanese guidelines · Biomarkers · Therapy

Abbreviations

AWGS	Asian Working Group for Sarcopenia
BCAA	Branched-chain amino acid
BIA	Bio-impedance analysis

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CT	Computed tomography
HCC	Hepatocellular carcinoma
JSH	Japan Society of Hepatology
L3	The third lumbar level
LC	Liver cirrhosis
MELD	Model for end-stage liver disease
MMD	Muscle mass decrease
NAFLD	Nonalcoholic fatty liver disease
OS	Overall survival
PEM	Protein energy malnutrition
PMI	Psoas muscle index
PS	Performance status
RCT	Randomized controlled trial

10.1 Introduction

Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and muscle strength, shown to be prevalent in patients with malignancies and common chronic diseases [1, 2]. Protein homeostasis is essential for the maintenance of skeletal muscle mass because skeletal muscle is the largest store of proteins in the body [3, 4]. The decreased muscle mass and muscle strength that accompanies aging is defined as primary sarcopenia, while the decreased muscle mass and muscle strength that accompanies an underlying disease is defined as secondary sarcopenia [1, 2]. Sarcopenia in liver disease reflects protein energy malnutrition (PEM) [5–7]. Although several potential biomarkers associated with skeletal muscle mass loss in liver disease have been reported to date, one of the major reasons for the limited understanding of sarcopenia in liver disease has been the difficulty in identifying the mediators of the liver-muscle axis [6–11]. Skeletal muscle mass has also recently been shown to be closely related to survival in patients with liver disease [12–20]. However, it seems that the consequences can vary according to the definition of sarcopenia. In 2016, the Japan Society of Hepatology (JSH) established its own assessment criteria for sarcopenia in liver disease as the number of liver disease patients with sarcopenia is expected to increase and there is compelling evidence to show patients with sarcopenia have poor clinical outcomes, and in subsequent several studies, its usefulness has been validated [8, 21–23]. Although sarcopenia is one of the most frequent complications associated with survival for LC patients, the newly proposed prognostic models lack an evaluation of nutritional status for LC patients. This is reflected by the lack of an optimal index for sarcopenia in terms of objectivity, reproducibility, and practicality. Quantifying skeletal muscle mass using cross-sectional abdominal imaging such as computed tomography (CT) is a useful tool for the assessment of sarcopenia. The JSH guidelines were created after full consideration of these points. On the other hand, exercise and branched-chain amino acid supplementation showed promising results in

sarcopenic patients with liver disease [24–31]. It may be clear that untreated sarcopenia can be associated with suboptimal clinical outcomes. Considering these backgrounds, in this article, we will summarize the current knowledge of sarcopenia in liver disease.

10.2 Mechanisms of Sarcopenia in Liver Disease

With the increasing age in humans, physical function starts to decline. Because skeletal muscle is the largest store of proteins in the body, protein homeostasis is essential for the maintenance of skeletal muscle mass [3, 4]. Aging disrupts the balance between protein synthesis and breakdown in skeletal muscle, resulting in muscle strength decline, walking disorders, falls, and other problems. All of these can negatively influence the daily activities of elderly persons [3, 4]. Muscle satellite cells exist between the sarcolemma and the basal lamina of muscle fibers. They are responsible for skeletal muscle regeneration and the function of muscle satellite cells worsens with the increasing age [6, 32, 33]. The positive regulatory effect of the Akt signaling pathway on muscle hypertrophy also declines with age, which is thought to be associated with muscle atrophy [6, 32, 33]. In persons aged 50 years or more, skeletal muscle mass is reported to decrease by about 1% per year [21]. On the other hand, Hanai et al. reported that in 149 liver cirrhosis (LC) patients, skeletal muscle mass declined by 2.2% per year [34]. These results suggest that sarcopenia in liver disease can also develop due to other reasons than aging. The most conceivable mechanism which can cause sarcopenia in patients with liver disease is PEM. Imbalances in amino acid metabolism and alterations of amino acid levels in human blood due to liver diseases, which can be closely associated with PEM, are demonstrated in the previous studies [5–7]. The incidence of PEM is high in LC patients because the liver is the primary organ involved in carbohydrate, lipid, protein, and energy metabolism [5]. LC is a state of accelerated starvation, with increased gluconeogenesis that requires amino acid diversion from other metabolic functions and protein homeostasis can be disturbed in LC patients due to multiple factors such as hyperammonemia, hormonal disorders, cytokine abnormalities, physical inactivity, and direct effects of ethanol and its metabolites [35]. In our data using indirect calorimetry ($n = 432$), six patients (2.8%) had PEM in patients with chronic hepatitis, 17 (13.8%) in patients with Child-Pugh A LC, 42 (52.5%) in patients with Child-Pugh B LC, and 10 (76.9%) in patients with Child-Pugh C LC ($P < 0.001$), and multivariate analysis revealed that Child-Pugh classification, age $\geq$64 years, aspartate aminotransferase $\geq$40 IU/L, and branched-chain amino acid to tyrosine ratio $\leq$5.2 were independent predictors associated with the presence of PEM [5]. PEM can lead to muscle atrophy and reduced muscle strength. Reduced glycogen storage in the liver can promote the degradation of skeletal muscle by requiring skeletal muscle to supply amino acids including branched-chain amino acids (BCAA) and glucose (from muscle glycogen) [36].

10.3 Definition, Epidemiology, and Clinical Impact of Sarcopenia in Liver Disease

The decreased muscle mass and muscle strength that accompanies aging is defined as primary sarcopenia, while the decreased muscle mass and muscle strength that accompanies an underlying disease, such as disuse atrophy from being bedridden, kidney disease, liver disease, inflammatory disease, advanced malignancies, and poor nutrition (insufficient caloric or protein intake), is defined as secondary sarcopenia [1, 2]. In our country, the incidence of sarcopenia among LC patients has been reported from 10% to 70% [12–16, 28, 37–42]. The wide range of incidence of sarcopenia may be due to the lack of clear definition for sarcopenia in liver disease patients and this was critical in the clinical settings among hepatologists in our country. Thus, consensus for the assessment of sarcopenia in liver disease had been eagerly awaited in our country.

Sarcopenia in liver disease is clinically of importance as it can influence the quality of life of LC patients [43–45]. Performance status (PS) is closely associated with clinical outcome in hepatocellular carcinoma (HCC), and is widely used in international guidelines [46, 47]. Patients with poorer PS and/or HCC may have sarcopenia [43–45]. A recent meta-analysis reported that the average prevalence rate of sarcopenia among participants (LC patients, 20 studies) was 48.1%, and appeared more among male with a rate of 61.6% whereas the rate was 36% for female, and regarding clinical outcomes, LC patients with sarcopenia had poorer survival rates and an increased risk of complications such as infection compared with those without sarcopenia [48]. Skeletal muscle mass is not only a good indicator of nutrition in LC patients, but also has recently been shown to be closely related to survival and postoperative complications in HCC patients. In most studies, sarcopenia was demonstrated to be an adverse predictor of clinical outcomes for HCC patients [12–20]. Recently, Montano-Loza et al. demonstrated that modification of model for end-stage liver disease (MELD) to include sarcopenia is associated with improved prediction of mortality in LC patients, primarily in patients with low MELD scores [49]. Limitations of the MELD score include lack of assessing the nutritional and functional status of LC patients [49]. On the other hand, the association between sarcopenia and nonalcoholic fatty liver disease (NAFLD) has been indicated by recent epidemiological reports. A recent meta-analysis demonstrated a significantly increased risk of NAFLD among patients with sarcopenia [50]. Sarcopenia and NAFLD have similar pathophysiological profiles [51]. Sarcopenia may occur simultaneously with obesity, particularly the accumulation of visceral fat, which can be associated with chronic inflammation, insulin resistance, and further decrease in the skeletal muscle mass, consequently leading to muscle catabolism [52, 53]. In some conditions, lean body mass is lost while fat mass may be preserved or even increased and LC patients may have a combination of loss of skeletal muscle and gain of adipose tissue. This state is defined as sarcopenic obesity and it can be a prognostic factor in LC patients [52, 54, 55]. A previous Korean study reported that visceral obesity was linked to future loss of skeletal muscle mass in Korean adults [56].

10.4 Japanese Guidelines for Sarcopenia in Liver Disease

Definitions and diagnostic criteria for sarcopenia in liver disease vary in the literature; there had been no agreement on diagnostic criteria for sarcopenia in liver disease. In 2016, the JSH established its own assessment criteria for sarcopenia in liver disease as the number of liver disease patients with sarcopenia is expected to increase and there is compelling evidence to indicate patients with sarcopenia have unfavorable clinical outcomes [21]. The major points in the JSH guidelines for sarcopenia in liver disease as compared with guidelines in Asian Working Group for Sarcopenia (AWGS) and European Working Group on Sarcopenia in Older People (EWGSOP) are: (1) in terms of handgrip strength, the same cutoff value as that in AWGS was adopted due to its prognostic impact in the JSH validation data; (2) elimination of age restriction from required evaluation items because there are several younger liver disease patients with sarcopenia due to PEM; (3) elimination of walking speed from required evaluation items because it may not be useful for assessing Japanese sarcopenic patients; (4) cutoff values for muscle mass in CT (the third lumbar level (L3)) were proposed because CT is frequently used for evaluation in liver disease; (5) psoas muscle mass on CT was also considered because it is easily calculated in the clinical settings [21, 57, 58].

10.5 Clinical Validation Data for the JSH Guidelines

In this section, we will present several validation data using the JSH guidelines.

In our recent study with the purpose of examining the influence of muscle mass decrease (MMD) as determined by data in bio-impedance analysis (BIA, cutoff values: <7.0 cm^2/m^2 for male and <5.7 cm^2/m^2 for female) in LC patients ($n = 382$, 204 in male and 178 in female) on survival and validating the utility of cutoff values in BIA recommended from the JSH guidelines, the 5-year cumulative overall survival (OS) rates were 59.8% in patients with MMD and 84.4% in patients without MMD ($P < 0.0001$), and in the multivariate analysis for survival, MMD revealed to be a significant adverse predictor for OS, indicating that cutoff values in the JSH guidelines were well validated [22].

In our previous study in LC patients, psoas muscle index (PMI, the sum of bilateral psoas muscle mass calculated by hand tracing at the L3 level on CT images divided by height squared (cm^2/m^2)) was used for survival analysis. A lower PMI was defined as <6.36 cm^2/m^2 for male patients and <3.92 cm^2/m^2 for female patients according to the recommendations of the JSH guidelines, and in the multivariate analysis of OS, PMI revealed to be a significant factor associated with OS [8]. In our another study for HCC patients undergoing radiofrequency ablation ($n = 182$), the median (range) value in PMI for male was 6.03 (1.63–9.90) cm^2/m^2 whereas that for female was 4.06 (1.21–7.32) cm^2/m^2 and the optimal cutoff points for PMI as determined by receiver operating characteristics analysis for survival were 6.31 cm^2/m^2 in male and 3.91 cm^2/m^2 in female, which were similar to cutoff values in the JSH guidelines (6.36 cm^2/m^2 in male and 3.92 cm^2/m^2 in female) [23].

10.6 The Clinical Impact of Biomarkers in Sarcopenic Patients in Liver Disease

Skeletal muscle mass is maintained by the balance between protein synthesis and protein breakdown, and sarcopenia can occur due to an increase in proteolysis or a reduction in protein synthesis, or both [6, 7]. Several potential biomarkers associated with skeletal muscle mass loss have been reported [6]. Of these, myostatin is a cytokine belonging to the transforming growth factor beta (TGFβ) family and the functional role of myostatin was first clarified in 1997 [9]. Myogenesis is facilitated by four myogenic regulatory factors and myostatin is a negative regulator of muscle protein synthesis which acts via the impaired mammalian target of rapamycin signaling, which strongly suppresses growth of skeletal muscle [10]. In our data of LC patients (108 males and 90 females with a median age of 67.5 years), the median myostatin level for males was 3419.6 pg/mL, whereas that for females was 2662.4 pg/mL ($P = 0.0024$). Median serum myostatin level for Child-Pugh A patients was 2726.0 pg/mL, whereas that for Child-Pugh B or C patients was 3615.2 pg/mL ($P = 0.0011$). The 5-year cumulative OS rates were 50.37% in the high-myostatin group and 73.60% in the low-myostatin group ($P = 0.0001$). Higher age ($P = 0.0111$) and lower PMI ($P < 0.0001$) were identified as adverse significant predictors of OS in the multivariate analysis, while higher serum myostatin ($P = 0.0855$) tended to be a significant adverse prognostic factor. Notably, in both genders, serum ammonia level showed a significantly positive correlation with serum myostatin level [8]. Thus, we concluded that higher serum myostatin level can be correlated with muscle mass loss, hyperammonemia, and impaired protein synthesis. Particularly, hyperammonemia mediated upregulation of myostatin in skeletal muscle is believed to be a mechanism of impaired protein synthesis and increased autophagy, which contribute to the development of sarcopenia and this is in consistent with our results [59]. Serum myostatin can be a useful biomarker for sarcopenia in liver disease; however, whether serial serum myostatin measurements will correlate with serial changes in muscle mass is an intriguing possibility as more rapid muscle loss worsens clinical outcome in LC patients [8, 59]. Although other potentially useful biomarkers such as follistatin (myostatin antagonist) for sarcopenia in liver disease have been reported, the prognostic impact of these is unclear [11, 60]. In this regard, further investigation will be needed.

10.7 Therapy for Sarcopenia in Liver Disease

There are several promising results regarding treatment for sarcopenia in liver disease.

10.7.1 Exercise

In general, exercise can improve skeletal muscle mass, muscle strength, and cardiopulmonary function. However, in LC patients, despite the increasing interest regarding the impact of exercise on sarcopenia, few clinical trials have been performed to date. Román et al. showed in their randomized controlled trial (RCT) (n = 17 (the exercise group (n = 8) and the control group (n = 9); all patients received leucine supplementation) that a program of moderate physical exercise together with leucine supplements in LC patients is safe and improves muscle mass [25]. In a similar study, Zenith et al. reported in their RCT for Child-Pugh A or B LC patients that thigh circumference and thigh muscle thickness were significantly higher in the exercise group (n = 9) compared with controls (n = 10) at week 8 [29]. In view of these results, exercise in LC patients may be promising. However, it should be emphasized that exercise under insufficient nutrients and protein intake could be dangerous in LC patients, given that it could accelerate further protein catabolism and muscle mass loss [30]. At the time of patient recruitment, a precise assessment for nutritional status and daily physical activities will be required in each subject. Currently, we are undergoing an RCT for examining the effect of exercise on sarcopenia for decompensated LC patients [31].

10.7.2 BCAA

BCAA granules were originally developed for the treatment of hypoalbuminemia associated with decompensated LC. However, subsequent studies found various other beneficial pharmacological effects of BCAA granules than albumin synthesis [26]. They include: (1) suppression of proteolysis in muscle; (2) energy source; (3) strengthening in immune function; (4) promotion in liver regeneration; and (5) suppression in carcinogenesis [27]. Considering these pharmacological effects of BCAA granules, positive therapeutic effect for sarcopenic LC patients can be expected. Hanai et al. demonstrated in their retrospective study that BCAA supplementation improved the survival of sarcopenic patients [28]. Kitajima et al. demonstrated that amelioration of hypoalbuminemia associated with BCAA supplementation correlated with decreased fat accumulation in skeletal muscle, maintenance of skeletal muscle mass, and improved glucose sensitivity [61]. However, further studies will be needed to confirm these results. Currently, we are undergoing a prospective study (Change in Muscle volume In patients with LivEr cirrhosis: prospective cohort Study (MILE study), UMIN-ID; UMIN000023256) for examining the effects of BCAA granules and other factors on muscle mass improvement (Fig. 10.1). If positive results of BCAA granules are confirmed in this trial, useful information will be provided for clinicians.

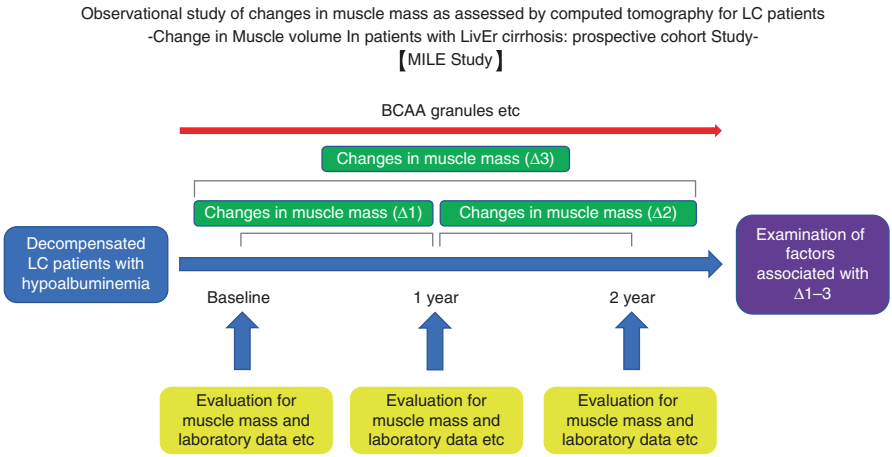

Fig. 10.1 Study design in our prospective study (change in Muscle volume In patients with LivEr cirrhosis: prospective cohort Study (MILE study), UMIN-ID; UMIN000023256)

10.7.3 BCAA and Exercise

Supplementation with BCAA administration and walking exercise combination therapy may be promising. Hiraoka et al. reported that in 33 LC patients treated with BCAA supplementation (protein 13.5 g, 210 kcal/day) as a late evening snack and walking exercise (additional 2000 steps/day prescribed), muscle volume and handgrip strength significantly increased at 3 months [24].

10.7.4 Testosterone

With the increasing age and other muscle wasting disorders, males and females undergo similar pathological changes in skeletal muscle. They include enhanced oxidative stress, mitochondrial dysfunction, satellite cell senescence, increased inflammation, elevated apoptosis and proteasome activity, and suppressed protein synthesis and myocyte regeneration [62]. Poor food intake and physical activity can be linked to skeletal muscle wasting. Sex hormones also play important roles in maintaining skeletal muscle homeostasis [62]. Testosterone is a potent anabolic factor accelerating muscle protein synthesis and muscle regeneration. Sinclair et al. demonstrated that administering testosterone to male LC patients who have low testosterone levels significantly increased their muscle mass, bone mass, reduced fat mass, and HbA1c [63].

10.8 Closing Remarks

We summarized the current knowledge of sarcopenia in liver disease. We also presented the JSH guidelines of sarcopenia in liver disease. This clinical entity will gain more attention among hepatologists in the future because the number of sarcopenic liver disease patients will increase. We hope that several unsolved issues of sarcopenia in liver disease are clarified in future studies.

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Conflicts of Interest None.

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Chapter 11
Esophagogastric Varices in Liver Cirrhosis

Hisashi Hidaka and Haruki Uojima

Abstract Bleeding esophagogastric varices (EV) represent the leading cause of death in patients with cirrhosis. The mechanism of EV bleeding has placed a special emphasis on the role of increased portal venous pressure. There are several treatments depending on the condition of patients, bleeding history, endoscopic findings, and hemodynamics.

Keywords Portal hypertension · Nonselective beta-blocker · Portosystemic shunt Endoscopic variceal ligation · Balloon-occluded retrograde transvenous obliteration

11.1 Introduction

Bleeding esophagogastric varices (EV) are the severest complications of portal hypertension (PH) and represent the leading cause of death in patients with cirrhosis. EV develop in 50% of patients with cirrhosis, and bleed in approximately 15–20% of patients per year [1, 2]. Variceal hemorrhage (VH) depends on the severity of liver disease, size of varices, and the presence of red color (RC) sign [2]. The mechanism of bleeding from EV places a special emphasis on the role of increased portal venous pressure [2]. On the other hand, in Japan an esophagogastroduodenoscopy (EGD) finding of EV is thought to be the most important finding. Furthermore, there are some differences in treatments for the EV between Japan and western countries. In this chapter, I will review diagnosis and treatments for EV based on these standpoints.

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11.2 Risk Stratification of Esophagogastric Varices

11.2.1 Hepatic Venous Pressure Gradient (HVPG)

As mentioned in the introduction, compensated cirrhosis can be divided into mild PH (HVPG >5 but <10 mmHg) and clinically significant PH (CSPH), defined by HVPG ≥10 mmHg [2]. CSPH is associated with an increased risk of developing EV and overt clinical decompensation (ascites, VH, and hepatic encephalopathy) [2, 3]. VH constitutes a decompensating event, but its mortality differs whether it presents as an isolated complication of cirrhosis (20% 5-year mortality) or whether it presents in association with other complications (>80% 5-year mortality). In patients with EV, an HVPG >12 mmHg identifies bleeding risk, mostly because there is clear evidence that shows that reducing the HVPG to levels of 12 mmHg or below is associated with protection from VH [4]. An HVPG >16 mmHg indicates a higher risk of death [5]. More than HVPG 20 mmHg predicts failure to control bleeding, early rebleeding, and death during acute VH [6, 7]. In patients with cirrhosis awaiting liver transplantation, each 1-mmHg increase in HVPG predicts a 3% increase in the risk of death in a median follow-up of 19 months [8]. Despite the crucial role of HVPG in the determination of CSPH and other outcomes, HVPG measurements require specific expertise, are invasive, relatively expensive, and not available in all centers. Therefore, noninvasive or surrogate indicators are increasingly utilized at most centers [2].

11.2.2 Noninvasive or Surrogate Indicators (Platelets, Ultrasound, CT, MRI, and Liver and Spleen Stiffness)

Among laboratory data, low platelet count is the most common laboratory sign of PH which correlates slightly with HVPG and with the presence of EV [2, 3].

Ultrasound provides safe imaging of morphological abnormalities associated with cirrhosis and PH. The presence of collateral circulation on ultrasound, CT, or MRI imaging (recanalized paraumbilical vein, splenorenal shunt, and dilated left and short gastric veins) or the finding of a reversal of flow within the portal system is 100% specific for CSPH [9]. Although splenomegaly taken alone is a sensitive, but nonspecific, PH and the size of the spleen should be routinely reported, because, when combined with platelet count and liver stiffness, they provide accurate data on the presence of CSPH and/or varices [10, 11].

The ability to assess liver stiffness (LS) has represented a major advance in this field [2]. LS by transient elastography (TE; Fibroscan) has proved very accurate for discrimination patients with or without CSPH [12]. Most studies have shown that the best LS cutoff to detect CSPH is >20–25 kilopascals (kPa), with a diagnostic accuracy over 90% [13, 14]. Furthermore, a sequential screening-diagnostic strategy, based on LS measurements assessed in the context of the presence of any ultrasound abnormality and/or a platelet count <150,000/mm^3, identified the subgroup of patients with compensate cirrhosis in whom CSPH would be more likely [14].

Spleen stiffness (SS) measurement by TE has been recently proposed as a novel index more tightly related to PH with promising results [15, 16]. In fact, SS

>54 kPa was better than LS and similar to HVPG in predicting first decompensation in one study [16].

Magnetic resonance elastography (MRE) has been shown to be accurate in the staging of liver fibrosis [17], but data regarding its diagnostic performance in the diagnosis of CSPH are still very limited [2]. Further studies are warranted in this field.

11.2.3 EGD Variceal Findings

There are some differences in EGD findings between esophageal varices and gastric varices (GV). Table 11.1 shows the criteria for recording EGD findings regarding EV according to the General Rules for Study of Portal Hypertension by the Japan

Table 11.1 General rules for recording endoscopic findings of esophagogastric varices

Category	Code subcategory
Location (L)	Ls: Locus superior
	Lm: Locus medialis
	Li: Locus inferior
	Lg-c: Adjacent to the cardiac orifice
	Lg-cf: Extension from the cardiac orifice to the fornix
	Lg-f: Isolated in the fornix
	Lg-b: Located in the gastric body
	Lg-a: Located in the gastric antrum
Form (F)	F0: No varicose appearance
	F1: Straight, small-caliber varices
	F2: Moderately enlarged, beady varices
	F3: Markedly enlarged, nodular or tumor-shaped varices
Color (C)	Cw: White varices
	Cb: Blue varices
	Cw-Th: Thrombosed white varices
	Cb-Th: Thrombosed blue varices
Red color sign (RC)	RWM: Red wale markings
	CRS: Cherry red spots
	HCS: Hematocystic spots
	Esophageal varices: RC0, RC1, RC2, RC3
	Gastric varices: RC0, RC1
	Te: Telangiectasia
Bleeding sign	Gushing bleeding
	Spurting bleeding
	Oozing bleeding
	Red plug
	White plug
Mucosal finding	E: Erosion
	Ul: Ulcer
	S: Scar

Society for Portal Hypertension [18, 19]. Especially, the RC sign on and form (F) of varices are the most important high-risk findings determined by EGD.

11.3 Management

EV treatment should be stratified according to the clinical stage (compensated or decompensated), past hemorrhage, variceal forms (small and without the RC sign, or others), and different hemodynamics (with or without a portosystemic shunt). The object of therapy for patients at the early stage is to prevent the development of a later stage [2]. An eliminating or suppressing etiological agent (e.g., HBV, HCV, and alcohol) is essential for management in patients with cirrhosis.

11.3.1 Prophylaxis for No Varices or Small EV (<F1 Without an RC Sign)

A large multicenter, placebo-controlled, double-blinded trial failed to show a benefit of the nonselective beta-blocker (NSBB), timolol, in the prevention of varices in patients with cirrhosis who had not yet developed varices [20]. Furthermore, a meta-analysis showed that NSBBs did not significantly reduce the first VH in patients with small varices [2]. Therefore, there is presently no indication that NSBBs prevent the formation of varices [2, 3].

11.3.2 Prophylaxis for the First Hemorrhage of Medium to Large EV (F2 or F3 Varices) Without a Portosystemic Shunt

Either NSBBs or endoscopic variceal ligation (EVL) is recommended for the prevention of the first VH of medium and large varices [2]. Traditional NSBBs (propranolol, nadolol) are valid first-line treatments [2]. Carvedilol, a non-cardioselective vasodilating beta-blocker, is more effective in reducing portal pressure than propranolol. Tripathi compared carvedilol and EVL for the prevention of the first variceal bleed in a randomized controlled multicenter trial [21]. Carvedilol had significantly lower rates of the first variceal bleed (10% versus 23%), with no significant differences in overall mortality (35% versus 37%), and bleeding-related mortality (3% versus 1%). Carvedilol might be more effective than traditional NSBBs in reducing HVPG [21, 22] but has not yet adequately been compared head-to-head to traditional NSBBs in clinical trials [2].

In Japan, Hashizume et al. reported the results of endoscopic injection sclerotherapy (EIS) performed in 1000 consecutively treated Japanese patients with EV, and they concluded that EIS and the close follow-up with endoscopy led to significant reduction in bleeding from EV and reduction of mortality related to this bleeding [23]. On the other hand, in western countries, EIS should not have been used for the primary prevention of VH because the mortality rate was significantly higher in the EIS group than that in the sham-therapy group [24, 25].

11.3.3 Acute EV Hemorrhage (Esophageal Varices Only or Lg-c)

EVL should be performed for patients with acute VH [2, 3]. Afterward, splanchnic vasoactive drugs should be used for up to 5 days [26] because HVPG significantly increases after EVL. Vasopressin is the most potent splanchnic vasoconstrictor. It reduces blood flow to all splanchnic organs, thereby leading to a decrease in portal venous inflow and in portal venous pressure. The clinical usefulness of vasopressin is limited by its multiple side effects, which are related to its potent vasoconstrictive properties, including cardiac and peripheral ischemia, arrhythmia, hypertension, and bowel ischemia [27]. Therefore, it can only be used continuously at the highest effective dose for a maximum of 24 h to minimize the development of side effects [27].

Furthermore, VH is associated with a high risk of bacterial infections [28]. The use of short-term prophylactic antibiotics in patients with cirrhosis and gastrointestinal hemorrhages with or without ascites has been shown to not only decrease the rate of bacterial infections but also to increase the survival rate [29].

11.3.4 Acute GV Hemorrhage With or Without a Portosystemic Shunt (Lg-cf or Lg-f)

GV bleeding is severe and is associated with high mortality [30]. Mishra et al. compared the efficacy of cyanoacrylate injection and NSBBs in primary prophylaxis of GV bleeding [31]. Cirrhotic patients with large EV type 2 (GOV2) (Lg-cf in Japan) with eradicated esophageal varices or large isolated gastric varix type 1 (IGV1) (Lg-f in Japan), who had never bled from GV, were randomized to cyanoacrylate injection (Group I, $n = 30$), NSBBs (Group II, $n = 29$), or no treatment (Group III, $n = 30$). Primary end-points were bleeding from GV or death. The actuarial probability of bleeding from GV over a median follow-up of 26 months was 13% in Group I, 28% in Group II, and 45% in Group III. The actuarial probability of survival was higher in the cyanoacrylate group compared to the no-treatment group (90% versus 72%). Although a single study suggested that cyanoacrylate injections

are more effective than NSBBs in preventing first bleeding in patients with large EV or isolated GV, further studies are warranted to evaluate the risk/benefit ratio of using cyanoacrylate in this setting [2, 30].

11.3.5 Secondary Prophylaxis for EV (Without a Portosystemic Shunt)

In western countries, the first-line therapy is the combination of NSBBs + EVL in patients with recurrent VH [2, 3]. On the other hand, in Japan, only endoscopic treatment (recently EVL) is the first-line therapy in this setting. However, there were more severe complications included bleeding from ligation-induced esophageal ulcers, chest pain, and dysphagia in EVL treatment [32]. Proton pump inhibitors (PPIs) are the most potent pharmacological agents for inhibition of gastric acid secretion [33]. Although PPIs will attenuate the effect that gastric acid plays in post-EVL complications, there is one short-term study (10 days) that evaluated the role of PPIs after EVL [26]. In that study, Sheenan et al. reported that PPI treatment for only 9 days after EVL did not significantly reduce complications or symptoms [26]. Hidaka et al. reported that long-term administration of PPIs significantly reduced the risk of treatment failure after EVL [34]. However, that study may have been limited because enrollment was not completed (i.e., only 21 patients for PPIs and 22 patients for placebo were included). Moreover, PPIs may modulate microbiome including spontaneous bacterial peritonitis in patients with liver cirrhosis, which was one of the most serious complications in cirrhosis patients with ascites [35].

11.3.6 Secondary Prophylaxis for GV with a Portosystemic Shunt

In western countries, transjugular intrahepatic portosystemic shunt (TIPS) is a treatment option for isolated GVs [36]. However, the risk of GV rebleeding within a year after TIPS has been reported to be around 10–20% [37]. Additionally, TIPS cannot always be the first-line therapy because of possible aggravation of hepatic encephalopathy [25]. In Japan, endoscopic treatment using cyanoacrylate is performed for GOV2 or IGV1 bleeding, then these GVs are treated by balloon-occluded retrograde transvenous obliteration (BRTO) in elective cases. BRTO was introduced by Kanagawa et al. [38] and has been performed most frequently in Asia, especially in Japan. This procedure involves the insertion of a balloon catheter into an outflow shunt, such as a gastrorenal shunt, via the femoral or the internal jugular veins. The sclerosing agent EO (ethanolamine oleate) is then injected through the catheter into the outflow shunt, under balloon occlusion. Several studies reported relatively

higher rates of complete eradication of GV (i.e., 70–90%) and good control of bleeding after BRTO [3, 39]. Further prospective studies are warranted to evaluate the long-term prophylactic effect and safety of the BRTO procedure.

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Chapter 12
Mechanisms and Treatment for Muscle Cramps in Liver Cirrhosis

Hiroyuki Nakanishi, Masayuki Kurosaki, and Namiki Izumi

Abstract Muscle cramps are an important complication of liver cirrhosis and impair quality of life. The prevalence of muscle cramps with liver cirrhosis ranges from 29% to 88%. The exact pathophysiology of muscle cramps continues to be poorly understood. And effective treatments have not been established. But several mechanisms and treatment option have been proposed. There are several reports pertaining to the treatment of muscle cramps including magnesium, zinc, 1-a-hydroxy vitamin D, vitamin E, eperisone hydrochloride, intravenous albumin, branched chain amino acids, taurine, quinidine, L-carnitine, and baclofen. L-carnitine improves the deterioration of energy metabolism. On the other hand, baclofen is effective for nerve dysfunction. There are needs for further study to determine which mechanistic targets have the highest value in developing effective therapies.

Keywords Muscle cramps · Carnitine · Baclofen · Liver cirrhosis · Quality of life

12.1 The Definition of Muscle Cramps Associated with Liver Cirrhosis

Muscle cramps are an important complication of liver cirrhosis [1–7]. Muscle cramps are defined as involuntary, visible, palpable, painful contractions of skeletal muscles, occurring at rest or strong enough to wake the patient from sleep [5, 6, 8].

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12.2 Prevalence of Muscle Cramps in Liver Cirrhosis or Chronic Hepatitis

The prevalence of muscle cramps with liver cirrhosis ranges from 29% to 88% depending on the inclusion criteria used by the investigators [1, 2, 9–12]. And in recent two reports comparing cirrhosis and chronic hepatitis, patients with cirrhosis had a significantly higher prevalence rate of muscle cramps than those with chronic hepatitis (51.8–52.0% vs. 7.5–43.7%) [1, 10]. These studies showed that muscle cramp in cirrhosis was an independent factor correlated with the severity of liver disease and worsening liver function [1].

12.3 Duration of Muscle Cramps

Muscle cramps can last for seconds to several minutes and may result in persistent tenderness and swelling for up to 72 h following an episode of cramping [7, 13]. Muscle cramps occur predominantly during the night; thereby patients suffer from a sleep disorder [10].

12.4 Muscle Cramps Impair *Quality of Life* (QOL) in Liver Cirrhosis Patients

Muscle cramps with liver cirrhosis are extremely painful [10, 14]. Patients find the symptom very uncomfortable, leading to decreased physical and social functioning. Moreover, muscle cramps significantly disturbed sleep in liver cirrhosis patients [10, 14–16]. General health-related quality of life, measured by instruments such as the Medical Outcome Study Short Form-36, the Nottingham Health Profile questionnaires, and the Chronic Liver Disease Questionnaire (CLDQ), is diminished in cirrhotic patients with muscle cramps [8, 10, 14, 17–21]. And muscle cramp was an independent risk factor of the impaired health-related quality of life. Muscle cramps impair sleep, physical functioning and mobility, general health, and mental health [20].

12.5 Etiology of Muscle Cramps in Liver Cirrhosis

The pathogenesis of muscle cramps in patients with liver disease remains largely unknown and there are no significant predictors of the occurrence of muscle cramps, including serum potassium levels, serum 25-(OH) vitamin D levels, diuretic use, the

presence of ascites, hepatic edema, Child-Pugh score, and model for end-stage liver disease score [3, 18, 20, 22, 23]. Konikoff et al. showed that the risk factors of muscle cramps were the presence of liver cirrhosis, higher total serum bilirubin levels, and lower serum albumin levels [3]. The exact pathophysiology of muscle cramps continues to be poorly understood. And effective treatments have not been established. But several mechanisms and treatment option have been proposed [5, 6, 8, 20, 24–26]. The impairment in energy metabolism reflected by a decrease in muscle adenosine triphosphate (ATP) production [27], nerve dysfunction, and changes in plasma print volume/electrolytes [20] are thought to be the three most common mechanisms [5]. There are needs for further study to clarify which mechanisms are the most important in causing muscle cramps.

12.6 Treatment Option of Muscle Cramps with Liver Cirrhosis

There are several reports pertaining to the treatment of muscle cramps including magnesium, zinc, 1-a-hydroxy vitamin D, vitamin E, branched chain amino acids, taurine, L-carnitine, eperisone hydrochloride, intravenous albumin, quinidine, and baclofen [2, 5, 6, 8, 9, 11, 24, 28–34]. Many agents showed a moderate benefit but have not been further studied (Table 12.1). Quinine sulfate was the most widely used agent for the relief of muscle cramps but has fallen out of favor because of its potential cardiotoxicity [35–37]. According to recent reports, there are some promising treatments such as L-carnitine and baclofen for muscle cramps in cirrhosis.

Table 12.1 Summary of reports about treatment of muscle cramps in liver cirrhosis patient

Treatment	Description	Outcome	Side effects	Comment	Author
Vitamin E	Prospective observation and treatment report 600 mg vitamin E/day ($n = 22$)	Improvement in cramps ($n = 22$)		No control group	Konikoff et al. [31]
	Pilot RCT; cross-over study vitamin E supplementation; dose unclear ($n = 9$)	No improvement ($n = 9$)	Worsening cramps reported in RCT	No improvement in muscle cramps	Chandok et al. [29]
Branched chain amino acids	Prospective treatment reports Variable doses BCAA 6 g–12 g/day ($n = 8, 37$)	Cramps resolved or reduced ($n = 45$)	None reported	Case-series reports No control group	Sako et al. [33] Hidaka et al. [30]

(continued)

Table 12.1 (continued)

Treatment	Description	Outcome	Side effects	Comment	Author
Taurine	Five prospective treatment reports (n = 12, 35) taurine 3 g/day	Cramps resolved or improved in all participants	None reported	Case-series reports No control group	Matsuzaki et al. [32] Yamamoto [34]
Eperisone hydrochloride	Prospective treatment report; variable doses (n = 21)	Cramps resolved or reduced (n = 17)	Fatigue, dizziness, epigastric discomfort	Case-series report No control group	Kobayashi et al. [11]
Intravenous albumin infusion	Cross-over study; not randomized Weekly intravenous albumin infusion (n = 20)	Cramps reduced or resolved (n = 20)	None reported		Angeli et al. [2]
Quinidine	RCT; quinidine 400 mg/day (n = 16)	Cramps resolved or reduced (n = 16)	Diarrhea (n = 5)	Restricted use due to side effects	Lee et al. [35]
L-Carnitine	Prospective treatment Report L-carnitine 900–1200 mg/day (n = 42)	Cramps reduced or resolved in 88.1% of patients (n = 42)	None reported	Case-series report No control group	Nakanishi et al. [6]
Baclofen	Prospective treatment report (n = 10)	Cramps resolved or reduced (n = 7)	None reported	Case-series report No control group	Henry and Northup [25]
	RCT; baclofen 30 mg vs placebo (n = 100)	Cramps reduced in 92% of patients (n = 100)	No difference comparing to placebo	Open-label dose increase until cramps disappearance	Elfert et al. [24]

However, there remains a need for further double-blinded, randomized, controlled clinical trials to show the effect of these treatments on muscle cramps with liver cirrhosis. And studies to draw clear conclusions about the most effective and risk-free treatment of muscle cramps in cirrhosis are needed.

12.7 L-Carnitine

L-carnitine, L-beta-hydroxy-gamma-*N*-trimethyl aminobutyric acid, is synthesized primarily in the liver and kidneys in conjunction with absorption from dietary sources such as meat and dairy products [38]. Moller et al. [27] performed skeletal

muscle biopsies in ten cirrhotic patients and found a reduction in ATP, phosphocreatine, and total adenine nucleotide levels. Carnitine plays an important role in lipid metabolism, being a cofactor for beta-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the mitochondrial membrane [39]. The fatty acid receives beta-oxidation and becomes acetyl CoA. The acetyl CoA is taken up into a TCA cycle. And the TCA cycle produces ATP in mitochondria (Fig. 12.1). Therefore, carnitine deficiency leads to a lack of ATP in skeletal muscles and this can cause malfunction of calcium ATPase pumps and a subsequent increase of intracellular calcium levels and inadequate muscle contraction [7, 40–44]. On this basis, carnitine deficiency may be a cause of muscle cramps in liver cirrhosis patients. On the contrary, the increase in ATP improves the diminished actin and myosin interaction and restores calcium into sarcoplasmic reticulum by calcium adenosine triphosphatase pumps [45–47], thereby preventing prolonged muscle contraction [5, 6]. The notable efficacy of L-carnitine administration also supports this hypothesis.

Nakanishi et al. evaluated the effects of L-carnitine supplementation in 42 consecutive cirrhosis patients with cramps [6]. The patients were treated with L-carnitine 300 mg three times a day (900 mg group) or four times a day (1200 mg group) for 8 weeks (at the discretion of attending physician). The frequency of muscle cramps was assessed by a questionnaire and the severity of muscle cramps was determined using a visual analog scale (VAS). In all patients, the frequency of muscle cramps significantly decreased in 88% of patients. And 29% of patients

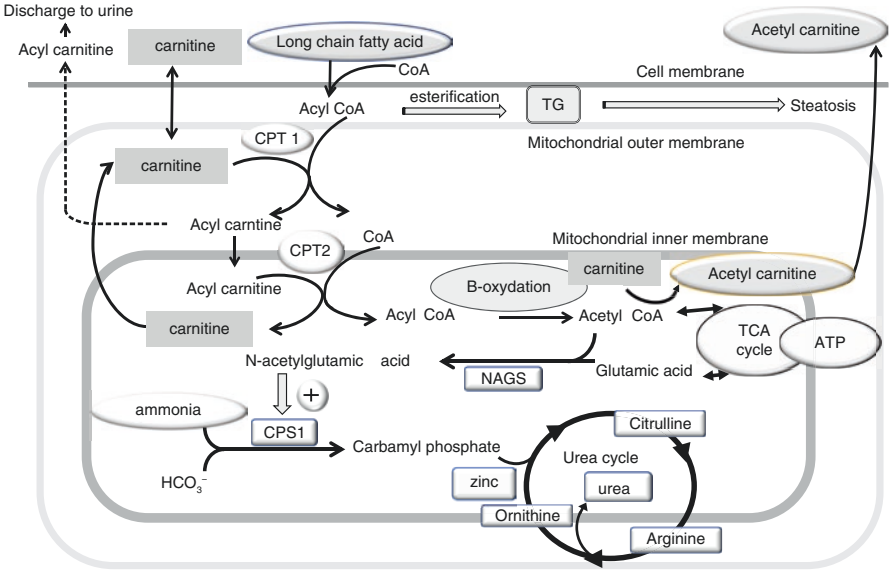

Fig. 12.1 L-carnitine metabolism (liver). *CPT* Carnitine palmitoyltransferase (carnitine rearrangement enzyme), *NAGS N*-acetylglutamate synthase, *CPS-1* Carbamoyl phosphate synthetase 1

achieved the disappearance of cramps after 8 weeks. The VAS score decreased in 87% of patients after treatment. The dose of L-carnitine was significantly associated with increased disappearance rate of cramps (43.5%, $n = 23$ in 1200 mg group vs 10.5%, $n = 19$ in 900 mg group; $p = 0.037$) and decreased severity assessed by VAS (9.9 ± 13.5 in 1200 mg group vs 39.6 ± 31.9 in 900 mg group; $p = 0.003$) at 8 weeks. No adverse effects were identified in any patient. However, it remains unclear whether the L-carnitine administration before going to bed was effective or increases in dose was effective. The optimal dose and duration of L-carnitine therapy should be determined in the future, larger scale studies.

12.8 Baclofen

Recently there were some reports showing the effect of baclofen on muscle cramps with liver cirrhosis. Henry et al. evaluated the effects of baclofen supplementation in ten cirrhosis patients with cramps [25]. The patients were treated with baclofen 5 mg three times a day for 1 week, and, if tolerated, the dose was then increased to 10 mg three times a day. The muscle cramps survey using a questionnaire was performed at initiation, termination of 4-week treatment, and after 2-week washout period. In seven patients without those dropped out, the frequency of muscle cramps significantly decreased from 5.5 ± 2.1 to 1.4 ± 2.0 days per week ($p = 0.01$), with a significant relapse after withdrawal ($p = 0.01$). And the severity estimated by 1–10 analog scales significantly improved from 8.5 ± 1.8 to 2.8 ± 2.7 ($p < 0.01$) [25]. Elfet et al. conducted a randomized placebo-controlled study of baclofen in the treatment of muscle cramps in patients with liver cirrhosis [24]. A total of 100 cirrhosis patients with muscle cramps were enrolled. They were randomized to receive either 30 mg/day of baclofen or placebo for 3 months. The frequency and severity of muscle cramps in subjects were evaluated monthly and 1 month after withdrawal. In the baclofen group, the frequency and severity of muscle cramps decreased significantly after 1 and 3 months of treatment, with a significant relapse after withdrawal. After 3 months of baclofen therapy, muscle cramps disappeared in 72% of patients. There was no significant difference in side effects between the baclofen group and placebo group [24]. Baclofen might be one of the treatment options of muscle cramps with liver cirrhosis that are caused by disruption of the nervous system.

12.9 Conclusion

According to recent studies, L-carnitine improves the deterioration of energy metabolism. On the other hand, baclofen is effective for nerve dysfunction. There are needs for further study to determine which pathological mechanistic targets have the highest value in developing effective therapies.

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Chapter 13
Management of Pruritus in Liver Cirrhosis

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Abstract The subjective symptoms of patients with liver cirrhosis should be carefully evaluated in clinical practice; the nature of pruritus in liver cirrhosis, especially with regard to disease progression and hepatic reserve, is still an elusive clinical question.

The identification of the putative pruritogen lysophosphatidic acid (LPA) in cholestatic liver disease sheds light on this expanding field of hepatic pruritus. Indeed, LPA likely plays a central role in the transmission of itching sensations from the peripheral tissues to the dorsal root ganglions, irrespective of the underlying disease (e.g., uremic pruritus).

Moreover, the demand for a pharmacological intervention for hepatic pruritus has been partially fulfilled with a newly available oral anti-pruritus agent, nalfurafine hydrochloride, making physicians in Japan much more aware of the clinical relevance of hepatic pruritus.

Evidence-based management of pruritus in liver cirrhosis is in its infancy. Much more attention to patients' reported outcomes, as well as meticulously planned clinical intervention, coupled with translational research, is needed for hepatologists to address this issue.

Keywords Liver cirrhosis · Pruritus · Lysophosphatidic acid (LPA) · Primary biliary cholangitis (PBC) · Cholestatic liver disease · Pruritogens · Cholestyramine · Rifampicin · Autotaxin (ATX) · Naltrexone · Sertraline · Nalfurafine hydrochloride · TRPA1 · TRPV1

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13.1 Introduction

A woman who had been suffering from intractable pruritus associated with HCV-related decompensated liver cirrhosis once recalled to the authors that on the very day of her undergoing orthotropic liver transplantation, "The most incredible change after waking up from systemic anesthesia in the ICU on the day of surgery was the complete absence of pruritus." This reminded us of a very similar conversation with a post-transplant patient of primary biliary cholangitis (PBC), even though their etiologies of liver disease were distinct.

Jaundice accompanies pruritus, as was first documented by the ancient Greek physician Aretæus, the Cappadocian in the second century BC. As such, pruritus is regarded as the hallmark of cholestatic liver disease but is also found in chronic liver disease, including liver cirrhosis.

In this chapter, we review recent progress in the understanding of pruritus in chronic liver disease, especially in liver cirrhosis. As it is still an area of uncertainty, we should continue to explore several clinical questions regarding the prevalence, characteristics, pathological mechanisms, and finally management strategy with reference to the accumulating knowledge about those with cholestatic liver disease.

13.2 Prevalence of Pruritus in Chronic Liver Disease

Data on the prevalence and severity of pruritus among contemporary chronic liver disease patients in the mid-2010s in Japan were the focus of two very recent reports: a multicenter study (Study A, $N = 1631$) [1] and a single center study (Study B, $N = 663$) [2]. Itching was qualitatively self-assessed in both studies: in Study A by visual analogue scale (VAS), ranging from 0 (no pruritus) to 10 (maximal pruritus) and in Study B by a numerical non-VAS Kawashima score, ranging from 0 (little or no itch) to 4 (intolerable itch). The prevalences of pruritus from Study A and Study B were quite comparable, 40.3% and 34.0%, respectively; no sex difference was observed in either study.

The prevalence of pruritus in liver cirrhosis was calculated as 43.9% in Study A; liver cirrhosis was more frequently observed in patients with pruritus than in those without (37.8% vs 32.7%, $p = 0.036$). Nevertheless, multivariate analysis did not identify liver cirrhosis as an independent risk factor associated with the prevalence of pruritus. However, AST greater than 60 U/ml, an indicator of chronic liver disease activity, was statistically associated (odds ratio = 2.306, $p = 0.011$).

Study B, on the other hand, was not structured to include liver cirrhosis as an explanatory variable for pruritus. However, low platelet number ($<10^4/mm^3$) was significantly associated with severe pruritus (Kawashima score ≥ 3; odds ratio = 2.39; $p = 0.017$), but not with overall pruritus. Even considering the different study designs of the two reports, pruritus itself is not likely a specific symptom of

Table 13.1 Distinct nature of pruritus in liver diseases; cirrhotic or cholestatic type

	Pruritus in liver cirrhosis [1, 2]	Pruritus in cholestasis [3]
Location	The abdomen and the back	The limbs, soles of the feet, and palms of the hands
Time (within day)	Severer in day than at night	Most intense in the late evening and early at night
Correlation to liver biochemistry	AST (with prevalence) The number of platelet (with severity)	No

liver cirrhosis. Its severity may advance in non-linear fashion during the course of disease progression, with some threshold point in the degree of portal hypertension, or in the state of decompensation. In other words, pruritus in liver cirrhosis might be distinct from that in non-cirrhotic chronic liver disease with regard to its characteristics and pathoetiology.

13.3 Characteristics of Pruritus in Chronic Liver Disease

Pruritus in cholestatic liver disease, the prototype in hepatic pruritus, has been characterized as follows: (1) typically localized at the limbs, soles of the feet, and palms of the hands, (2) no primary skin lesion, except secondary excoriation after scratching, (3) diurnal variation most intense in the late evening and early at night, and (4) no apparent correlation with liver biochemistry [3].

The top two common localizations of pruritus revealed from patient interviews in the aforementioned multicenter study [1] were, in contrast to the above characterization, the back (63.1%) and the abdomen (29%), the former of which was most common even among PBC patients. Moreover, pruritus was reported to be more severe by day than by night.

As such, certain differences likely exist between the features of cholestatic and non-cholestatic pruritus among chronic liver disease patients. Which of the two is more similar in nature to liver cirrhosis-related pruritus remains an elusive clinical question; there has thus far been no report regarding the features of liver cirrhosis-specific pruritus (Table 13.1).

13.4 Pathological Mechanisms of Pruritus in Chronic Liver Disease

The potential "pruritogens" in cholestasis have long been postulated as follows: they are probably (a) in systemic circulation, (b) physiologically secreted into the bile, and (c) transformed in the liver/intestine. Those assumptions were made by the

clinical observations that pruritus has been shown to be alleviated by (a) plasmapheresis, albumin dialysis, or plasma separation/anion absorption, (b) oral administration of the anion exchange resin cholestyramine and nasobiliary drainage, and (c) potent pregnane X receptor (PXR) rifampicin [3]. Correspondingly, there is a long list of candidate pruritogens in cholestatic pruritus, namely bile salts, endogenous opioids, histamines, serotonin, progesterones/estrogens, and the recently reported lysophosphatidic acid (LPA). Nevertheless, the plasma concentrations of these factors in cholestatic patients have not in general been found to be correlated with itch intensity; some are regarded as modulators (e.g., endogenous opioids, serotonin, and progesterones/estrogens), but not as causative agents [3].

LPA, on the other hand, was identified by translational research as a putative pruritogen, and has gained increasing attention, especially with regard to its potent role in the promotion of liver fibrosis and of hepatocarcinogenesis [4]. In key breakthrough research, Kremer et al. [5] screened the cytosolic free Ca ($[Ca^{2+}]_i$) response of various neuronal cell lines, as a simple marker of cell activation, to sera of pruritic and nonpruritic cholestatic patients as well as healthy volunteers. They detected a particularly strong $[Ca^{2+}]_i$ response induced by sera of women with intrahepatic cholestasis of pregnancy (ICP) and finally identified the non-peptide endogenous chemical compound LPA as the neuronal activator in these sera samples as a possible trigger of unmyelinated itch-neuron endings. Indeed, intradermally injected LPA induced a scratch response in mice. LPA is unstable in sera and its concentration is increased in storage due to its formation from its precursor, lysophosphatidic acid (LPC). Accordingly, they then examined the activity of the enzyme responsible for the formation of LPA from LPC, lysophospholipase D (LPD), commonly called autotaxin (ATX).

The activity and protein levels of ATX were examined in three groups of patients—cholestatic patients with pruritus, those without, and healthy volunteers—who were found to have descending serum levels [5]. Likewise, serum ATX activity was revealed to be decreased after successful treatment of cholestatic pruritus by therapeutic interventions, including administration of the anion exchange resin cholestyramine or the PXR agonist rifampicin, or nasobiliary drainage, among other treatments [5]. Complicating the issue is the fact that ATX is not excreted into bile [5] and is reported to be partly cleared from plasma by scavenger receptors present on liver sinusoidal endothelial cells [6]. An as-yet unidentified factor ("factor X") that is either excreted in the bile or retained in systemic circulation likely causes transcriptional upregulation of ATX, again in unidentified tissue ("tissue Y"), resulting in a net increase in circulating ATX levels.

Assuming that a dysregulated LPA-ATX axis exists in cholestasis patients with pruritus, we might hypothesize that pruritus in liver cirrhosis occurs by a similar mechanism, i.e., accompanied by elevated serum ATX. Recent analysis has shed light on LPA and ATX as novel serum markers of liver fibrosis, irrespective of etiology. Watanabe et al. originally described that both plasma LPA and serum ATX levels are increased in chronic hepatitis C [7]. Nakagawa et al. extended the former study, demonstrating that serum ATX antigen levels or activity were significantly correlated with both liver fibrosis stage and stiffness [8]. Although the exact

mechanisms for the elevation of serum ATX may differ between cholestatic liver disease and liver cirrhosis, ATX may play a common role as pruritogen in both clinical entities. Direct comparison of the levels of serum ATX in cholestatic liver disease and liver cirrhosis should be performed in the future.

13.5 Treatment of Pruritus in Chronic Liver Disease

The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) recommend an evidence-based treatment strategy in which the anion exchange resin cholestyramine, the PXR agonist rifampicin, the opioid μ antagonist naltrexone, and the selective serotonin reuptake inhibitor (SSRI) sertraline are prescribed in a stepwise manner to adequately gain control over cholestatic pruritus. In Japan, however, the use of those drugs for pruritus is "off-label" in each case; only some cholestatic patients with concomitant hypercholesterolemia, such as symptomatic PBC, have found benefit from cholestyramine treatment.

With regard to the current status of empirical treatment for pruritus among all-cause liver disease patients, the aforementioned multicenter pruritus study documented that 45.7% of patients with pruritus received therapy with anti-pruritus agents; of them, 70.7%, 7.3%, and 12.3% received external medications only (e.g., skin moisturizer, anti-histamine, or corticosteroid ointment), oral medicine only (probably anti-allergic drugs), and both, respectively [1]. As patients' subjective reports of the efficacy of responses included partial (41.2%) or even null response (16.6%), unmet needs for appropriate treatment are still present in this clinical area.

The opioid system is regarded as the central system for the perception of itching [9]. Countering the itch-inducible μ-opioid system, the κ-opioid pathway is considered to suppress itching from clinical observations; continuous epidural infusion of butorphanol (a partial κ agonist) was reported to decrease pruritus caused by epidural morphine in postoperative children [10]. Moreover, intranasal administration of butorphanol was also effective for reducing intractable chronic itch due to various diseases, including PBC [11]. Kumagai et al. validated the "yin and yang" dual modulation of itching by μ and κ opioid receptors with findings that the serum ratio of endogenous opioids β-endorphin (a μ agonist) to dynorphin-A (a κ agonist) increased according to the itch intensity in hemodialysis patients complaining of pruritus [12].

Nalfurafine hydrochloride ((2E)-N-[(5R,6R)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]-3-(furan-3-yl)-N-methylprop-2-enamide monohydrochloride), which was originally developed by TORAY, Japan, has been shown to be a selective κ-opioid receptor agonist in vitro, and exerts a broad range of antipruritic effects in both anti-histamine-effective and -ineffective models of itch in preclinical settings. After approval for the treatment of uremic pruritus resistant to conventional drugs in Japan and Korea [13], a placebo-controlled double-blind phase III study for refractory pruritus in patients with chronic liver disease,

which included 142 liver cirrhosis patients, was undertaken in Japan [14]. Nalfurafine was given orally once a day for 12 weeks, at a dose of 2.5 μg or 5 μg. Changes in the mean VAS scale from the baseline value at week 4 were significantly greater in the groups treated with either 2.5 μg ($p = 0.0022$) or 5 μg ($p = 0.0056$) nalfurafine than in placebo groups, while a dose-response pattern was not apparent. The subsequent subgroup ANCOVA analysis based on the primary disease revealed a significant difference in VAS only in PBC patients treated with 2.5 μg of nalfurafine ($p = 0.0106$), but neither in liver cirrhosis nor chronic hepatitis patients, likely in part because the statistical power was low due to the small sample size of the subgroup. The major adverse drug reactions included pollakiuria, somnolence, and insomnia, but most such reactions were mild. In 2015, nalfurafine became the first drug approved for the treatment of pruritus in chronic liver disease in Japan.

After 3 years of clinical experience in Japan, at least two post-market surveys of the clinical efficacy of nalfurafine among patients with chronic liver disease are now available in the literature. A single center study among patients with various etiologies of liver disease [2] revealed that 93 out of 138 patients (67.4%) had improvement of itch, defined as a self-reported decrease in VAS of 50 mm or more, after a median duration of 6.4 weeks. Of note, there were no significant differences in treatment efficacy between those with low ($<10.0 \times 10^4/mm^3$) and high ($\geq 10.0 \times 10^4/mm^3$) platelet count ($p = 0.170$), implicating that pruritus in liver cirrhosis was indeed an indication for nalfurafine. The second study focused on the long-term (longer than 12 weeks) efficacy and utility of nalfurafine [15]. Nine out of eleven patients showed continuous improvement of symptoms, and this progress was still apparent at 20 weeks after starting administration ($p < 0.0001$).

Finally, the very recent report of a multicenter, post-marketing, single-arm prospective study to investigate the efficacy of nalfurafine (2.5 μg, once daily for 12 weeks) in PBC patients with refractory pruritus gives us some mechanistic insights into nalfurafine, regarding the dysregulated LPA-ATX axis. Yagi et al. asked patients to complete questionnaires (the Japanese version of PBC-40 and the SF-36) to assess their symptoms and health-related quality of life (HRQOL), and to evaluate pruritus severity using the VAS [16]. The mean PBC-40 itch domain scores and VAS declined significantly from baseline during the study period ($p = 0.041$ and $p = 0.001$, respectively), while at the same time serum ATX levels were significantly increased ($p < 0.001$). The fact that nalfurafine, a κ agonist, could mask these increases may indeed indicate that the opioid system for itch-sensing is upstream from the peripheral itch-causing LPA-ATX axis. The role of ATX in non-cholestatic pruritus in liver cirrhosis should continue to be evaluated cautiously in Japanese patients, especially in terms of the kinetics of ATX during long-term administration of nalfurafine; we cannot totally rule out the possibility that an increase in ATX in liver cirrhosis patients might have a negative impact on overall disease status.

The very recent publication by Kittaka et al. convincingly demonstrated that cytoplasmic LPA produced de novo could activate TRPA1 and TRPV1 in dorsal root ganglion neurons to cause itching in mice, through LPA5 receptor-phospholipase D signaling [17]. Is this cascade also a target for the inhibition of pruritus in liver

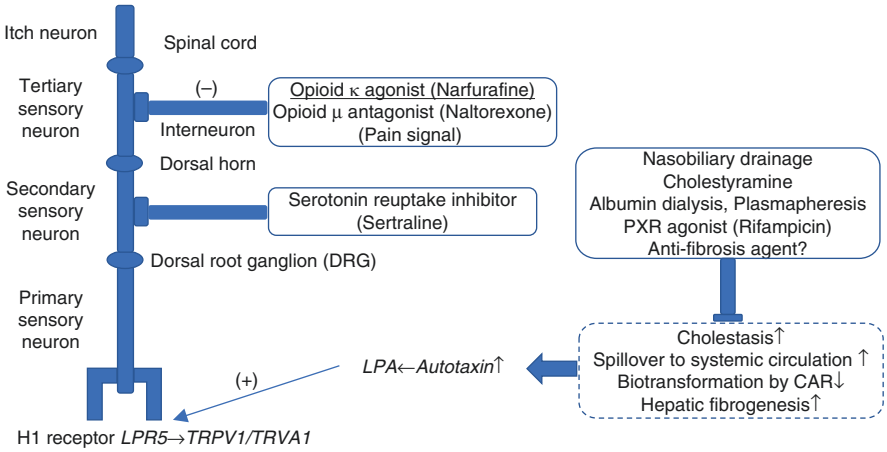

Fig. 13.1 Hepatic pruritus: from pruritogen(s) to treatment

cirrhosis? This will continue to be an open question until the central role of the LPA-ATX axis in pruritus in liver cirrhosis is validated (Fig. 13.1).

Patient-reported outcomes in liver cirrhosis should be more thoughtfully evaluated in clinical practice; precise documentation of the nature of pruritus in liver cirrhosis, especially with regard to disease progression and hepatic reserve, will greatly assist the advancement of understanding in this field.

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Chapter 14
Prevention of Hepatocarcinogenesis in Liver Cirrhosis

Kyoko Hoshikawa and Yoshiyuki Ueno

Abstract Hepatocellular carcinoma (HCC) is still the second leading cause of cancer-related deaths in men worldwide. Most cases of HCC arise in the cirrhotic liver by various causes of hepatitis. In this chapter, we describe about the prevention of HCC incidence in cirrhosis. For viral hepatitis including hepatitis B and C virus hepatitis, use of the proper antiviral therapy is the most important way to reduce the risk of HCC. For other non-viral (NASH and alcoholic steatohepatitis) hepatitis, elimination of the causal substance is critical, such as reduction of alcohol consumption or body weight. However, careful surveillance is also essential for patients with cirrhosis.

Keywords Cirrhosis · Hepatocellular carcinoma · Hepatocarcinogenesis

14.1 Introduction

Despite the recent development of antiviral therapy for viral hepatitis, hepatocellular carcinoma (HCC) is still the second leading cause of cancer-related deaths in men worldwide [1]. Moreover, the mortality rate of HCC is increasing in North America, Oceania, and in Central and Northern Europe [2] due to an increasing number of patients with non-viral liver disease, especially with non-alcoholic steatohepatitis (NASH), and lifestyle changes that result in obesity.

Most cases of HCC arise in the cirrhotic liver and occur as a result of long-term chronic hepatic inflammation induced by various causes of hepatitis [3]. Although fibrosis in the liver is closely associated with HCC, the mechanism underlying hepatocarcinogenesis from fibrotic liver comprises multiple factors and remains uncertain. Generally, various irritants including hepatitis B virus (HBV), hepatitis C virus (HCV), and non-viral (lipotoxicity and alcohol) stimuli induce apoptosis, which leads to inflammation in the liver. Chronic inflammation then induces the activation

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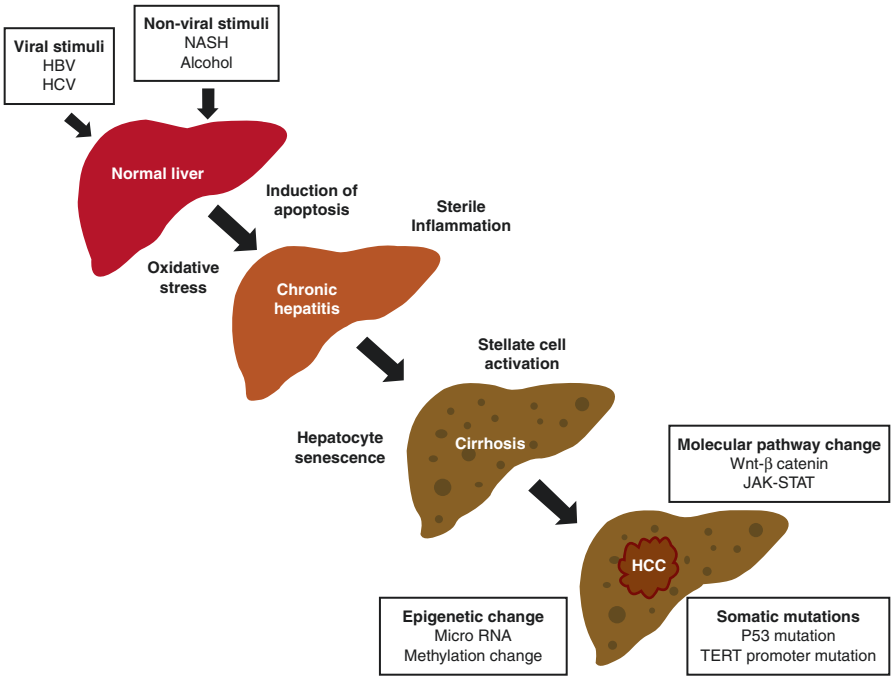

Fig. 14.1 The pathogenetic mechanism of HCC in cirrhosis

of hepatic stellate cells, which is followed by fibrosis and cirrhosis. Within the fibrotic liver, alternations of molecular pathways, epigenetic changes, and somatic mutations can occur and contribute to hepatocarcinogenesis (Fig. 14.1). This chapter mainly focuses on the prevention of hepatocarcinogenesis associated with HBV, HCV, and non-viral (NASH and alcoholic steatohepatitis) hepatitis.

14.2 Prevention of Hepatocarcinogenesis in HBV-Related Cirrhosis

Regardless of the universal development of hepatitis B vaccination and the use of antiviral therapy for the chronic hepatitis phase, HBV is still the leading cause of HCC [4, 5]. A recent publication reported that almost half of the individuals with HCC worldwide are infected with HBV [6]. This proportion varies with region and country, as a very high percentage (up to 60%) is observed in Asian countries (e.g., China), whereas a relatively low percentage (20%) is observed in the USA [6]. Therefore, better control of HBV infection is crucial to reduce the number of HCC cases, particularly those in high-prevalence areas of HBV.

It is well known that HBV-related HCC occurs in a non-cirrhotic liver, while most HCV-related HCC occur in a cirrhotic liver. This difference is due to the mech-

anism of the development of HCC; the part of HBV DNA such as HBx coding region is integrated into the host DNA as a possible oncogene and contributes to the onset of HCC. However, the risk of HBV-related HCC is still higher in individuals with cirrhosis than in those without cirrhosis. To date many studies have been performed that describe the relationship between antiviral therapy and the prevention of HCC, although the beneficial effect of the antiviral therapy for HBV-related cirrhosis is inconsistent. Summaries of the results of the previous studies on antiviral therapy for HBV with respect to the therapy's contribution to HCC incidence and recent consensus are described below.

In the last decade, new antiviral therapies such as nucleo(s)tide analogs (NAs) have been approved for use in HBV treatment. Thus far, interferon (IFN) therapies (IFN-α and pegylated IFN-α) and several NAs (lamivudine, adefovir, entecavir, and tenofovir) have been approved for the treatment of HBV infection. The overall reported effect of IFN-α treatment on the development of HCC in the setting of HBV is inconsistent [7]. However, some studies have shown that IFN-α treatment may be beneficial in reducing HCC incidence in patients with preexisting cirrhosis [8, 9].

A meta-analysis performed for initial NAs, such as lamivudine and adefovir, in patients with HBV and early cirrhosis showed a significant reduction in the risk of HCC [10–12]. More recently approved NAs including entecavir and tenofovir have shown more promising effects for the prevention of HCC. In one study, entecavir treatment significantly reduced the incidence of HCC in individuals with HBV infection and cirrhosis [13, 14]. Furthermore, the efficacy of entecavir with respect to HCC suppression was greater than that with lamivudine treatment [13]. However, a limited number of studies have been published on the efficacy of tenofovir therapy on HCC. A recent study reported that ETV/TDF therapy reduced the risk of HCC in patients with HBV and compensated cirrhosis [15].

Besides viral infection, other factors should also be considered in the development of HBV-related HCC. Host factors are also important in the development of HCC; according to a recent study, older age, male gender, and high HBs-Ag or HBV DNA titer are thought to be independent risk factors for HCC. Currently, several scoring systems are used to assess the risk of HBV-related HCC [16, 17].

In a summary, the application of the proper antiviral therapy and subsequent suppression of the viral load are the most important ways to reduce the risk of HCC due to HBV-related cirrhosis, but careful surveillance is also needed, particularly for older males with high HBs-Ag or HBV DNA viral load.

14.3 Prevention of Hepatocarcinogenesis in HCV-Related Cirrhosis

Recently, several direct-acting antivirals (DAAs) that provide a highly desired effect of sustained virologic response (SVR) in chronic hepatitis C have been approved. However, HCV infection is still the major cause of HCC particularly in patients with cirrhosis. Unlike HBV, most cases of HCV-related HCC occur in the cirrhotic

liver. Recently published studies regarding the long-term effect of SVR on the incidence of HCC have also revealed the mechanisms of HCC prevention. On the contrary, some studies have shown the opposite result, and cases with early occurrence and recurrence of HCC after DAA treatment have been reported. Below, the details regarding the efficacy of antiviral treatment including past IFN-based therapy and current IFN-free DAA therapy are described; other non-antiviral therapies are also mentioned.

Historically, randomized controlled trials have been conducted to evaluate the efficacy of IFN-α in HCV-related HCC in patients with cirrhosis in Japan. These studies revealed that IFN-α decreased the incidence of HCC (4% in IFN-α patients vs. 38% in controls) [18]. The international hepatitis C antiviral long-term treatment against cirrhosis trial revealed that long-term peginterferon therapy reduced the risk of HCC in patients with cirrhosis [19]. Accompanied by using antiviral therapy, a high SVR is even achieved in the patients with cirrhosis. One study that included a large cohort assessed the long-term efficacy of SVR after IFN-based treatment and demonstrated a reduction in the incidence rate of HCC in patients with cirrhosis (SVR 6.4 vs. no SVR 21.0/1000 per year) [20]. More recently, treatment with several IFN-free DAA therapies that have been approved led to a high SVR. However, the beneficial effect of DAAs on the incidence of HCC remains controversial. A group known as HCV Research UK determined the short-term effect of IFN-free DAA on SVR in patients with cirrhosis and concluded that antiviral therapy improves liver function with no indication of the development of adverse effects of HCC [21]. On the contrary, research groups from Italy and Spain reported an early occurrence and recurrence of HCC after DAA therapy in such patients [22, 23]. They speculate that this conflicting phenomenon occurs because of the immune system changes within the liver and an incomplete detection of early HCC before DAA therapy. A study on the long-term efficacy of IFN-free DAA therapy for the reduction in the incidence of HCC is warranted to resolve this issue.

In addition to those discussed above, other therapies may be used to prevent HCV-related HCC. Ursodeoxycholic acid and glycyrrhizin were broadly used as primary drugs before the advent of the current efficient antiviral therapy. These drugs exert anti-inflammatory effects on the liver, and longitudinally prevent fibrosis and HCC. A study of glycyrrhizin on a large cohort revealed that glycyrrhizin reduces the incidence of HCC among patients with HCV who are resistant to IFN therapy. Another study reported the efficacy of angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers for the prevention of fibrosis and HCC [24–26]. These drugs are broadly used as antihypertensive agents, and their safety is well established. The efficacy of these drugs against fibrosis may possibly be due to the relationship between the activation of stellate cells and angiotensin II receptor expression [27]. It has also been confirmed that branched-chain amino acids (BCAAs) prevent the development of HCC in the cirrhotic liver [28, 29]. Since the development of HCC is closely related to insulin resistance (IR) and sometimes occurs in individuals with diabetes [30], it is important to maintain a normal response to insulin to prevent the development of HCC. BCAAs could prevent the occurrence of HCC by improving IR in the cirrhotic liver [31].

Although several therapies can be used to prevent the development of HCC from HCV-related cirrhosis, older age is still the strongest risk factor [32]. Thus, to prevent the development of HCC from HCV-related cirrhosis, the use of antiviral therapy for patients with compensated cirrhosis and concomitant, careful surveillance is needed, particularly in elderly populations, even if SVR is achieved.

14.4 Prevention of Hepatocarcinogenesis in Non-viral-associated Cirrhosis

14.4.1 NASH-Associated HCC

With lifestyle changes followed by an increased number of individuals with obesity, non-alcoholic fatty liver disease and its severe phenotype, NASH, have become a significant public health problem. Currently, NASH is the second leading cause of liver transplantation among adults who are on the transplant waiting list. However, NASH treatment is still limited to weight reduction due to a lack of effective pharmacological therapy. Under such circumstances, the incidence of HCC due to NASH has increased and has become an important issue.

As in other cancers (colorectal, breast, kidney, and esophageal cancer), obesity is associated with an increased risk of HCC [33, 34]. The pathogenesis of NASH is closely related to the condition of obesity, and thus weight control is pivotal in such patients. Recently, one study revealed that weight reduction by lifestyle modification and bariatric surgery improved the condition of fibrosis in the liver [35, 36].

Other important risk factors for the development of HCC are IR followed by diabetes mellitus. Most NASH patients exhibit some degree of IR and/or diabetes. It is well known that diabetes increases the risk of HCC [30, 37]. Among the treatment of diabetes, metformin, a drug that inhibits hepatic gluconeogenesis reduces the risk of HCC in patients with diabetes [38].

Although weight reduction and maintenance of a better metabolic status are crucial in the prevention of HCC development from NASH, urgent development of pharmacological therapy is still needed for NASH patients.

14.4.2 Alcohol-Associated HCC

A meta-analysis demonstrated that alcohol intake is linked to an increased risk of HCC [39]. Furthermore, the HCC incidence rates are higher in patients with cirrhosis [40, 41]. The most effective and important method of HCC prevention is the reduction of alcohol consumption. However, since these patients also tend to have alcoholism, it is sometimes challenging for them to stop their alcohol intake. Therefore, at an appropriate time, these patients should be introduced to groups such as alcoholics anonymous and/or should be informed regarding a consultation with a psychiatrist.

14.4.3 Other Causes of HCC

Although the occurrence of HCC is rare in women with primary biliary cirrhosis (PBC), an increased risk of HCC is observed in men with PBC [42]. Among patients with autoimmune hepatitis, the development of cirrhosis and HCC is rare. Similarly, the occurrence of HCC as a result of primary sclerosing cholangitis is low.

14.5 Conclusion

In the last decade, the treatment for chronic hepatitis has rapidly evolved, and the disease proportion is shifting from viral to non-viral hepatitis. However, HCC mortality rates are still relatively high compared with those of other cancers, and thus HCC prevention has become even more important in patients with cirrhosis. In addition to the appropriate therapy for individual liver diseases, careful surveillance is essential, especially in patients with cirrhosis.

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Chapter 15
Antifibrotic Therapy for Liver Cirrhosis

Le Thi Thanh Thuy, Hoang Hai, Vu Ngoc Hieu, Ninh Quoc Dat, Dinh Viet Hoang, and Norifumi Kawada

Abstract Liver cirrhosis plays a main cause of morbidity and mortality, especially for those at an advanced decompensated stage. The development and progression of cirrhosis involve a diffuse *hepatic* process characterized by fibrosis and the conversion of normal *liver* architecture into structurally abnormal nodules. There have been a number of recent advances in our understanding of the pathogenesis of hepatic fibrosis, including evidence of the reversibility of fibrosis and the inactivation of hepatic stellate cells (HSCs) and/or myofibroblasts (MFs), and increasing numbers of small molecules and biological agents have been developed to explore new means of treating this condition. Here, we focus on the main approaches for antifibrotic therapy: (1) elimination of the cause of liver cirrhosis; (2) inhibition of the accumulation of inflammatory cells in the liver; (3) deactivation of HSCs and MFs; (4) control of key signal transduction pathways; and (5) antioxidant therapy.

Keywords Liver fibrosis · Cirrhosis · Hepatic stellate cells · Myofibroblast Antioxidant · Pro-oxidant · Cytoglobin · Antifibrosis

15.1 Introduction

Cirrhosis is an advanced stage of liver fibrosis that occurs with progression from chronic liver disease and results in liver inflammation, fibrogenesis with dense extracellular matrix (ECM), distortion of the hepatic vasculature, and collapse of the liver structure [1]. The ECM in cirrhosis is composed of a complex assembly of different molecules, including the fibril-forming interstitial collagens type I and III, basement membrane collagen type IV, non-collagenous glycoproteins, such as fibronectin and laminin, elastic fibers, and glycosaminoglycans and

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proteoglycans [2]. The major hepatic ECM-producing cells are myofibroblasts (MFs), which are derived from activated hepatic stellate cells (HSCs) or portal fibroblasts, other inflammatory cells, and bile duct epithelial cells. The ECM also binds and secretes growth factors and cytokines that drive morphogenesis, cell function, and metabolism [3].

Rapid progress in our understanding of the molecular mechanisms underlying cirrhosis, and its potential reversal, has resulted in the development of a number of antifibrotic drugs. This chapter will discuss therapeutic approaches to the reversal of fibrosis, focus on treatment of the underlying disease, and the development of intrinsic antifibrotic drugs that specifically target the mechanism of fibrogenesis, regardless of the cause of liver disease.

15.2 Elimination of the Cause of Liver Cirrhosis

The earlier chapters of this book described the etiology of liver diseases that induce liver cirrhosis. These include chronic viral hepatitis B and C, autoimmune diseases, biliary disorders, drug-induced liver diseases, and alcoholic steatohepatitis (ASH), as well as nonalcoholic steatohepatitis (NASH). Fibrosis without control of the underlying causative factors can result in advanced stage cirrhosis with high levels of ECM deposition, causing morbidity and mortality. Therefore, to prevent fibrosis, the causative factors should be eliminated. Efficient therapies have been developed to control and alleviate the underlying causes, which are capable of not only slowing down fibrosis progression, but also of inducing regression of fibrosis. About 80% of the 143 million people exposed to hepatitis C virus (HCV) worldwide develop chronic infection [4, 5], and the risk of cirrhosis progression is 10–30% over 30 years [6]. Fortunately, highly effective therapies using direct-acting antiviral (DAA) can achieve remarkably high sustained virologic response (SVR) rates of 94–100% [7–10].

NASH and obese individuals that manage to lose 10% of their body weight can show reductions in liver inflammation [11] and gradually weight loss of 7% per year is recommended [12]. In addition, treatment with vitamin E or pioglitazone improved NASH in adults without diabetes [13]. ASH is induced by excessive and prolonged alcohol use. To prevent ASH, therefore, alcohol consumption should be less than 60–80 ml/day for men or 40–50 ml/day for women, for no longer than 5 years. Autoimmune hepatitis and primary biliary cholangitis are common autoimmune diseases, which may progress to cirrhosis, and are treated by immunosuppression and ursodeoxycholic acid, respectively. Another etiology of fibrosis is drug-induced liver injury, including all classes of adverse drug reactions [14]. In most cases, drug withdrawal based on monitoring of liver enzyme parameters can improve the disease [15].

15.3 Inhibition of Accumulation of Inflammatory Cells to the Liver

15.3.1 Damaged Hepatocytes Recruit Inflammatory Cells Secreting Cytokines and Chemokines to the Liver

Hepatocyte necrosis and apoptosis triggered by any etiology are prominent drivers of chronic liver inflammation and fibrosis [16–18]. Apoptotic bodies and cellular contents derived from damaged hepatocytes can activate quiescent HSCs and Kupffer cells, and lead to the accumulation of neutrophils and other immune cells. These activated cell populations can release the proinflammatory cytokines, interleukin (IL)-1, IL-6, IL-8, IL-12, and tumor necrosis factor-alpha (TNF-α), and the chemokines C-C motif chemokine ligand 2 (CCL2) and CCL5 [16, 19]. These cytokines/chemokines can stimulate activity in nearby cells (paracrine action), in distant cells (endocrine action), and in the cells from which they are produced in an autocrine manner [20]. The roles of these cytokines and chemokines in activating HSCs, amplifying inflammation, and driving liver fibrosis and cirrhosis have been well characterized in both human and animal studies [16, 21, 22]. Current therapies target these small proteins, either to neutralize them or inhibit them from accelerating liver damage and fibrosis.

15.3.2 Cytokines and Chemokines Inhibitors

If cytokines can drive HSC activation, targeting a single or multiple cytokines may provide a way to block or reverse at least some of the fibrotic processes occurring in liver disease. A number of drugs target multiple cytokines, including anti-IL-4/13 (ClinicalTrials.gov ID # NCT 01529853) and anti-IL-17A/R [23, 24], which have been used for other indications such as idiopathic pulmonary fibrosis and plaque psoriasis. However, these drugs also have antifibrotic potential in liver fibrosis. In a recent study, 55 adults with biopsy-confirmed NASH were randomized to receive pentoxifylline (anti-TNF-α, PTX) at a dose of 400 mg three times a day ($n = 26$) or placebo ($n = 29$) over 1 year. PTX significantly improved steatosis and lobular inflammation. PTX also improved liver fibrosis (mean change in fibrosis score − 0.2 for PTX vs. +0.4 for placebo, $p = 0.038$) [25].

Chemokine-directed therapies investigated in animal models of NASH and fibrosis, such as the CCL2 blocker NOX-E36 [26, 27], have recently entered phase I and II clinical trials in patients with diabetes. Another interesting dual oral CCR2/CCR5 inhibitor, cenicriviroc, has been used successfully in rodent models of NASH and fibrosis [28], but clinical trials in nonalcoholic fatty liver disease or hepatic fibrosis have not yet been performed.

Further investigations are required regarding which cytokines should be chosen and how they affect the whole body or organ-specific targets [29].

15.3.3 Infection in Liver Cirrhosis and Gut Microbiome-Based Therapies

In parallel with a state of excessive activation of proinflammatory cytokines, patients with cirrhosis are in a state of immune dysfunction, referred to as cirrhosis-associated immune dysfunction syndrome, which predisposes them to infection [30, 31]. Chapter 8, "Microbiome in liver cirrhosis," described in detail these alterations. Here, we summarize gut microbiome-based therapies, including probiotics, prebiotics, synbiotics, and antibiotics, which may control complications of liver cirrhosis, such as hepatic encephalopathy (HE). Many studies have shown that probiotics have effects on HE by reducing blood ammonia levels, improving minimal hepatic encephalopathy (MHE), and preventing overt HE [32]. Shulka et al. reported that the administration of prebiotics, probiotics, and synbiotics was related to improvement of MHE [33]. A systematic review and meta-analysis of randomized trials indicated that probiotics and synbiotics improved HE to a greater extent than placebo and lactulose [34]. Rifaximin, a minimally absorbed oral antimicrobial agent, has broad-spectrum in vitro activity against gram-positive and gram-negative aerobic and anaerobic enteric bacteria, reduces the risk of HE recurrence and HE-related hospitalization [35], and improves systemic hemodynamics and renal function [36, 37]. Among 12 promising clinical trials (http://www.ClinicalTrials.gov) in phase IV trials of single or combination microbiome therapies for liver cirrhosis and its complications, the most promising results were seen for rifaximin (Table 15.1).

15.4 Targeting Activated Hepatic Stellate Cells and Myofibroblasts

15.4.1 Apoptosis, Senescence, or Reversion of Activated HSCs

Hepatic fibrosis is reversible and cirrhosis may regress in some patients with reduction in the number of activated HSCs [38]. There are three main ways to eliminate activated HSCs, i.e., apoptosis, senescence, and reversion to an inactivated phenotype.

Apoptosis: Several studies in animal models of liver fibrosis in the recovery phase suggested a vital contribution of HSC apoptosis to the resolution of fibrosis [39, 40]. Many regulators of HSC apoptosis have been discovered, such as anti-apoptotic factors TNF-α, TGF-β, and interferon-alpha (IFN-α). In addition, the

Table 15.1 Microbiome-base therapies for liver cirrhosis patients in phase IV of clinical trials

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
1	Effect of VSL#3 (original De Simone formulation) on cognitive function, risk of falls, and quality of life in patients with cirrhosis	IV	40	Cirrhosis, varices, esophageal	Dietary supplement: VSL#3 (original De Simone formulation) Other: placebo	No results reported	NCT01686698
2	Rifaximin reduces the complications of decompensated cirrhosis: a randomized controlled trial	IV	200	Cirrhosis	Drug: rifaximin	No result available	NCT02190357
3	Comparison of efficacy of cefotaxime, ceftriaxone, and ciprofloxacin for the treatment of SBP in patients with LC	IV	261	SBP, liver cirrhosis	Drug: cefotaxime Drug: ceftriaxone Drug: ciprofloxacin	No results reported	NCT01265173
4	Albumin administration in patients with cirrhosis and infections unrelated to spontaneous bacterial peritonitis	IV	110	Cirrhosis	Drug: human albumin	Beneficial effects on the renal and circulatory function and shows a potential survival benefit	NCT00124228
5	Randomized controlled trial of mechanistic effects of rifaximin in cirrhosis and chronic hepatic encephalopathy	IV	38	Liver cirrhosis, hepatoencephalopathy, early fatal progressive	Drug: rifaximin-#, Drug: placebo oral tablet	No results reported	NCT02019784
6	L-ornithine L-aspartate in overt hepatic encephalopathy	IV	200	Cirrhosis of liver hepatic encephalopathy	Drug: L-ornithine L-aspartate drug: placebo	Improve hepatic encephalopathy and prevent serious side effects compared with placebo or no treatment	NCT01722578

(continued)

Table 15.1 (continued)

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
7	Steroid free immunosuppression in liver transplantation	IV	40	Liver cirrhosis liver transplant disorder	Drug: steroids Drug: basiliximab Drug: tacrolimus Drug: enteric-coated mycophenolic acid	Safe and effective as CS-containing immunosuppressive regimen in adult post-liver transplant	NCT00296244
8	Intestinal decontamination with rifaximin. The inflammatory and circulatory state in patients with cirrhosis	IV	54	Liver cirrhosis ascites	Drug: rifaximin Drug: placebo	No impact on the inflammatory state and only minor effects on bacterial translocation	NCT01769040
9	Norfloxacin in the primary prophylaxis of spontaneous bacterial peritonitis	IV	70	Spontaneous bacterial peritonitis ascites liver cirrhosis	Drug: oral norfloxacin	Significant increase in the 3-month and 1-year probability of survival	NCT00359853
10	The safety/efficacy of rifaximin with/without lactulose in subjects with a history of recurrent hepatic encephalopathy	IV	222	Hepatic encephalopathy cirrhosis	Drug: rifaximin Drug: rifaximin and lactulose	Combination is more efficacious than rifaximin alone	NCT01842581
11	Lactulose, L-ornithine L-aspartate, or rifaximin versus placebo for preventing hepatic encephalopathy in variceal bleeding	IV	87	Hepatic encephalopathy	Drug: lactulose Drug: L-ornithine L-aspartate Drug: rifaximin Drug: placebo	Prevent the development of HE in those with variceal bleeding	NCT02158182
12	Efficacy, safety, and pharmacokinetics of rifaximin in subjects with severe hepatic impairment and hepatic encephalopathy	IV	100	Hepatic encephalopathy	Drug: placebo Drug: rifaximin	No results reported	NCT01846663

expression of death receptors in activated HSCs, including Fas/CD95, TNF receptor 1 (TNFR1), p75NTR, and TRAIL receptors, could represent another way of stimulating HSC apoptosis [41–43].

Senescence: Some HSC-derived MFs may transform into senescent cells [44], which constitute a barrier to liver fibrosis due to stable cell cycle arrest. Several mechanisms have been suggested for induction of HSC senescence, including replicative exhaustion, overstimulation, and oxidative stress [41]. Recently, insulin-like growth factor-I (IGF-I) and IL-22 were found to induce HSC senescence, thus limiting liver fibrosis [45, 46].

Reversion: Recent studies suggested a new phenotype of HSCs, in which aHSCs undergo reversal to a quiescent-like phenotype [16, 47, 48]. Reverted HSCs exhibit downregulation of the fibrogenic genes collagen-α1, α-SMA, TGF-βRI, and TIMP1, and upregulation of some quiescence-associated genes to levels comparable to those seen in qHSCs. Human HSCs are also able to adopt an inactivated phenotype similar to the above results in mouse models [49]. This phenomenon may inform new therapeutic perspectives for liver cirrhosis.

15.4.2 Targeting Activated Myofibroblast and HSC-Specific Drug Delivery

Fibrolysis pathways target MFs and drug delivery to fibrogenic cells within the liver represents a major priority [38]. A number of clinical trials are currently in progress to assess the efficacy of potential agents (Table 15.2), including galectin 3, farnesoid X receptor (FXR), and combined peroxisome proliferator-activated receptor (PPAR)-α–PPAR-δ agonist.

Galectin 3 is required for TGF-βmediated MF activation and matrix production. Disruption of the galectin 3 gene [50] or treatment with galectin inhibitors significantly reduced fibrosis and reversed cirrhosis [51]. A phase I clinical trial of GR-MD-O2 (galectin 3 inhibitors) in patients with NASH and a phase II study to evaluate efficacy in patients with advanced fibrosis have been completed.

FXR signaling stimulates sensitivity to insulin, as well as fatty acid beta oxidation, and also reduces gluconeogenesis and lipogenesis in hepatocytes [52]. A large randomized phase II clinical trial of the FXR ligand obeticholic acid (OCA), for 72 weeks in 283 participants with biopsy-confirmed NASH, indicated clear improvement of NAFLD activity score and fibrosis stage [53].

A combined peroxisome proliferator-activated receptor (PPAR)-α–PPAR-δ agonist, GFT505, which showed high efficacy in multiple animal models [54], has entered phase IIb of a large randomized clinical trial in 270 participants. This agent resolves NASH without exacerbating fibrosis in patients with moderate to severe NASH [55], and a phase III trial conducted in 2000 participants began in 2016 (Table 15.2).

Despite potent activities of many antifibrotic drugs in vitro, only minor effects are observed in vivo due to nonspecific delivery. Therefore, many new therapies

Table 15.2 Current clinical trials phase I–IV for therapy targeting myofibroblasts and HSC

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
1	Safety and efficacy of selonsertib, GS-0976, GS-9674, and combinations in participants with bridging fibrosis or compensated cirrhosis due to nonalcoholic steatohepatitis	II	350	Nonalcoholic steatohepatitis	Drug: SEL Drug: GS-0976 Drug: GS-9674 Drug: SEL placebo Drug: GS-0976 placebo Drug: GS-9674 placebo	Pending	NCT03449446
2	Rollover study of cenicriviroc for the treatment of liver fibrosis in participants with nonalcoholic steatohepatitis	II	200	Nonalcoholic steatohepatitis Liver cirrhosis Non-alcoholic fatty liver disease	Drug: cenicriviroc (CCR2/CCR5 antagonist)	Pending	NCT03059446
3	AURORA: Phase 3 Study for the efficacy and safety of cenicriviroc (CVC) for the treatment of liver fibrosis in adults with NASH	III	2000	Nonalcoholic steatohepatitis	Drug: cenicriviroc (CCR2/CCR5 antagonist) Drug: placebo	Pending	NCT03028740
4	Phase 3 study to evaluate the efficacy and safety of elafibranor versus placebo in patients with nonalcoholic steatohepatitis (NASH)	III	2000	Nonalcoholic steatohepatitis (NASH) with fibrosis	Drug: elafibranor (PPAR-α and PPAR-δ agonist) Drug: placebo	Pending	NCT02704403

Table 15.2 (continued)

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
5	Randomized global phase 3 study to evaluate the impact on NASH with fibrosis of obeticholic acid treatment	III	2370	Non-alcoholic steatohepatitis (NASH)	Drug: obeticholic acid Drug: placebo	Pending	NCT02548351
6	Safety, tolerability, and efficacy of GS-4997 alone or in combination with simtuzumab (SIM) in adults with nonalcoholic steatohepatitis (NASH) and fibrosis stages F2-F3	II	72	Non-alcoholic steatohepatitis (NASH)	Drug: GS-4997 (ASK1 inhibitor) Biological: SIM	Improvement in fibrosis with reductions in liver stiffness, serum biomarkers of apoptosis and necrosis	NCT02466516
7	Phase 1 study to evaluate safety of GR-MD-02 in subjects with non-alcoholic steatohepatitis (NASH) and advanced fibrosis	I	31	Non-alcoholic steatohepatitis (NASH)	Drug: GR-MD-02 (galectin inhibitor) Drug: placebo	No results reported	NCT01899859
8	Clinical trial to evaluate the safety and efficacy of GR-MD-02 for the treatment of liver fibrosis and resultant portal hypertension in patients with Nash cirrhosis	II	162	Hypertension, portal	Drug: GR-MD-02 (galectin inhibitor) Drug: placebo	No results reported	NCT02462967

(continued)

Table 15.2 (continued)

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
9	Clinical trial to evaluate efficacy of GR-MD-02 for treatment of liver fibrosis in patients with NASH with advanced fibrosis	II	30	Nonalcoholic steatohepatitis	Drug: GR-MD-02 (galectin inhibitor) Drug: placebo	No results reported	NCT02421094
10	Efficacy and safety study of cenicriviroc for the treatment of NASH in adult subjects with liver fibrosis	II	289	Nonalcoholic steatohepatitis	Drug: cenicriviroc (CCR2/ CCR5 antagonist) Drug: placebo	No results reported	NCT02217475
11	Study of INT-747 as monotherapy in patients with PBC	II	59	Liver cirrhosis, biliary	Drug: placebo Drug: INT-747	Reduction of alkaline phosphatase levels, GGT ($p < 0.0001$), ALT ($p < 0.01$) compared to control	NCT00570765
12	Study of INT 747 in combination with URSO in patients with primary biliary Cirrhosis	II	165	Liver cirrhosis, biliary	Drug: INT-747 Drug: URSO Drug: placebo	Significant reduction of ALP, GGT, ALT	NCT00550862

have been developed targeting HSCs, a key component in the development of fibrosis, through receptors expressed on these cells, to increase the efficacy and reduce the disadvantages of nonspecific targeted therapies [56, 57]. Promisingly, Vitamin A-coupled liposomes specifically targeting HSCs completely reversed fibrosis in different experimental mouse models of liver injury [58]. Thus, the development of drug delivery systems targeting HSCs may represent a useful approach for inhibiting or reversing fibrosis in a therapeutic setting in clinical practice.

15.5 Control of Key Signal Transduction Pathways

15.5.1 Intracellular Signaling Pathways Mediating Liver Fibrogenesis

All in vitro and in vivo studies in cultured HSCs and experimental fibrogenesis using knockout mice, or human studies, indicated several intracellular pathways regulating fibrogenesis [59, 60], i.e., ERK, JNK, PI3K-Akt, TGF-β1, PDGF, PPAR-γ, and TLRs. Extracellular-regulated kinase (ERK) was induced in acute liver damage by CCL4 which mediates proliferation and chemotaxis of HSC, and modulates nuclear signaling [61]. c-Jun N-terminal kinase (JNK) activation occurs during toxic, metabolic, and autoimmune liver injury [62, 63] involved in HSC activation and fibrogenesis [64]. The focal adhesion kinase (FAK)-phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway is important for HSC migration, cell attachment, and collagen production [65]. The transforming growth factor (TGF)-β1-activated Smad signaling pathway stimulates experimental hepatic fibrosis [66], shows a close correlation between increased TGFβ-1 gene expression and the high-level collagen type I mRNA expression in the liver tissue of patients with cirrhosis [60], and is major pathway induced HSCs activation and fibrosis development [67]. Overexpression of TGF-β1 in transgenic mice results in fibrosis of multiple organs [21], and TGF-$\beta1^{-/-}$ mice show strong resistance to the development of liver fibrosis [68]. Platelet-derived growth factor (PDGF) is the most potent factor involved in stimulating HSC proliferation, differentiation, and migration [69]. The ligand-dependent transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ) pathway regulates HSC activation and experimental liver fibrosis. PPAR-γ ligands inhibit the fibrogenic actions in HSCs and attenuate liver fibrosis in vivo [70, 71]. Recent studies suggested a role for intracellular pathways signaled by toll-like receptors (TLRs) in liver fibrosis associated with hepatitis C infection [72], NASH and ASH [73], primary biliary cirrhosis [74], and cirrhosis [75, 76].

15.5.2 Targeting the Receptor–Ligand Interaction

Inhibition of the signal transduction pathways involved in liver fibrogenesis, as mentioned above, likely has the potential to treat liver fibrosis [77]. Blockade of TGF-β1 synthesis or signaling is a primary target for the development of antifibrotic approaches [68]. However, because TGFβ also regulates homeostatic functions including growth suppression, systemic inhibition of TGFβ could enhance the development of neoplasia. Therefore, selective blockade of the TGFβ pathway by targeting cell surface molecules involved in its activation is especially

appealing. An inhibitory antibody to αvβ6 integrin (an activator of latent-TGF-β1) (STX-100, ClinicalTrials.gov ID # NCT 01371305) is currently being tested in idiopathic pulmonary fibrosis (IPF). Antifibrotic activity of another drug approved for IPF, pirfenidone, is also being tested in cirrhotic patients in a phase II clinical trial [78].

Targeting the PDGF signaling pathway also holds therapeutic promise [69]. Sorafenib and nilotinib are the typical representatives of fibrosis inhibitors through the PDGF pathway [69, 79]. Sorafenib is a first-line oral chemotherapy drug for patients with advanced hepatocellular carcinoma, sorafenib has previously been demonstrated to be a potential antifibrotic agent, due to its multi-targeting of the Ras/MEK/ERK pathway [79].

Targeting of intracellular signaling via nuclear receptors, such as PPAR-γ and FXR, was described in Sect. 15.4.2. The discovery of membrane and nuclear receptors expressed by HSCs, which have been previously identified in other tissues, has opened new avenues for antifibrotic therapies, including those targeting the renin–angiotensin system, and serotonin, cannabinoid, and endothelin 1 receptors [80, 81].

15.5.3 Combination Therapy

Combination therapies that address liver fibrosis via a multipronged approach hold a great deal of promise for future treatment, ideally by targeting interactions between cells, soluble mediators, the ECM and its receptors, and/or relevant intracellular signaling pathways [80]. Under conditions wherein the etiology of liver fibrosis cannot be eradicated, therapies for liver fibrosis may help to restrict disease progression to cirrhosis and reduce the risk of cirrhosis-related complications [82]. Table 15.3 lists ongoing phase III and IV clinical trials for liver cirrhosis using combination therapies (http://www.ClinicalTrials.gov).

15.6 Antioxidant Therapy

15.6.1 The Antioxidant System in Liver Cirrhosis

Oxygen free radicals, more generally known as reactive oxygen species (ROS), along with reactive nitrogen species (RNS) represent the most important group of radical species generated in living systems [83]. ROS and RNS play an important role in the establishment of fibrosis and subsequently in cirrhosis [84]. Both animal models of chronic liver injuries/fibrosis and human studies in fibrotic/cirrhotic patients showed direct detection of ROS/RNS in liver specimens [85–89]. Furthermore, fibrogenic progression is associated with a significant decrease and/or

Table 15.3 Current clinical trials phase II–IV for combination therapies

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
1	Treatment of liver cirrhosis due to hepatitis B virus with Fuzheng Huayu and entecavir	IV	700	Liver cirrhosis due to hepatitis B virus	Drug: entecavir + placebo Drug: entecavir + Fuzheng Huayu tablet	No result reported	NCT02241590
2	Traditional Chinese medicine combined with entecavir to treat refractory liver fibrosis in liver cirrhosis due to HBV	IV	350	Hepatitis B virus Related cirrhosis	Drug: entecavir+Fuzheng Huayu+TCM granule	No result reported	NCT02241616
3	Antifibrotic activity of GI262570 in chronic hepatitis C subjects	II	265	Cirrhosis, liver	Drug: GI262570 0.5 mg (PPARγ agonist) Drug: GI262570 1.0 mg Drug: placebo	No result reported	NCT00244751
5	The effectiveness and safety for mesenchymal stem cell for alcoholic liver cirrhosis	II	12	Alcoholic liver cirrhosis	Biological: mesenchymal stem cell injection	Recruiting	NCT01741090
6	Pirfenidone, an antifibrotic and anti-inflammatory drug	II	150	Fibrosis hepatitis C chronic	Drug: pirfenidone Drug: matched equivalent placebo	Improves inflammation, fibrosis and steatosis	NCT02161952
7	Safety and efficacy study of interferon gamma-1b in hepatitis C patients with liver fibrosis or cirrhosis	II	502	Liver fibrosis Cirrhosis	Drug: interferon gamma-1b	Not able to reverse fibrosis in patients with advanced liver disease for 1 year	NCT00043303

(continued)

Table 15.3 (continued)

No	Title	Phase	Participants	Conditions	Interventions	Results	ID
8	Phase 4 study of obeticholic acid Evaluating clinical outcomes in patients with primary biliary cholangitis	IV	428	Liver cirrhosis, biliary	Drug: obeticholic acid (OCA) Drug: placebo	Recruiting	NCT02308111
9	Prevention of decompensation in liver cirrhosis	IV	100	Alcoholic liver cirrhosis Ascites	Drug: losartan (drug)	No result reported	NCT00239096
10	Hemodynamic effect of simvastatin with beta blockers in clinical portal hypertension	IV	60	Liver cirrhosis Portal hypertension	Drug: simvastatin Drug: placebo	No result reported	NCT01282385
11	Effect of candesartan in alcoholic liver fibrosis	II	85	Alcoholic liver disease	Drug: candesartan for hepatic fibrosis	Improvement of fibrosis in histological and quantitative measurements	NCT00990639

depletion of antioxidant defenses. Vitamin E depletion was found in both carbon tetrachloride-injured rats [90] and in patients with parenchymal liver cirrhosis [91]. Therefore, the use of molecules with antioxidant properties has been proposed to treat fibrosis and cirrhosis caused by oxidative stress.

15.6.2 Antioxidant Therapy in Liver Fibrosis and Cirrhosis

In experimental models of liver fibrosis/cirrhosis, antioxidant compounds include food supplements and drugs, such as polyunsaturated phosphatidylcholine (PPC) [92], peroxisome proliferator-activated receptor (PPAR) α ligand [93], ursodeoxycholic acid [94], and resveratrol [95–98] have been tested. A recently discovered vertebrate globin, cytoglobin (CYGB) [99], the molecular characteristics of which are similar to those of myoglobin, is also an antioxidant due to its NO scavenging activity. CYGB may facilitate diffusion of oxygen through tissues, scavenge NO or other ROS, or serve a protective function during oxidative stress [100].

In human trials, *S-Adenosylmethionine* (SAMe), silymarin, and vitamin E are used in liver fibrosis/cirrhosis patients. SAMe has already shown beneficial effects in liver transplantation patients with alcoholic liver cirrhosis, improving survival or delaying the need for operation [101], and improves bilirubin and alkaline phosphatase levels of cholestasis [102]. Use of silibinin, the major active constituent of *silymarin***,** in hepatic cirrhosis results in improvement in antioxidant status, cytoprotection, reversal of fibrosis, and regeneration [103], greater total glutathione concentrations and concurrent decreases in N-terminal propeptide of type III collagen, a biomarker for hepatic fibrosis [104], and decreased mortality rates [105]. The effect of vitamin E in fibrosis/cirrhosis patients was reported in ASH- or NASH-induced fibrosis, in which the histological findings, such as steatosis, inflammation, and fibrosis, of the NASH patients were improved [106]. The most promising results were from PIVEN trial [13] performed in 247 patients for 96 months in which vitamin E led to clear histological regression, with no fibrosis progression.

Currently, the ClinicalTrials.gov website lists 14 early and phase I–IV clinical trials of current antioxidative therapies using antioxidants for liver cirrhosis. In these studies, vitamins, especially vitamin E, are the most frequently studied antioxidants as dietary supplements (Table 15.4).

In summary, antioxidant therapy targets (1) recovery of antioxidant enzymes/compounds and (2) reduction in the production of ROS and RNS. Despite the clear effects of antioxidant therapy in animal models, human trials still showed inconsistent results. However, silymarin (or silybin) and vitamin E, in both single and combination therapy, were the most successful antioxidant approaches for liver fibrosis and cirrhosis patients.

Table 15.4 Current clinical trials phase I–IV for liver cirrhosis using antioxidant therapies

No	Title	Phase	Participants	Conditions	Intervention	Results	ID
1	Effects of dark vs. white chocolate on the postprandial increase in portal pressure in cirrhosis	II	22	Cirrhosis; portal hypertension	Dietary supplement: dark chocolate|dietary supplement: white chocolate	Improving flow-mediated hepatic vasorelaxation and ameliorated systemic hypotension	NCT01408966
2	Vitamin E supplement in patients with cirrhosis and acanthocytosis	II	27	Cirrhosis	Dietary supplement: vitamin E supplement (tocofersolan)	Overall well tolerated but did not affect erythrocyte membrane lipid composition	NCT01463735
3	Simvastatin in preventing liver cancer in patients with liver cirrhosis	II	80	Cirrhosis	Drug: simvastatin	Recruiting	NCT02968810
4	Defined green tea catechin extract in preventing liver cancer in patients with cirrhosis	I	48	Cirrhosis	Drug: defined green tea catechin extract Other: laboratory biomarker analysis Other: pharmacological study	Recruiting	NCT03278925
7	Ropinirole for the treatment of muscle cramps in patients with cirrhosis	IV	60	Muscle cramp cirrhosis	Drug: vitamin E Drug: ropinirole Other: muscle cramp survey	Recruiting	NCT03176966
8	Treatment of severe alcoholic hepatitis with corticoids plus N acetyl cysteine versus corticoids alone	III	174	Alcoholic hepatitis	Drug: corticoids plus N acetyl cysteine	Increased 1-month survival among patients with severe acute alcoholic hepatitis, 6-month survival	NCT00863785

9	Sorafenib in treating patients with locally advanced or metastatic liver cancer and cirrhosis	I	6	Liver cancer	Drug: sorafenib tosylate	No result reported	NCT00767468
10	Silybin—vitamin E—phospholipids complex reduces liver fibrosis in patients with chronic hepatitis C treated with Peg-IFN-a and RBV	III	64	Liver fibrosis	Drug: silybin 94 mg + vitamin E 90 mg + phospholipids 194 mg complex Drug: placebo	Reduced serum levels of markers of liver fibrosis	NCT01935817
11	Efficacy and safety of pentoxyphilline and tocopherol on the fibrosis in patients with chronic hepatitis C	III	100	Hepatitis C, chronic liver fibrosis	Drug: pentoxyphilline Drug: tocopherol	No result reported	NCT00119119
12	Pilot study of pentoxifylline for hepatopulmonary syndrome	I	9	Hepatopulmonary syndrome	Drug: pentoxifylline	No significant change in PaO(2) or A-a PaO(2) with treatment	NCT00593658
13	Pegfilgrastim in patients with alcoholic hepatitis	II	78	Alcoholic hepatitis	Drug: standard of care + pegfilgrastim Drug: standard of care	Recruiting	NCT02776059
14	Efficacy and tolerance of treatment with DHA, choline and vitamin E in children with non-alcoholic steatohepatitis	III	60	Fatty liver; liver fibrosis; obesity; metabolic syndrome; nonalcoholic fatty liver disease	Drug: docosahexaenoic acid plus vitamin E plus CHOLINE Drug: placebo pearls	Improve steatosis and reduce ALT and glucose levels in children with NASH	NCT01934777

15.7 Conclusion

Currently, antifibrotic drug is a matter of concern with more than 600 clinical trials are now ongoing studies. One of the trigger for this interest is the recognition of the central role of hepatic stellate cells in liver fibrosis. Promising approaches to removing fibrogenic cells are being evaluated, including development of drug delivery systems that target activated HSCs. In parallel, inhibition of intracellular signaling pathway by target receptor-ligand is of interest. Furthermore, it is interesting to use the well-known safety drugs which use for other indicators but also have the antifibrotic activities, such as anti-IL4/13, and recently new direct anti-viral agents for hepatitis C and B originally used to target viral replication but now being explored as potential antifibrotic therapies. Most importantly, the efficacy of antifibrotic drugs known to attenuate experimental liver fibrosis should be tested in humans. Hopefully, the best drug for antifibrotic therapy will be discovered in the near future.

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Chapter 16
Liver Transplantation for Liver Cirrhosis

Yuhei Hamaguchi and Toshimi Kaido

Abstract Liver transplantation (LT) is the only curative treatment that can increase the chances of long-term survival in patients with end-stage liver disease including liver cirrhosis (LC), acute liver failure, or advanced hepatocellular carcinoma (HCC). Especially in patients with LC and HCC, LT is an ideal treatment, allowing not only resection of the HCC, but also provision of normal liver in place of damaged liver that would promote multicentric carcinogenesis. LT includes deceased donor LT and living donor LT. In this chapter, we first introduce current status and results of LT, and then discuss recent issues in LT for decompensated liver cirrhosis.

Keywords Liver cirrhosis · Living donor liver transplantation · Deceased donor liver transplantation · Hepatocellular carcinoma · Sarcopenia · Nutritional therapy Rehabilitation

16.1 Introduction

Recent advances in medical treatment have improved quality of life and prognosis in patients with liver cirrhosis. However, many patients still suffer from pathologies ranging from decompensated liver cirrhosis to liver failure without responding to medical treatment. Liver transplantation (LT) is the only curative treatment that can increase the chances of long-term survival in patients with end-stage liver disease, acute liver failure, or advanced hepatocellular carcinoma (HCC). Especially in patients with HCC, LT is an ideal treatment, allowing not only resection of the HCC, but also provision of normal liver in place of damaged liver that would promote multicentric carcinogenesis. LT includes deceased donor LT (DDLT) and living donor LT (LDLT). DDLT is carried out using the whole liver or only a part (split-liver transplantation) donated after brain death. On the other hand, only the right or

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left lobe from the living donor is generally used in LDLT. Although DDLT accounts for most LTs in Europe and the USA, approximately 90% of LTs are LDLTs in Asia, especially in Japan. In this chapter, we first introduce current status and results of LT, and then discuss recent issues in LT for decompensated liver cirrhosis.

16.2 Current Status and Results of LT

Indications for LT are end-stage liver diseases that cannot be treated with internal medicines or other surgical procedures, including hepatitis B virus (HBV)- or hepatitis C virus (HCV)-associated liver cirrhosis, progressive intrahepatic cholestatic diseases including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), alcoholic liver cirrhosis, biliary atresia, autoimmune hepatitis, hereditary metabolic diseases, HCC in liver cirrhosis without distant metastasis and vascular invasion within Milan criteria, and acute liver failure. In the USA, a total of 7127 adult LTs were performed in 2015, including 6768 DDLTs and 359 LDLTs, and HCV-associated liver cirrhosis was the most common indication for LT [1]. According to that report, 6-month and 1-year graft failure rates in DDLT were 7.8% and 10.3%, respectively. Among patients who underwent LT in 2008–2010, the 5-year overall survival (OS) rate was 73.6%. On the other hand, about 450 LTs have been recently performed per year in Japan, and the total number of LTs by the end of 2016 was 8825; of these, 8447 cases (95.7%) involved LDLT and 378 (4.3%) involved DDLT [2]. Decompensated liver cirrhosis including HBV- or HCV-associated liver cirrhosis, progressive intrahepatic cholestatic diseases, and HCC accounted for about 50% of the underlying diseases in LT recipients. One- and 5-year OS rates were not significantly different between LDLT and DDLT (84.7% vs. 87.4% in 1-year OS, 78.2% vs. 81.6% in 5-year OS). In LDLT, OS rates in male recipients, adult cases, and re-transplantations were significantly lower than in female recipients, pediatric case, and primary transplantations, respectively. In analyses according to original diseases, 5-year OS rates in patients with cholestatic disease (86.1%) or metabolic disease (86.1%) were significantly higher than in patients with hepatocellular disease (74.8%), neoplastic disease (70.1%), or acute liver failure (71.1%). By donor age, prognosis in recipients from older donors was significantly worse than in recipients from younger donors. Although the OS in ABO-incompatible cases was significantly lower than in ABO-compatible cases, recent advances in peri-transplant treatment including rituximab prophylaxis have improved the prognosis in ABO-incompatible cases [2].

Since this increasing demand for LT has resulted in a worldwide shortage of available liver grafts, construction of a prognostic model for use in patients undergoing LT is crucial to optimize the allocation of the limited number of liver grafts to appropriate recipients. During 2015, 1673 patients died without LT and another 1227 were removed from the waiting list as too sick for LT [1]. In DDLT, the model for end-stage liver diseases (MELD) scoring system, which is calculated using three objective parameters of international normalized ratio (INR) of prothrombin time,

serum bilirubin, and serum creatinine, has been adopted to allocate organs in the USA since 2002 [3, 4]. In 2003, MELD score was shown to be superior to the Child-Pugh score in the ability to predict 3-month mortality among patients awaiting LT [3, 5]. MELD score has thus replaced the Child-Pugh score for predicting prognosis and for allocation of liver grafts in patients with liver cirrhosis [3, 4].

On the other hand, because recipients for LDLT have specific donors, a strict allocation system like the MELD score in DDLT is not necessarily required in LDLT. Many hospitals therefore evaluate and determine the indications of recipients and donors for LDLT according to selection criteria specific to the institution, including factors such as patient age, relationship between recipients and donors, ABO-compatibility, and lower limit of graft size.

16.3 Modulation of Portal Venous Pressure

Donor safety and favorable outcomes of recipients after LT are the most important priorities of LDLT. In LDLT, the incidence of all-donor complications including donors for pediatric recipients has been reported to be higher when using right ($n = 500$, 44.2%), compared with left or extended lateral lobe grafts ($n = 762$, 18.8%; $p < 0.001$) [6]. Left lobe grafts are thus preferable because of the lower complication rates and larger remnant liver, ensuring donor safety. However, the lower limit of graft-to-recipient weight ratio (GRWR) and the risk of small-for-size syndrome are critical problems that need to be overcome when using left lobe grafts. To use smaller size grafts, intentional modulation of portal venous pressure (PVP) during LDLT has been introduced, and we have previously reported that modulation of PVP to <15 mmHg enabled safe transplantation of smaller grafts [7]. Figure 16.1

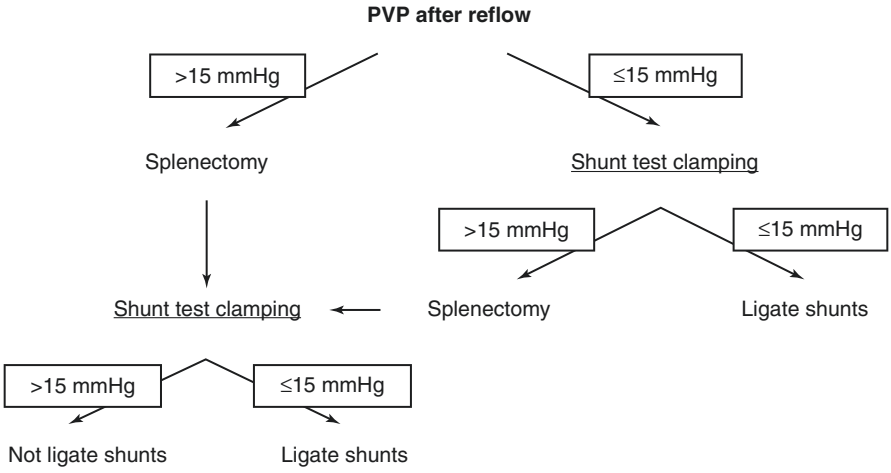


Fig. 16.1 Current strategy for intentional portal pressure control

shows a flowchart for PVP control. If portal pressure is >15 mmHg after reflow of the liver graft, intentional portal pressure control is used by concurrent splenectomy. Portosystemic collaterals with diameter larger than approximately 1 cm, such as splenorenal shunts, gastric/esophageal varices, and inferior mesenteric venous varices, are temporarily clamped and then ligated if PVP is ≤15 mmHg. If PVP is >15 mmHg on temporary clamping of portosystemic collaterals, we perform a splenectomy. After the splenectomy, portosystemic collaterals are ligated when PVP is ≤15 mmHg on temporary clamping of portosystemic collaterals. When PVP is >15 mmHg on temporary clamping of portosystemic collaterals after splenectomy, the collaterals are not ligated. We have gradually lowered our criteria for GRWR to 0.6% with PVP modulation [8].

16.4 LT for HCC

LT is a radical treatment for HCC because the procedure not only resects the disease, but also replaces the underlying damaged liver with normal tissue, simultaneously addressing both intrahepatic metastases and multicentric carcinogenesis. In Western countries, with the advent of the Milan criteria [9], DDLT for HCC has achieved favorable survival rates comparable to those for nonmalignant liver diseases. In contrast, due to the critical shortage of deceased donor organs, LDLT has great significance in Eastern countries including Japan, Korea, and China. The category of LT and the concept of selection criteria in LT for HCC thus differ markedly between Western and Eastern countries. Since the introduction of the Milan criteria, some expanded criteria focusing on tumor size and number have been proposed, including the University of California San Francisco (UCSF) criteria (a single lesion ≤6.5 cm in diameter or 2–3 lesions ≤4.5 cm with total diameter ≤ 8 cm on the basis of preoperative radiological data) [10], the 5–5 rule (up to five nodules with a maximum diameter of 5 cm) [11], and the up-to-7 rule (HCC with seven as the sum of the diameter of the largest tumor in centimeters and the number of tumors) [12]. In addition, new expanded criteria considering tumor biology have been established using tumor markers and ^{18}F-fluorodeoxyglucose position emission tomography. Based on target outcomes of a 5-year survival rate ≥80% and a 5-year recurrence rate ≤10%, the Kyoto group in Japan proposed new selection criteria, the Kyoto criteria, combining three independent significant risk factors for recurrence (tumor number and tumor size based on the findings from pretransplant imaging, and concentrations of tumor markers): tumor number ≤10; maximal diameter of each tumor ≤5 cm; and serum des-γ-carboxyprothrombin level ≤400 mAU/ml [13]. In a retrospective analysis, 147 patients who met the Kyoto criteria showed significantly lower 5-year recurrence rates (4%) than 49 patients who exceeded them (51%, $p < 0.001$; Fig. 16.2a). Similarly, 5-year survival rates in patients within the Kyoto criteria (82%) were significantly higher compared with patients exceeding the criteria (42%; $p < 0.001$; Fig. 16.2b) [14].

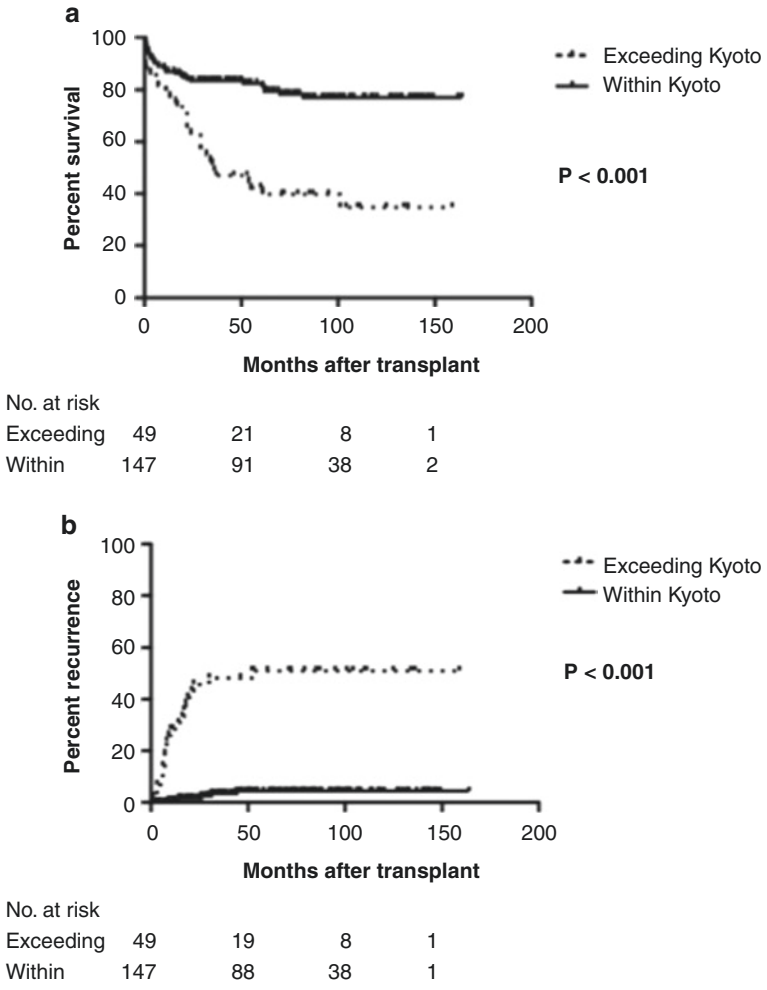


Fig. 16.2 Overall survival rates (**a**) and recurrence rates (**b**) after liver transplantation for hepatocellular carcinoma according to the Kyoto criteria

16.5 Recurrence of Primary Liver Disease

Cirrhosis secondary to HCV infection is one of the main indications for LT. Recurrent hepatitis C infection of the allograft is universal if HCV is detectable at the moment of LT. Approximately one-third of patients progress to liver cirrhosis in the graft within only 5 years after LT, and graft and patient survivals are significantly worse in patients undergoing LT for HCV-related cirrhosis than in those undergoing transplant for other reasons [15, 16]. Two strategies, including pre- and post-transplant treatment of HCV infection, can be adopted for

achieving sustained virologic response (SVR), virus eradication and finally improving clinical outcomes of recipients with HCV infection. With direct antiviral agents, almost all HCV-infected patients can now be cured either before or after LT [17, 18]. In the future, HCV infection may no longer affect long-term outcomes after LT.

Long-term administration of anti-HBs immunoglobulins (HBIGs) is a major treatment for the prevention of HBV recurrence after LT [19]. Most LT centers use antiviral agents (entecavir or tenofovir) with or without concomitant administration of HBIGs. These therapies have drastically reduced HBV recurrence, resulting in excellent long-term outcomes [20].

Recurrence of PBC is uncommon and rarely responsible for graft loss [21]. On the other hand, PSC recurrence has been reported to occur in 10–30% of recipients after LT [21]. One recent study found PSC recurrence in 40% of patients at a median of 30 months after LT [22]. Cumulative incidences of PSC recurrence were 24.5% at 3 years, 39.3% at 5 years, and 45.8% at 6 years. No specific intervention has been found to be effective in addressing PSC recurrence, with re-transplantation as the only option in patients developing such recurrence.

16.6 Sarcopenia in LT

Protein energy malnutrition (PEM) is a common problem in patients with end-stage liver disease awaiting LT. PEM is more prevalent in those with decompensated liver disease, that is, those with ascites, portosystemic hepatic encephalopathy (HE), or portal hypertensive bleeding. PEM is also more frequent among patients hospitalized for alcoholic liver disease than among patients with nonalcoholic liver disease [23]. PEM can significantly increase the operative risk at the time of surgery and represents a risk factor for morbidity and short- and long-term mortality in patients undergoing LT, and decreased graft survival after LT [23–25]. Recently, sarcopenia has been defined as a pathology characterized by a progressive and generalized loss of skeletal muscle mass and strength. This concept has attracted much attention and sarcopenia has been shown to represent an independent risk factor for lower overall and disease-free survivals in various diseases [26]. Sarcopenia is classified according to cause as primary when no cause other than aging is evident, or as secondary. With secondary sarcopenia, disease-related sarcopenia is associated with advanced organ failure, including failure of the liver or heart. Nutrition-related sarcopenia results from insufficient dietary intake of energy or protein. Patients with end-stage liver disease requiring LT usually have liver failure and poor nutrition. As a result, most patients undergoing LT meet the criteria for secondary sarcopenia. To date, several studies have shown that sarcopenia in LT is associated with mortality, increased stay in hospital and in the intensive care unit, failure to achieve rescue, post-transplantation sepsis, and infection [27]. Kaido et al. evaluated skeletal muscle mass (SMM) using bioelectrical impedance analysis in 124 patients undergoing LDLT and identified patients with low SMM before transplant had significantly

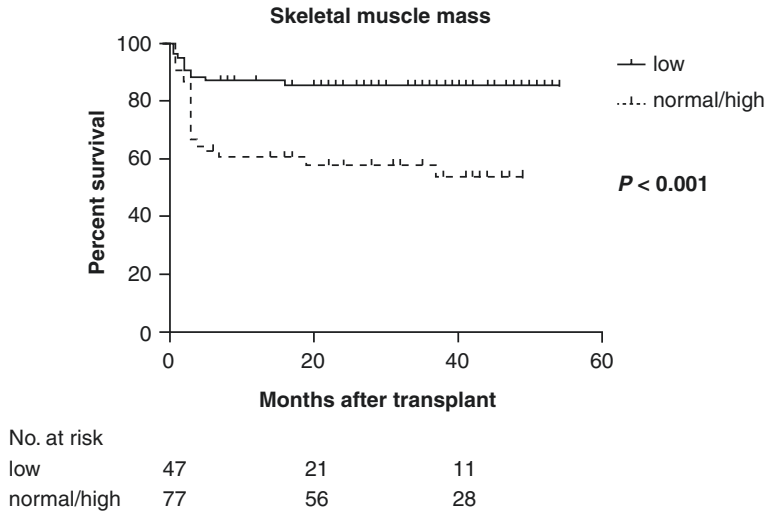
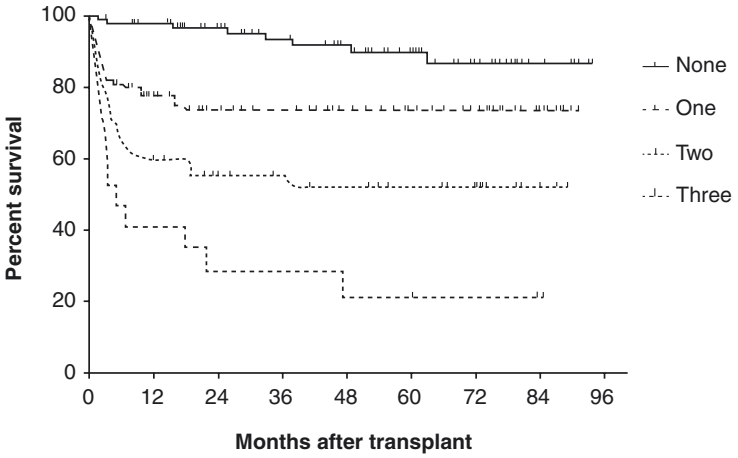


Fig. 16.3 Overall survival rates according to skeletal muscle mass in patients undergoing living donor liver transplantation

worse survival compared with patients with high SMM (Fig. 16.3) [28]. On the other hand, the increase of intramuscular adipose tissue (IMAT) with aging has been identified as a potential contributor to declining strength and quality of muscle, one of the components of sarcopenia [29]. Skeletal muscle steatosis as measured by intramuscular adipose tissue content (IMAC) has been linked to the pathogenesis and severity of non-alcoholic steatohepatitis [30, 31]. Using preoperative computed tomography (CT), we evaluated IMAC and psoas muscle mass index (PMI) in 200 adult patients undergoing LDLT [32]. OS rates in patients with a high IMAC (muscle steatosis) or low PMI (low SMM) were significantly lower than in patients with normal IMAC or PMI ($p < 0.001$ each).

Based on our previous findings regarding the impact of pretransplant nutritional status, including skeletal muscle mass, in January 2013 we added the new criterion of "inability to walk unaided" to exclude patients with severe sarcopenia from LT. After implementation of the new criteria, the 1-year OS rate after LT significantly improved to 94% compared with the rate under the previous criteria (71%) [33]. Interestingly, patient background, including MELD score and Child-Pugh classification, did not significantly differ between patients in this cohort and that of our previous retrospective cohort. In other words, outcomes have dramatically improved with the addition of only one criterion, excluding patients who cannot walk unaided, even though the severities of patient condition and underlying liver disease did not differ between before and after revision of our criteria. The new exclusion criterion of inability to walk unaided is a simple criterion to exclude patients with severe sarcopenia without using any devices. However, the criterion is somewhat lacking in objectivity. We therefore tried to establish a more objective criterion to judge sarcopenia. Recently, using CT from 657 donors for LDLT,



No. at risk				
None	102	70	47	22
One	108	52	37	23
Two	50	22	17	11
Three	17	5	4	3

Fig. 16.4 Overall survival rates in patients classified by the number of the body composition variables

skeletal muscle mass, muscle quality, and visceral adiposity were evaluated using skeletal muscle mass index (SMI), IMAC, and visceral-to-subcutaneous adipose tissue area ratio (VSR). Sex-specific cut-offs for SMI, IMAC, and VSR were determined, and correlations with outcomes after LDLT in 277 recipients were examined with the aim of establishing new selection criteria for LDLT [34]. The OS rate was significantly lower for each group of patients with low SMI ($p < 0.001$), high IMAC ($p < 0.001$), or high VSR ($p < 0.001$) compared to the respective normal groups. In addition, low SMI, high IMAC, and high VSR contributed to an increased risk of post-LDLT mortality in an additive manner (Fig. 16.4). Patients beyond all three cut-offs ($n = 17$, 6.1%) showed the lowest survival rate after LDLT (1-year OS rate, 41.2%; $p < 0.001$). Based on these results, we have excluded patients beyond all 3 cut-offs (low SMI, high IMAC, and high VSR) as candidates for LDLT since October 2016. The 1-year OS rate after LT further improved to 96% after adopting this new selection criterion.

16.7 Nutritional and Rehabilitation Therapy in LT

Nutritional status can worsen rapidly during the postoperative period due to preoperative malnutrition, surgical stress, immunosuppressive therapy, post-interventional complications, postoperative protein catabolism, and fasting periods. The main goals

of pre-LT nutritional therapy are to prevent further nutrient and muscle depletion and to correct any vitamin and mineral deficiencies present to minimize the risks of infection and debility. An early, planned, preoperative nutritional intervention can be performed in most cases of LDLT, since the date of LT is known in advance, unlike in DDLT. Nutritional therapy, as well as rehabilitation at the time of referral of the potential recipient, should start a few months before LT to most effectively increase the SMM [23]. Kaido et al. reported that perioperative nutritional therapy significantly increased OS in patients with low skeletal muscle mass ($p = 0.009$) [28]. For adult recipients preparing for LDLT, Kaido et al. described a detailed preoperative nutritional therapy regimen [28]. This regimen starts approximately 2 weeks before LDLT after the bioelectrical impedance analysis (BIA) assessment. The therapy consists of three components: a nutrient mixture enriched with branched-chain amino acids (BCAAs) or BCAA nutrients as a late evening snack; synbiotics using a supplementation product enriched with glutamine, dietary fiber, and oligosaccharide three times daily, and a lacto-fermented beverage containing 5×10^8/mL of *Lactobacillus casei* strain Shirota once a day via feeding tube or orally until discharge. Additionally, patients with a low serum zinc level receive 1.0 g/day of polaprezinc. Dietitians adjust the type and amount of food for each patient to maintain a total caloric intake of 30–35 kcal/kg/day and a protein intake of 1.2–1.5 g/kg/day, including BCAA nutrients, in accordance with the guidelines of the European Society of Parenteral and Enteral Nutrition [35]. A tube jejunostomy for enteral nutrition is placed in the proximal jejunum using a 9-French enteral tube in all recipients at the time of surgery. Postoperative early enteral nutrition is started within the first 24 h after surgery through the tube jejunostomy. We gradually increase the total daily caloric intake until postoperative day (POD) 3, from 10–15 kcal/kg/day to 25–35 kcal/kg/day. As an enteral nutrient, we prefer a new immunomodulating diet, MEIN, which is a protein complex derived from milk and enriched with hydrolyzed whey peptide (Meiji Dairies Co., Tokyo, Japan). Enteral feeding is stopped when the patient can tolerate adequate oral intake containing solid food. All patients are supplemented with synbiotics via the feeding tube or orally until the patient can consume a sufficient diet.

All patients undergo preoperative rehabilitation including pulmonary rehabilitation, evaluation of swallowing function, and physical therapy. All patients also routinely undergo postoperative rehabilitation delivered by physical therapists at the bedside in the intensive care unit, usually from POD 2 or 3 until the patient is able to walk.

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Chapter 17
Regenerative Therapy for Liver Cirrhosis

Toshihiko Matsumoto, Taro Takami, and Isao Sakaida

Abstract Liver cirrhosis is the end stage of chronic liver disease and causes serious complications such as ascites, encephalopathy, and portal hypertension. The only radical treatment currently available is liver transplantation, but issues such as a shortage of donors, long-term immunosuppression, and high lifelong medical costs limit the feasibility of liver transplantation. To overcome these limits to the feasibility of liver transplantation, liver regeneration therapy through cell and stem cell transplantation, bioartificial liver systems, and organ bioengineering are advancing. This chapter will describe the current status and perspective of liver regeneration therapy for liver cirrhosis.

Keywords Liver cirrhosis · Liver regeneration · Stem cell · Granulocyte colony-stimulating factor · Hepatocyte-like cell · Bioartificial liver system · Bioengineered liver

17.1 Introduction

Recent advancements in antiviral drugs have enabled viral clearance and control of hepatitis in many viral cirrhosis patients [1–4]. However, the incidence of liver cirrhosis (LC) caused by alcohol consumption or nonalcoholic steatohepatitis continues to increase, and it is one of the main factors behind the number of patients awaiting liver transplants in the USA [5]. Liver transplantation is the only treatment with which a radical cure can be expected, but issues such as a shortage of donors,

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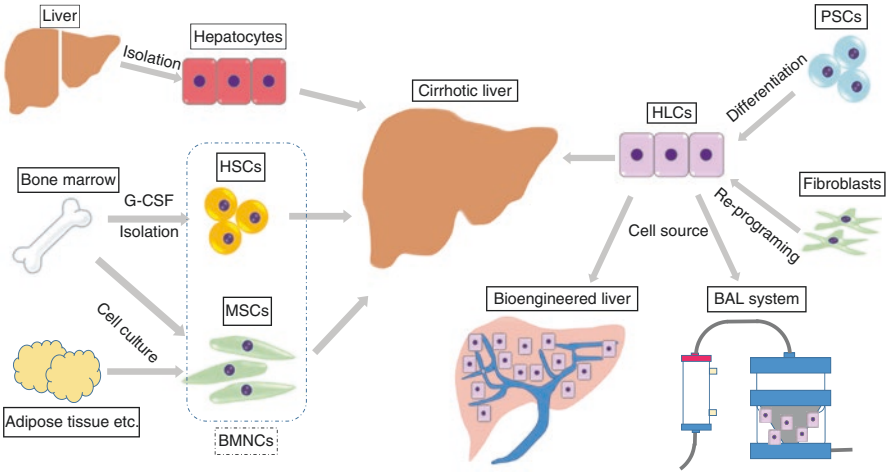

Fig. 17.1 Current status and perspective of liver regeneration therapy for LC. Cell and stem cell therapies for LC patients and new technologies including stem cell-derived hepatocyte-like cells, BAL systems, and organ bioengineering as the next step in liver regenerative medicine. *LC* liver cirrhosis, *G-CSF* granulocyte colony-stimulating factor, *HSC* hematopoietic stem cell, *MSC* mesenchymal stem cell, *BMNC* bone marrow mononuclear cell, *HLC* hepatocyte-like cell, *PSC* pluripotent stem cell, *BAL* bioartificial liver

long-term immunosuppression, and high lifelong medical costs limit the feasibility of liver transplantation. To overcome these limits to the feasibility of liver transplantation, liver regeneration therapy through cell and stem cell transplantation, bioartificial liver (BAL) systems, and organ bioengineering are advancing. These therapies are more convenient than organ transplantation in a number of respects, including less invasiveness, use of autologous cells without immunological rejection, and multiple transplants. This chapter will initially describe the current state of cell and stem cell therapy for LC patients, and then introduce stem cell-derived hepatocyte-like cells, BAL systems, and organ bioengineering as the next step in liver regenerative medicine (Fig. 17.1).

17.2 Cell and Stem Cell Therapy

The healthy liver has a high regenerative capacity and is notably resistant to damage. However, LC causes a decrease in the number of liver parenchymal cells and architectural distortion due to deposition of the extracellular matrix (ECM), and the resulting excessive scar formation prevents the proliferation of hepatocytes [6]. Consequently, the regenerative capacity of the cirrhotic liver is decreased. To recover this lost regenerative capacity, cell transplantation or stem cell transplantation can be performed. Supplementation of hepatocytes and resolution of liver fibrosis are important therapeutic targets when attempting to promote liver regeneration.

17.2.1 Hepatocyte Transplantation

Hepatocyte transplants are performed with the aim of supporting the synthetic functions of the liver, as well as to achieve detoxification. The general approaches are autologous transplantation of hepatocytes isolated from a single lobe of the recipient's cirrhotic liver or allogeneic transplantation of donor hepatocytes from noncirrhotic liver donors. In initial hepatocyte transplantation for LC, autologous hepatocytes isolated from resected liver were transplanted by means of intrasplenic injection [7]. Intrasplenic injection has been the most common method of hepatocyte delivery, because the risk of portal hypertension increased after portal infusion of cells into a cirrhotic liver. Following autologous hepatocyte transplantation, many cases of allogeneic hepatocyte transplantation for LC have been reported, and while the effects of hepatocyte transplantation are modest, its clinical safety has been confirmed [8, 9]. The reasons for the modest effects include the difficulty of isolating high-quality hepatocytes from suboptimal donor livers, the difficulty of achieving long-term effects due to low survival and growth rates of transplanted hepatocytes, the inability to increase cells through culturing, and the difficulty of cryopreservation. Transplantation of hepatocytes into extrahepatic sites, such as a lymph node, may be effective, but this approach has not been tested clinically [10]. Due to these difficulties, attempts are currently being made to transplant stem cells that can stimulate endogenous liver regeneration and fibrolysis, rather than directly supplementing liver parenchymal cells through hepatocyte transplantation.

17.2.2 Stem Cell Transplantation

The existence of Y chromosome-positive hepatocytes in female recipients of therapeutic bone marrow transplantations with male donors was reported in 2000, suggesting that human hepatocytes can be derived from stem cells originating in the bone marrow [11]. Subsequently, stem cells have attracted significant attention as a cell source for liver regenerative therapies. The bone marrow contains stem cells, such as hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Although an effective mechanism for infusion of HSCs and MSCs to improve LC remains to be elucidated, activation of endogenous hepatocyte growth, fibrosis resolution, immune regulation, or differentiation into hepatocytes have been suggested. The infusion of harvested bone marrow cells, mobilizing bone marrow-derived stem cells by granulocyte colony-stimulating factor (G-CSF), and transplantation of stem cells expanded in vitro are common methods that have been used in stem cell therapy for LC (Table 17.1).

17.2.2.1 Autologous Bone Marrow Mononuclear Cells (BMNCs)

Bone marrow mononuclear cells (BMNCs) are isolated from whole bone marrow and contain a diverse cell population, including lymphocytes, monocytes, hematopoietic stem cells, and mesenchymal stem cells. In animal models, bone marrow

Table 17.1 Randomized controlled studies using stem cell transplantation or G-CSF in liver cirrhosis

Reference	Therapy	Dose of G-CSF	No. of cells, route	No. of patients, etiology	Follow-up	Result
Spahr et al. [18]	• G-CSF + Autologous BMNCs • SMT	G-CSF 10 μg/kg/day for 5 days	$0.47 \pm 0.15 \times 10^8$ BMNCs/kg Hepatic artery	G-CSF + BMNCs: 28 ALD (MELD score 19 ± 3.8) SMT: 30 ALD (MELD score 19.1 ± 3.9)	3 months	No significant difference between the groups (7and 11 patients in the BMNC and control groups, respectively, returned to moderate drinking)
Verma et al. [22]	• G-CSF • G-CSF + GH • SMT	G-CSF 5 μg/kg every 12 h for 5 days then every three monthly for 3 days till 12 months GH 1 U/day for 12 months	–	G-CSF: 21 (MELD score 13: 8–22) 15 ALD, 2 HCV, 1 ALD + HBV, 1 ALD + HCV, 2 NAFLD G-CSF + GH: 23 (MELD score 16: 10–22) 14 ALD, 3 HCV, 1 ALD + HBV, 4 NAFLD, 1 cryptogenic SMT: 21 (MELD score 16: 8–22) 9 ALD, 3 HCV, 1 ALD + HBV, 7 NAFLD, 1 cryptogenic	12 months	Improved transplantation-free survival, CP score, MELD score, nutrition, fibrosis, ascites control, and QOL score, reduced infections (GH was not found to have any additional benefit)

Newsome et al. [23]	• G-CSF • G-CSF + $CD133^+$ cells • SMT	G-CSF 15 μg/kg/day for 5 days	0.6×10^6 $CD133^+$ cells/kg Peripheral vein	G-CSF: 26 (MELD score 12.7: 12.0–13.1) 12 ALD, 3 HCV, 3 NAFLD, 7 PBC, 1 cryptogenic G-CSF + $CD133^+$ cells: 28 (MELD score 13.2: 12.1–13.9) 14 ALD, 3 HCV, 5 NAFLD, 3 PBC, 2 cryptogenic, 1 mixed SMT: 27 (MELD score 13.1: 12.4–13.8) 12 ALD, 4 HCV, 5 NAFLD, 5 PBC, 1 mixed	3 months	No significant difference between the groups
Kedarisetty et al. [24]	• G-CSF + DPO • SMT	G-CSF 5 μg/kg/day for 5 days and then every third days until day 28 DPO 40 μg/week for 4 weeks	–	G-CSF + DPO: 29 (MELD score 22: 11–38) 21 ALD, 1 HBV, 2 HCV, 5 Cryptogenic SMT: 26 (MELD score 20: 10–30) 14 ALD, 2 HBV, 1 HCV, 9 Cryptogenic	12 months	Improved survival rate, CP score, and MELD score, reduced septic shock

(continued)

Table 17.1 (continued)

Reference	Therapy	Dose of G-CSF	No. of cells, route	No. of patients, etiology	Follow-up	Result
Mohamadnejad et al. [29]	• Autologous BMSCs • Placebo	–	$1.20–2.95 \times 10^8$ BMSCs/body Peripheral vein	BMSC: 14 (MELD score 15.4 ± 5.4) 1 HBV, 2 PBC, 4 AIH, 7 Cryptogenic Placebo: 11 (MELD score 14.5 ± 3.7) 1 HBV, 1 HCV, 5 AIH, 4 Cryptogenic	12 months	No significant difference between the groups
Xu et al. [30]	• ETV+ Autologous BMNCs • Including BMSC • ETV	–	$8.45 \pm 3.28 \times 10^8$ BMNCs /body ($0.75 \pm 0.5 \times 10^6$ BMSCs /body) Hepatic artery	ETV + BMNCs: 20 HBV (MELD score 14.3 ± 3.5) ETV: 19 HBV (MELD score 13.9 ± 2.7)	6 months	Improved MELD score. (ETV + BMNC including BMSC group improved better)
Suk et al. [31]	• Autologous BMSCs one-time • Autologous BMSCs two-time • SMT	–	5×10^7 BMSCs / body, one-time or two-time Hepatic artery	BMSCs one-time: 18 ALD (MELD score 4.5 ± 3.4) BMSCs two-time: 19 ALD (MELD score 4.5 ± 3.9) SMT: 18 ALD (MELD score 7.1 ± 4.2)	12 months	Improved CP score and reduction in the proportion of collagen. (No significant difference between one-time and two-time BMSC transplantation groups)

G-CSF granulocyte colony-stimulating factor, *BMNC* bone marrow mononuclear cell, *SMT* standard medical treatment, *GH* growth hormone, *DPO* darbepoetin α, *BMSC* bone marrow mesenchymal stem cell, *ETV* entecavir, *MELD* model for end-stage liver disease, *ALD* alcoholic liver disease, *HCV* hepatitis C virus, *HBV* hepatitis B virus, *NAFLD* nonalcoholic fatty liver disease, *PBC* primary biliary cholangitis, *AIH* autoimmune hepatitis, *CP* Child-Pugh

cells infused through a tail vein efficiently migrated and repopulated cirrhotic liver. In this process, bone marrow cells produced matrix metalloproteinases and ameliorated liver fibrosis. Microenvironmental improvement produced liver regeneration and resulted in an improved survival rate [12]. A clinical trial of autologous bone marrow cell infusion (ABM*i*) therapy for decompensated LC was started in 2003. With ABM*i* therapy, the autologous BMNCs purified from 400 ml of bone marrow fluid were infused through a peripheral vein into a patient with decompensated LC. Significant improvements in serum albumin levels and Child-Pugh (CP) score, increased liver volume, and decreased ascites were observed, and no major adverse effects were noted [13–15]. The results of clinical trials conducted in Brazil, in which fewer BMNCs than in ABM*i* therapy were infused into the hepatic artery of patients with decompensated LC, have suggested that hepatic artery infusion is more effective than peripheral vein infusion [16]. A recent report described the long-term efficacy of autologous BMNC transplantation for hepatitis B virus (HBV)-related decompensated LC [17]. At 5-year follow-up, liver volume was significantly greater, cirrhosis was sustained, and collagen content was decreased at 6-month follow-up, and liver function, including serum albumin levels and CP scores, was improved at 1-year follow-up. Five years after cell infusion, 26.3% of patients maintained improved liver function. To date, one adequately randomized, controlled study has been performed using autologous BMNC infusion following G-CSF treatment in alcoholic LC patients, but had no effect [18]. In order to clearly demonstrate the efficacy of autologous BMNC therapy, more randomized, controlled studies in decompensated LC patients of diverse etiologies are needed.

17.2.2.2 Hematopoietic Stem Cells (HSCs) and Granulocyte Colony-Stimulating Factor (G-CSF)

Hematopoietic stem cells (HSCs) are characterized by their extensive self-renewal capacity and pluripotency with expression of CD34 and CD133. G-CSF is a potent inducer of HSC proliferation and mobilization from the bone marrow into the peripheral blood. The two general approaches that have been taken are G-CSF treatment followed by autologous transplantation of HSCs isolated from peripheral blood or bone marrow of the recipient, and G-CSF treatment alone. In an animal model and a clinical trial, HSCs mobilized by G-CSF were shown to migrate into damaged liver and to accelerate liver regeneration in a paracrine manner or by direct stimulation of hepatic progenitor cells [19, 20]. However, many of the previous studies were unable to determine the efficacy of HSC transplantation for LC, because of the size and nature of the trial design. Garg et al. reported that G-CSF treatment improved mortality of patients with acute-on-chronic liver failure due to alcohol consumption or HBV in a randomized, controlled study, by preventing the development of sepsis [21]. However, acute-on-chronic liver failure is a different pathophysiological state to that seen in chronic liver failure. In 2017, Verma et al. reported the efficacy of multiple courses of G-CSF treatment in patients with decompensated LC in a randomized, controlled study [22]. In their study, G-CSF

was injected for 5 days and then every third day until day 28, and improved survival rate, CP score, and MELD score, and reduced risk of septic shock were confirmed. Meanwhile, an adequately powered, multicenter, open-label, randomized, controlled phase 2 trial, the "REALISTIC trial," has been reported more recently [23]. In this study, patients with compensated LC were randomly assigned to receive standard care, treatment with subcutaneous G-CSF for 5 days, or treatment with G-CSF for 5 days followed by leukapheresis and intravenous infusion of three doses of CD133-positive HSCs. G-CSF with or without HSC infusion did not improve liver dysfunction or fibrosis and might have been associated with an increased frequency of adverse events, including ascites, sepsis, and encephalopathy, compared with standard care. Both studies showed reliable data, but the severity and etiology of LC and the dose of G-CSF were different between these studies. These findings must be confirmed in large cohorts of patients with decompensated cirrhosis who essentially need liver regeneration therapy. Recently, improvement in outcomes of patients with decompensated LC by combined treatment with G-CSF and erythropoietin has been reported [24]. Like this study, the combination of G-CSF and a potent inducer of liver regeneration could be a new field of liver regeneration therapy using G-CSF. Finally, a point to be aware of in the G-CSF treatment for LC patients is rupture of the spleen, because rupture of the spleen during peripheral blood stem cell mobilization by administration of G-CSF has been reported even in healthy individuals [25].

17.2.2.3 Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) are non-hematopoietic, multipotent stem cells with the capacity to differentiate into mesodermal lineage, as well as ectodermal and endodermal lineages. The defining characteristics of MSCs are: (1) adherence to plastic in standard culture conditions; (2) expression of CD73, CD90, and CD105, and lacking expression of CD14, CD11b, CD34, CD45, CD79α, CD19, and HLA-DR surface molecules; and (3) the ability to differentiate into osteoblasts, adipocytes, and chondroblasts under standard in vitro differentiating conditions [26]. MSCs can be isolated from bone marrow fluid, adipose tissue, umbilical cord, dental pulp, synovium, and many others. MSCs have positive effects during liver regeneration such as inhibiting apoptosis in hepatocytes and suppression of a variety of immune cells and hepatic stellate cells, through secretion of soluble factors, including prostaglandin E2, interleukin 10, and hepatocyte growth factor [27, 28]. MSCs also have therapeutic benefits, including proliferative capacity that allows in vitro expansion of the number of cells, and immune evasion capacity, which forms the basis of allogenic use.

In 2013, an initial randomized, controlled study in patients with decompensated LC in Iran was reported and suggested that autologous bone marrow-derived MSC transplantation through a peripheral vein has no beneficial effect in decompensated LC [29]. However, this study was limited in that LC patients with diverse etiologies were enrolled, which may have resulted in selection bias caused by the heterogene-

ity of their pathophysiological states. Meanwhile, there have been two randomized trials of patients with HBV-related LC or alcoholic LC that reported a beneficial effect of MSC transplantation [30, 31]. Xu et al. reported the efficacy of autologous bone marrow-derived MSC transplantation in patients with HBV-related LC [30]. In that study, patients with HBV-related LC were randomly assigned to the transplantation group that was administered entecavir (ETV) followed by MSC transplantation and the control group that was administered ETV, and the transplantation group showed greater improvement in liver function. In 2016, an adequately powered, multicenter, open-label, randomized, phase 2 trial in alcoholic LC patients was reported [31]. In this study, patients with alcoholic LC were randomly assigned to one control group and two autologous bone marrow-derived MSC groups that underwent either one-time or two-time hepatic arterial injection of bone marrow-derived MSCs. As a result of autologous bone marrow-derived MSC transplantation, reduction of collagen deposition and improvement of CP scores were confirmed, and the proportion of patients with adverse events did not differ among the three groups. No significant differences were seen between the one-time and two-time bone marrow-derived MSC groups. Clinical trials have been performed in patients with different etiologies, and varying numbers of MSCs have been transplanted through different routes. It will be necessary to standardize future clinical trials in terms of cell numbers and injection route. From the perspective of adverse effects, promotion of carcinogenesis and fibrosis by MSC transplantation remains an important concern. However, long-term 192-week observation of patients with HBV-related decompensated LC treated with BMNCs including MSCs revealed no differences in the incidence of hepatocellular carcinoma between the transplantation group and the control group [32]. The contribution of endogenous MSCs to hepatic fibrogenesis remains contentious as a result of conflicting reports. In contrast, there are no convincing reports of exogenous MSCs contributing to liver fibrosis [27].

17.3 Potential Cell Sources and New Technologies for Liver Regeneration Therapy

17.3.1 Stem Cell-Derived Hepatocyte-Like Cells

There are limited sources of primary human hepatocytes, which make it difficult to supply the abundant quantities that liver regeneration therapy requires. As alternatives to primary human hepatocytes, the generation of highly functional stem cell-derived hepatocyte-like cells (HLCs) is being attempted by differentiation from pluripotent stem cells (PSCs) or direct reprogramming of fibroblasts to HLCs [33, 34]. Human induced pluripotent stem cells (iPSCs) have been used to create an organ bud capable of liver-specific protein production and drug metabolism [33]. Furthermore, a culture platform for massive and reproducible production of liver

buds entirely from human iPSCs has been developed [35]. Direct converted hepatocytes from human fibroblasts by overexpression of FOXA3, HNF1α, and HNF4α displayed functions characteristic of mature hepatocytes [36]. Important advances are being made to overcome the limitations of stem cell-derived HLCs, including scale-up limitations, heterogeneous cultures, and incomplete gene expression. Stem cell-derived HLCs are promising cell sources for liver regeneration therapy.

17.3.2 Bioartificial Liver Systems

A bioartificial liver (BAL) system incorporates hepatocytes into a purely mechanical, albumin dialysis-based, artificial liver support device that is capable of performing synthetic functions, as well as blood detoxification. BALs need hollow-fiber cartridges or reservoir loaded with over 100 g of hepatocytes. Ideally, a BAL system would use primary human hepatocytes. However, large amounts of high-quality human hepatocytes are not readily available. Therefore, different cell lines or porcine hepatocytes are currently used. Human trials of BAL in treating acute liver failure have been conducted, but no BAL system has been shown to improve survival in acute liver failure patients to date [37]. However, in 2016, a BAL system using human HLCs induced from human fibroblasts was reported [38]. In a porcine acute liver failure model, this device restored liver functions, corrected blood levels of ammonia and bilirubin, and prolonged survival. When functional BAL systems using abundant high-quality HLCs become available, they could extend the wait time for a suitable liver donor for patients with end-stage liver disease.

17.3.3 Organ Bioengineering

A bioengineered liver through the recellularization of a three-dimensional liver scaffold, including synthetic matrices, such as biodegradable polymer matrices and natural matrices, such as decellularized xenogeneic liver matrices, has been developed [39, 40]. This whole-organ bioengineering approach could overcome the limitations of liver transplantation, such as a shortage of donors and long-term immunosuppression, because autologous HLCs show significant promise as a readily available and functional cell source. In this bioengineering approach, ECM plays an important role in the phenotypic stability and differentiation of HLCs through biochemical and molecular signaling. This approach also has several important advantages over the limitation of in vitro creation of liver buds, including lack of an external bile tree and inability to transplant such buds orthotopically, as well as size restriction [41]. However, further research is required, particularly concerning the cells used for repopulation and cell volumes required to sustain function, before this complex procedure can be applied to humans.

17.4 Conclusion

The efficacy and safety of using stem cells for advanced liver disease have been suggested by the results of clinical trials. Furthermore, new less-invasive treatment methods using stem cell-derived HLCs have been developed. Physicians are eagerly awaiting definitive evidence of the safety and efficacy of regenerative therapy for LC.

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