Myostatin as a Prognostic Biomarker in Hepatic Cirrhosis Patients with Sarcopenia

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Abstract

Cirrhosis represents the advanced stage of numerous chronic liver conditions. Sarcopenia is a frequently overlooked complication in cirrhotic patients, resulting from factors such as insufficient protein intake, malabsorption, diminished muscle growth, and increased muscle breakdown. Sarcopenia is a multifaceted, chronic condition associated with elevated risks of morbidity and mortality. In the field of hepatology, sarcopenia is typically described as a phenotypic indication of muscle mass loss, affecting approximately 30-70% of liver cirrhosis patients. Sarcopenia has been scientifically linked to an increased risk of falls, reduced quality of life, the emergence of acute decompensated liver failure, and mortality in cirrhotic patients. Myostatin, classified as a cytokine within the transforming growth factor beta (TGF-β) family, is recognized for its role in disrupting protein synthesis. It functions as a negative regulator of muscle growth, inhibiting myogenesisand is linked to the onset of sarcopenia. Extensive literature supports the prognostic importance of sarcopenia in cirrhosis, and serum myostatin levels have the potential to serve as a valuable biomarker. Elevated myostatin concentrations have been linked to the presence of sarcopenia and decreased survival rates in individuals diagnosed with liver cirrhosis.

Keywords: *Sarcopenia, Liver cirrhotic, Myostatin, Prognostic Biomarkers.*

A. INTRODUCTION

Hepatic cirrhosis is a chronic liver disease that results from progressive liver disease caused by, including viral infections, autoimmune disorders, cholestatic and metabolic diseases. Hepatic cirrhosis is identified by the alteration of the liver's structure, leading to the development of regenerative nodules. This state arises as a result of hepatocellular necrosis, collapse of the reticulin connective tissue accompanied by connective tissue deposits, distortion of the vascular network, and nodular regeneration of the liver parenchyma.[1,2]

Cirrhosis of the liver represents a significant public health challenge in numerous nations, as indicated by findings from the Global Burden of Disease Study, in 2015, there were 10.6 million instances of decompensated liver cirrhosis, and the incidence rate for liver cirrhosis and chronic liver disease stood at 20.7 per 100,000 individuals, increased by 13% in 2000. The estimated incidence of cirrhosis in East Asia ranges from 16.5/100,000 to 23.6/100,000 in Southeast Asia. Globally, in 2017,

chronic liver disease affected 1.5 billion individuals, with the primary culprits being NAFLD (60%), HBV (29%), HCV (9%), and alcohol-related liver disease (2%). Cirrhosis is the leading cause of liver-related deaths worldwide. Death consequence due to cirrhosis accounted for 2.4% of all deaths worldwide in 2017. No data on the prevalence of liver cirrhosis in Indonesia, there's only several reports from central educational hospitals.[2-4]

The liver plays a critical role in overseeing the metabolism of nutrients and energy, controlling anabolic hormones, eliminating ammonia, and generating cytokines. When liver function is compromised, it can lead to malnutrition, elevated ammonia levels, and persistent inflammation, disrupting the equilibrium between muscle protein synthesis and proteolysis. One of the complications related to metabolic changes in cirrhosis is sarcopenia and its prevalence is high in cirrhosis patients.[15] Increased rate of sarcopenia is observed in accordance to severity worsening of liver disease based on the Child-Pugh score.[5,6,7] Myostatin belongs to the transforming growth factor-β (TGF-β) family and serves as a crucial factor in the control of skeletal muscle development. Elevated serum myostatin levels have been observed in patients with liver cirrhosis, and a correlation exists between high myostatin levels and heightened mortality rates among individuals diagnosed with liver cirrhosis.[8]

B. PATHOPHYSIOLOGY OF LIVER CIRRHOSIS

Several cell types participate in the pathophysiology of liver cirrhosis, including hepatocytes and cells that line the sinusoids, such as hepatic stellate cells (HSCs), sinusoidal endothelial cells (SECs), and Kupffer cells (KCs). Activation of HSCs is a crucial stage in the progression of liver fibrosis. Hepatic stellate cells transform into a fibrogenic phenotype due to the presence of DAMPs (Damage Associated Molecular Patterns) released by damaged hepatocytes. Once activated, hepatic stellate cells continue to proliferate and remain in an activated state, releasing a substantial amount of fibrogenic cytokines and generating excessive extracellular matrix (ECM). This leads to an imbalance between pro-fibrosis and anti-fibrosis mechanisms, with pro-fibrosis mechanisms ultimately resulting in abnormal scar formation and the development of liver fibrosis.[9,10,11]

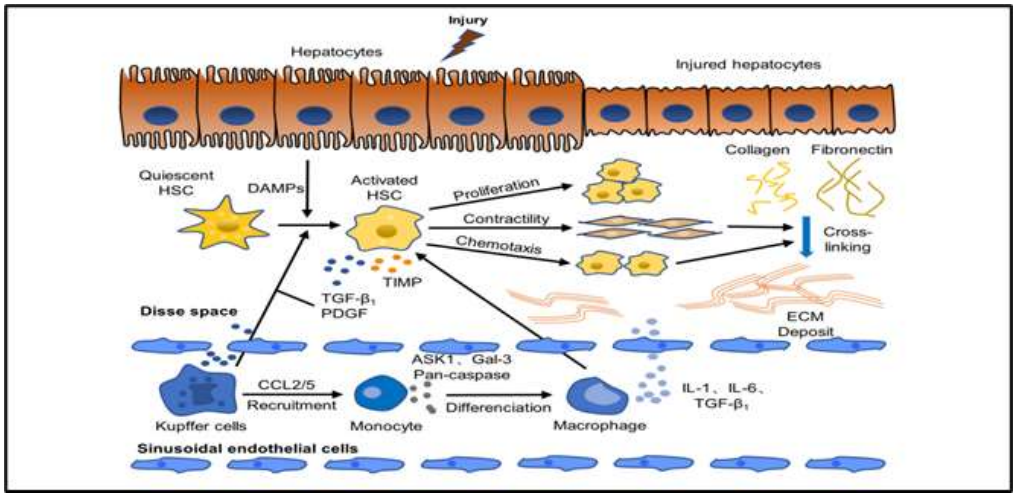

Figure 1. Pathogenesis of Liver Fibrosis[10]

In decompensated cirrhosis, Metabolic alterations transpire and are marked by a decrease in oxidative glucose metabolism within the mitochondria, coupled with an elevated reliance on extramitochondrial glucose utilization via glycolysis. Systemic inflammation elevates energy expenditure, causing mitochondria to malfunction. This leads to a decrease in ATP production in peripheral organs, favoring a less efficient glycolytic process and generating reactive oxygen species (ROS), which can cause damage to cells and tissues. To meet the increased energy requirements, the processes of lipolysis and proteolysis are accelerated, this results in a decrease in both adipose tissue and skeletal muscle mass, a condition known as sarcopenia. Simultaneously, lipids and amino acids are released into the bloodstream. Lipids function as immune activators and contribute to the escalation of systemic inflammation. Additionally, glutaminase plays a pivotal role in generating ammonia from amino acids derived from proteolysis and intestinal translocation. Elevated ammonia levels lead to mitochondrial dysfunction and tissue damage, further intensifying the loss of skeletal muscle mass (sarcopenia) and compromising neutrophil function. This impairment of neutrophil function can facilitate secondary infections, which in turn exacerbate systemic inflammation.[12]

C. SARCOPENIA IN LIVER CIRRHOSIS

Sarcopenia has been delineated by the European Working Group on Sarcopenia (EWGS) as a syndrome characterized by a gradual and widespread reduction in both muscle mass and strength, resulting in physical impairment, diminished quality of life, and potentially mortality. The Asian Working Group of Sarcopenia (AWGS) also characterizes sarcopenia as the age-related decline of skeletal muscle in conjunction with low muscle strength and/or reduced physical performance. In the context of cirrhosis, most research has primarily focused on assessing muscle mass alone. Consequently, a consensus has been reached to operationalize sarcopenia in cirrhotic patients as a phenotypic indication of muscle mass loss.[13,14]

Primary sarcopenia is a condition linked to aging and characterized by the reduction of the mass of skeletal muscles, strength, and function. On the other hand, secondary sarcopenia is characterized as a state in which the underlying disease leads to the deterioration of skeletal muscle mass, strength, and function. Sarcopenia in liver cirrhosis is part of secondary sarcopenia. Sarcopenia has proven to be a strong and reliable predictor of hepatic decompensation, quality of life, susceptibility to infections, and mortality among patients with liver cirrhosis, post-liver transplant, and hepatocellular carcinoma. Assessment of sarcopenia in cirrhosis is recommended because it affects on prognosis, which sarcopenia will increase the risk of complications related to cirrhosis.[13-15]

Frequency of Sarcopenia in Liver Cirrhosis

The prevalence of sarcopenia in individuals with cirrhosis varies significantly, spanning from 30% to 70%. The specific prevalence relies on the diagnostic method employed and the gravity of the underlying liver ailment. As the Child-Pugh score defines the severity of liver disease (CP A = 10%; CP B = 34%; CP C = 54%), it was noted that the incidence of sarcopenia, as determined by computed tomography (CT) values, escalates with the worsening of liver disease severity.[6,7]

Etiology of Sarcopenia in Liver Cirrhosis

Several common things can trigger development of sarcopenia in cirrhosis are described in table 1. In the UK, over the last decade, the most common causes of sarcopenia have been ALD and NAFLD.[6,16]

Table 1. Mechanisms contributing to cirrhotic sarcopenia[16]

Malnutrition and malabsorption o Anorexia, nausea, dysgeusia o Hypermetabolism o Reduced intake
Altered lipid and amino acid metabolism o Decreased gluconeogenesis o Increased ketogenesis o Increased protein turnover
Decreased clearance of ammonia in the liver → hyperammonemia
Increased inflammatory markers (TNF-α, IL-6)
Increased myostatin
Decreased anabolic hormones (IGF-1 and testosterone)
Inactivity

Sarcopenia Assessment

There are several different modalities for assessing sarcopenia, summarized in Table 2, technically there is no single, safe and reliable assessment tool, as well as *gold standard* to define sarcopenia.[6]

Table 2. Summary of tests used to assess sarcopenia[6]

Muscle Mass	Muscle Function	Muscle Strength
Computed tomography (CT)	*Short physical performance battery test* (SPPB)	*Handgrip strength* (HGS)
Skeletal muscle index L3	Walking speed	*Knee flexion/extension peak torque strength*
Magnetic resonance imaging (MRI)	*Timed get-up-and-go test*	*Liver frailty index* (LFI)
Mid-arm muscle circumference (MAMC)	*Liver frailty index* (LFI)	
Dual-energy X-ray absorptiometry (DEXA)	*Aerobic exercise capacity: 6-minute walking distance* (6MWD)	
Muscle ultrasound	*Cardiopulmonary exercise testing* (CPET)	
Bioimpedance analysis (BIA)		

Computed tomography (CT) is the most widely used and validated modality for evaluating muscle mass, with a special focus in cirrhotic patients on the skeletal muscle index (SMI) of the 3rd lumbar vertebrae, gender-specific cut-off value of <50 cm2/m2 in men and <39 cm2/m2 in women. Challenges with CT examination include cost and repeated radiation exposure, so its usefulness in sarcopenia would be limited to patients undergoing imaging as part of routine care (i.e. screening for liver cancer or portal vein thrombosis).[6] Table 3 displays the parameter values and diagnostic standards for the identification of sarcopenia.

Table 3. Reference parameter values and diagnostic criteria for sarcopenia based on EWGSOP, JSH, and AWGS[17]

Parameter	Measurements	Revised EWGSOP	JSH	Revised AWGS
Muscle mass	DXA	M: 7.0 kg/m²		M: 7.0 kg/m²
		F: 5.5 kg/m²		F: 5.4 kg/m²
	BIA		M: 7.0 kg/m²	M: 7.0 kg/m²
			F: 5.7 kg/m²	F: 5.7 kg/m²
	CT (L3 level)		M: 42 cm²/m²	
			F: 38 cm²/m²	
Muscle strength or function	Grip strength	M: 27 kg	M: 26 kg	M: 28 kg
		F: 16 kg	F: 18 kg	F: 18 kg
	Walking speed	6 min (400m)		1.0m/s
	Chair stand (5 rises)	15 s		12 s
	SPPB	8 points		10 points

M: Male, F: Female

Pathogenesis of Sarcopenia in Liver Cirrhosis

Skeletal muscles serve as the primary reservoir for proteins. In regular circumstances, there exists a precise equilibrium between protein synthesis (anabolism) and their degradation (catabolism or proteolysis) to sustain skeletal muscle mass and protein turnover. A reduction in protein synthesis and an elevation in proteolysis result in muscle atrophy.[5]

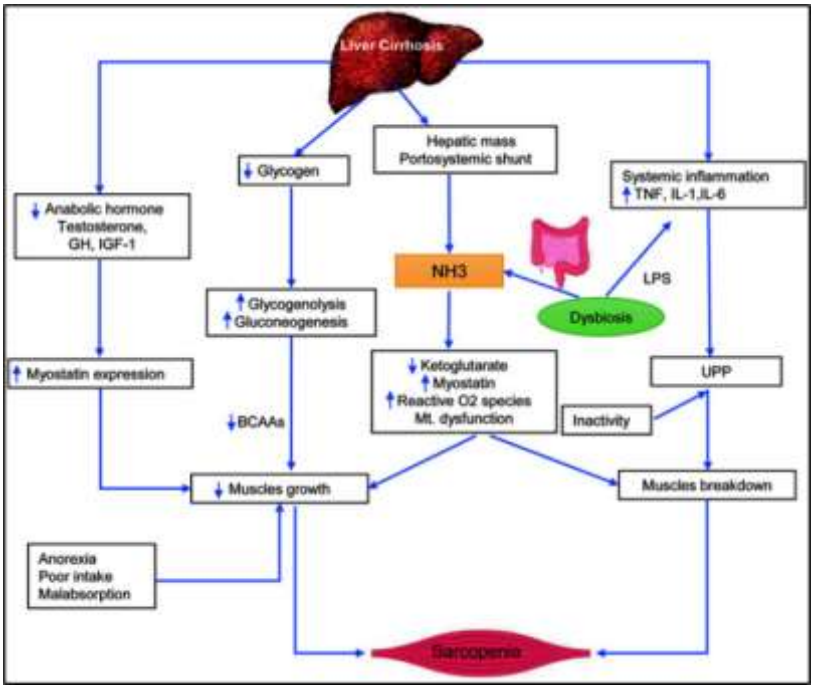

Figure 2. Illustrated Description of the Primary Factors and Processes Underlying Sarcopenia in Liver Cirrhosis [19]

Sarcopenia frequently arises as a complication in individuals diagnosed with liver cirrhosis, stemming from various factors. These factors encompass compromised liver function, resulting in decreased protein synthesis, depleted glycogen stores that stimulate increased gluconeogenesis, Heightened muscle protein breakdown, malnutrition associated with the disease, compromised detoxification, Reduced levels of anabolic hormones such as IGF-1 and testosterone, coupled with increased concentrations of inflammatory cytokines.

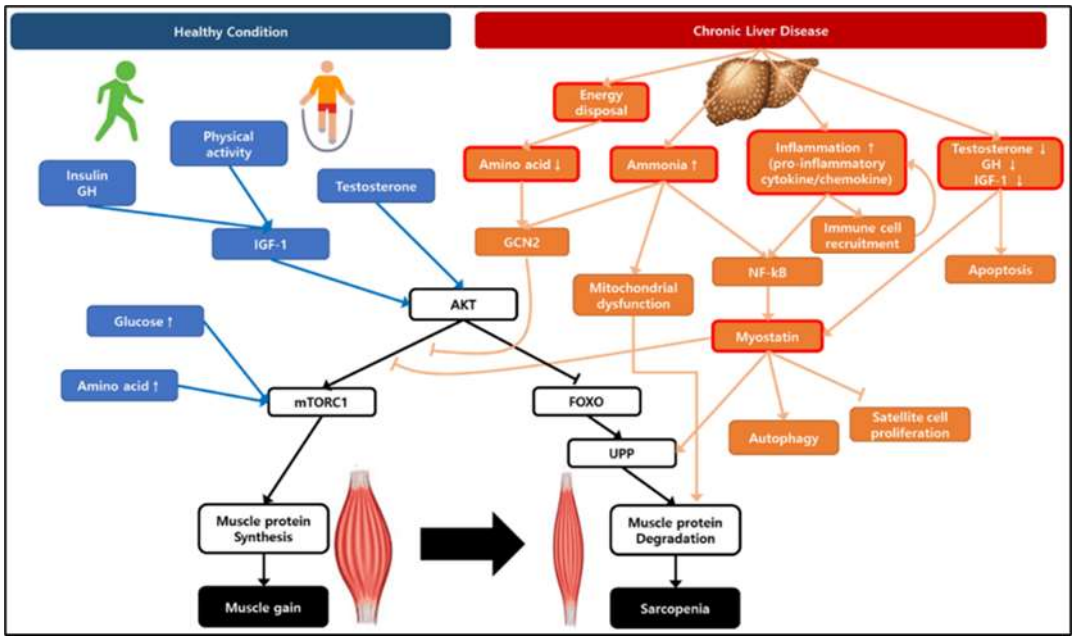

Figure 3. A Diagram Demonstrating How Muscle Protein Synthesis and Degradation are Controlled in Individuals with Chronic Liver Disease and Sarcopenia.[18]

D. MYOSTATIN AND SARCOPENIA

Myostatin, belonging to the transforming growth factor-beta (TGF-β) family, is one of the myokines that skeletal muscle cells generate and release. It plays a crucial role in restraining the growth of skeletal muscles, impacting both muscle enlargement (hypertrophy) and the proliferation of muscle cells (hyperplasia). IGF-1 and testosterone play roles in suppressing myostatin. In liver cirrhosis patients, Decreased IGF-1 and testosterone levels trigger an increase in myostatin expression, resulting in diminished protein synthesis and heightened protein degradation. The myostatin signaling pathway triggers the phosphorylation of the SMAD2/3 complex through the utilization of the activin receptor type IIB (ActRIIB), a receptor that is expressed in skeletal muscle cells. This SMAD2/3 complex, in turn, hinders the AKT-mediated phosphorylation of FOXO, retains FOXO within the myocyte nucleus. In the absence of this inhibition, FOXO experiences phosphorylation by AKT, leading to its migration from the nucleus to the cytoplasm, where it is eventually broken down. Inside the nucleus, FOXO governs the transcription of specific genes, facilitating functions like autophagy and protein degradation through the Ubiquitin Proteasome System (UPS),

while concurrently impeding processes like protein synthesis, cell proliferation, and differentiation. These actions ultimately result in growth inhibition and the development of skeletal muscle atrophy. Furthermore, FOXO contributes to gluconeogenesis and lipid absorption, concurrently inhibiting processes like lipogenesis and glycolysis. Simultaneously, the AKT/mTOR signaling pathway, which is subdued by the SMAD2/3 complex, continues to impede the growth of skeletal muscles. The activation of the SMAD pathway signaling obstructs the multiplication of satellite cells (tissue stem cells) and the transformation of myoblasts into myotubes through a process that depends on SMAD, suppressing factors such as MYOD1, MYF5, and MYOG.[5,20]

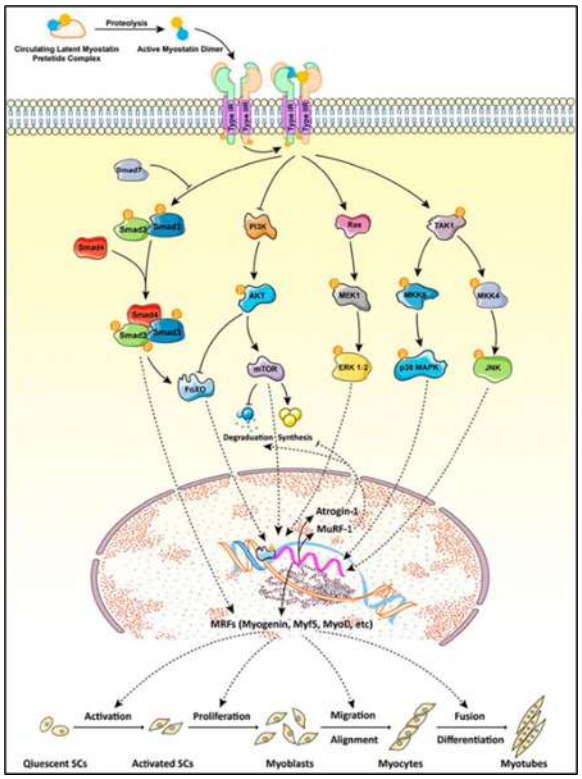

Figure 4. Myostatin and Signaling Pathways Involved in Myogenesis.[21]

Notably, it has been observed that the levels of myostatin in the bloodstream are markedly elevated in individuals with liver cirrhosis when compared to healthy individuals. Furthermore, elevated levels of myostatin in the blood have been linked to the presence of hyperammonemia and muscle atrophy among patients with liver cirrhosis. Elevated serum myostatin concentrations are likewise correlated with an increased mortality rate in individuals with liver cirrhosis.[8]

Myostatin as a Prognostic Biomarker

Liver biopsy and hepatic venous pressure gradient (HVPG) measurement can reflect the prognosis of liver cirrhosis patients, but these examinations have limitations in clinical application due to their invasive procedures. Child-Pugh and MELD scores

are noninvasive methods that can be used to predict the prognosis of liver cirrhosis, but other indicators are still needed. Ideal fluid biomarkers needed to predict the prognosis of cirrhosis can be obtained in a simple, noninvasive and reproducible manner. Recently, many efforts have been made to explore the prognostic value of fluid biomarkers at various stages of liver cirrhosis and one of them is serum myostatin, which the levels of serum myostatin in liver cirrhosis patients are significantly higher than in healthy control.[22]

Numerous investigations have highlighted the substantial increase in serum myostatin levels among patients with end-stage liver disease or those diagnosed with sarcopenia. This heightened myostatin presence has been linked to decreased survival rates in liver cirrhotic patients, particularly when compared to individuals with lower serum myostatin levels. Studies conducted within cirrhotic patients who have chronic hepatitis B or C have further confirmed the significant connection between serum myostatin and both survival rates and the progression of hepatocellular carcinoma (HCC).

In a multicenter cohort study led by Ji Hyun Kim et al., a noteworthy correlation emerged between serum myostatin levels and the risk of developing HCC in patients with Alcohol Liver Cirrhosis (ALC). This investigation underscores the relevance of elevated baseline serum myostatin in predicting an increased risk of HCC within a five-year timeframe among ALC patients. Elevated serum myostatin levels are additionally associated with a greater likelihood of hepatic decompensation and a diminished chance of survival in ALC patients. Consequently, serum myostatin levels may serve as a valuable prognostic marker for assessing the risk of HCC in ALC patients, providing a useful tool for appropriate risk assessment in this patient group.[23]

An inquiry conducted by Nishikawa and colleagues delved into the utility of myostatin as a prognostic tool in individuals with liver cirrhosis. The primary aim of this study was to elucidate the relationship between serum myostatin levels and an array of markers, encompassing skeletal muscle mass, and to assess how serum myostatin levels influenced the survival of liver cirrhosis patients. The investigation involved a cohort of 198 subjects diagnosed with liver cirrhosis. Skeletal muscle mass was evaluated by determining the psoas muscle index (PMI) from the initial CT scans. Following this, the study cohort was divided into two groups according to the median myostatin levels specific to each gender.[23]

The research encompassed a group of 108 male and 90 female participants, with an average age of 67.5 years. The male participants demonstrated a median myostatin level of 3419.6 pg/mL, while the female participants displayed a median myostatin level of 2662.4 pg/mL ($P = 0.0024$). Additionally, for patients categorized as Child-Pugh A ($n = 123$), the median serum myostatin level was 2726.0 pg/mL, whereas Child-Pugh B or C patients ($n = 75$) exhibited a median level of 3615.2 pg/mL ($P = 0.0011$).

In the high myostatin group, the recorded Overall Survival rates at 1, 3, 5, and 7 years were 93.94%, 72.71%, 50.37%, and 38.47%, respectively. Conversely, the low

myostatin group exhibited Overall Survival rates at 1, 3, 5, and 7 years of 96.97%, 83.27%, 73.60%, and 69.95%, respectively (P = 0.0001). Furthermore, advanced age and reduced Psoas Muscle Index (PMI) emerged as notable indicators associated with a less favorable Overall Survival. Elevated levels of serum myostatin were found to be indicative of an adverse prognosis. In both male and female subjects, PMI, serum albumin, prothrombin time, and the ratio of branched-chain amino acids (BCAA) to tyrosine (BTR) displayed significant adverse associations with myostatin levels. Notably, serum ammonia levels exhibited a pronounced positive correlation with myostatin levels.[23]

Nishikawa and colleagues discerned a notable association between elevated serum myostatin levels and various clinical parameters, including sarcopenia, hyperammonemia, and compromised protein synthesis. The latter, in turn, may be manifested in diminished serum albumin concentrations among individuals afflicted with liver cirrhosis. The authors of this study proposed that serum myostatin levels hold promise as a plausible biomarker, substantiating their findings by revealing the concurrence of heightened myostatin levels with the presence of sarcopenia and the reduced survival rates of liver cirrhosis patients.[23]

Nishikawa and colleagues further elucidated that serum myostatin levels exhibit a discernible correlation with the liver's functional capacity. A significant association was noted between serum myostatin levels and the levels of serum ammonia. These findings lend support to the conjecture that the reduction in skeletal muscle mass might be attributed to compromised ammonia detoxification and an elevated myostatin expression.[23]

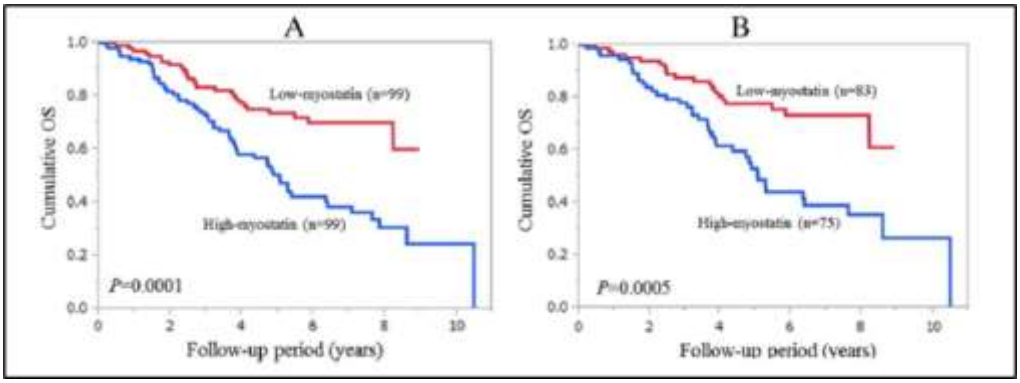

Figure 4. (A) Cumulative OS Rate (n = 198). (B) Cumulative OS Rate After Excluding Hepatocellular Carcinoma Patients at Baseline (n = 158).[23]

E. CONCLUSION

Sarcopenia frequently manifests in individuals diagnosed with liver cirrhosis, reflecting an irregularity in the equilibrium between the synthesis and breakdown of muscle proteins due to diminished liver function. Sarcopenia is a complicated and multifactorial disease. Sarcopenia assessment is recommended because affecting the prognosis. There are several tools and examination techniques can be used to assess

sarcopenia in cirrhosis. The gold standard is VL3 CT scan to determine muscle mass, but this examination is relatively expensive and cannot be done repeatedly. Increased myostatin levels serve as one of the contributory mechanisms in the genesis of sarcopenia within the context of liver cirrhosis. Myostatin, categorized as a cytokine within the transforming growth factor beta (TGF-β) family, is extensively distributed in skeletal muscle and operates as an inhibitory agent for protein synthesis, subsequently retarding the growth of skeletal muscles. The manifestation of hyperammonemia in individuals afflicted with liver cirrhosis precipitates the activation of p65-nuclear factor kappa B (NF-kB), resulting in the transcriptional enhancement and upsurge in myostatin expression. Notably, patients with liver cirrhosis are characterized by significantly elevated serum myostatin levels compared to healthy controls, and these heightened levels are associated with diminished survival rates when compared to those with lower serum myostatin concentrations. Elevated serum myostatin levels are also linked to muscle mass loss and disrupted protein synthesis in individuals with liver cirrhosis. As a result, the analysis of serum myostatin is anticipated to serve as a valuable biomarker for prognostic purposes in patients with liver cirrhosis and sarcopenia.

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