SARCOPENIA, FRAILTY AND NUTRITION IN LIVER DISEASES

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SARCOPENIA, FRAILTY AND NUTRITION IN LIVER DISEASES

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EDITORIAL
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Editorial: Sarcopenia, Frailty and Nutrition in Liver Diseases

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Keywords: sarcopenia, cirrhosis, fatty liver (see NAFLD), complications, chronic liver disease (CLD)

Editorial on the Research Topic

Sarcopenia, Frailty and Nutrition in Liver Diseases

Sarcopenia is a multifactorial process, representing a progressive and diffuse loss of skeletal muscle mass, strength, and function, with a high prevalence in chronic liver diseases and a significant negative impact on survival, quality of life, and development of other complications of cirrhosis and post-liver transplant outcome (1).

This Research Topic aimed at collecting papers suitable to improve our knowledge about the interplay between malnutrition, sarcopenia, and frailty in chronic liver diseases as well as prevalence, risk factors, nutritional screening, outcome and prognosis, nutritional interventions, and exercise in non-alcoholic fatty liver disease (NAFLD), in advanced chronic liver diseases, and before and after liver transplantation (LT).

In this special e-book there are 13 papers covering the above mentioned aspects, the majority of them referring to liver cirrhosis and the others to NAFLD.

Due to the high prevalence of sarcopenia in cirrhotic patients (48.1%) (2) and the potential of sarcopenia in predicting diseases outcomes, identifying the risk factors of sarcopenia in patients with chronic liver diseases has become a critical clinical issue.

Hui et al. explored the relationship between sleep–wake disturbance and malnutrition risk in hospitalized patients with cirrhosis, and the Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT) score was observed to be significantly higher in poor sleepers. Moreover, the Pittsburgh Sleep Quality Index has been demonstrated to be an independent risk factor positively correlated with RFH-NPT, meaning a high risk of malnutrition. The article by Wang et al. evaluated and confirmed that cirrhotic patients with a higher visceral to subcutaneous adipose tissue area ratio have a higher risk of malnutrition compared to patients with low subcutaneous adiposity or high visceral adiposity.

The study by Yin et al. showed that body mass index (BMI) cannot act as an independent prognostic predictor of liver cirrhosis, as already expected. However, in this paper, patients with cirrhosis and acute gastrointestinal bleeding may have a slightly lower short-term survival if they are overweight or obese. On the other hand, in the study by Geng et al., the fat mass and rate of the total body and trunk were significantly higher in Wilson disease (WD) patients, while the muscle and skeletal muscle mass of the total body and trunk were significantly lower in these patients. This finding may have implications for a higher rate of atherosclerosis and acute cardiovascular disease in WD patients. Also, the paper by Lv et al. concluded that the higher the serum albumin before continuous renal replacement therapy (CRRT), the lower the mortality of critically ill patients (a large proportion of cirrhotic patients) with acute kidney injury (the majority of them due to sepsis) treated with CRRT, and the higher the clearance efficiency of serum phosphorus.

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Due to the association between sarcopenia in cirrhotic patients and increased mortality, sepsis complications, hyperammonemia, hepatic encephalopathy, and increased hospital length of stay after LT, multiple studies have focused on management strategies. Several potential therapeutic targets were identified: branched chain amino acid (BCAA) supplementation, myostatin inhibitors, and mitochondrial protective agents (2). The article by Ismaiel et al. of our collection explained how supplementation with BCAAs such as leucine, valine, and isoleucine could ameliorate protein synthesis, lipid and glucose metabolism, insulin resistance, and hepatocyte proliferation, and reduce oxidative stress in hepatocytes in liver cirrhosis. On the other hand, they emphasized the fact that administration timing, dose, and nutritional education regarding BCAA supplementation are considered essential factors that might lead to good or suboptimal results. We, as hepatologists, should be aware of this when prescribing BCAA to cirrhotic patients in order to ameliorate sarcopenia.

One of the papers from our collection (Topan et al.) prospectively demonstrated the high prevalence of sarcopenia in cirrhosis (57.2%) and the association between sarcopenia and portal hypertension-related complications (ascites), infectious complications (urinary tract infection and spontaneous bacterial peritonitis), and the risk of hepatocellular carcinoma (HCC). The first study (3) demonstrated that sarcopenia is a significant independent factor for HCC development in male patients with cirrhosis by multivariate competing risk analysis and could be explained by multiple pathways such as aging, physical inactivity, insulin resistance, vitamin D or zinc deficiency, and chronic inflammation.

The first study (Iacob et al.) evaluating the SarQoL® questionnaire in cirrhotic patients revealed that it can evaluate quality of life and, at the same time, identify subjects with sarcopenia and altered QoL. The SarQoL® questionnaire could identify patients that would benefit the most following a multidisciplinary approach and therapeutic interventions.

A very recent meta-analysis (4) showed that sarcopenia was independently associated with an ∼2-fold higher risk of mortality in patients with cirrhosis, mortality that increased with greater severity, or longer durations of sarcopenia. The CONUT (Controlling Nutritional Status) score proved to be a reliable and easy-to-calculate tool in predicting the development of 3-month complications after LT that could be integrated in clinical practice, as proved in the article by Spoletini et al.. Malnutrition and immunologic compromise increase the risk of post-LT complications and this study showed a correlation between the CONUT score and the development of severe complications and 90-day and long-term mortality after LT.

The importance of these considerations is even more critical in light of the evolving epidemiology of LT candidates due to the increased prevalence of non-alcoholic steatohepatitis (NASH). A recent study investigating the relationship between frailty and cirrhosis etiology revealed that NASH patients were among the frailest category of LT candidates, justifying specific consideration to the liver functional reserve and malnourishment and immunologic impairment when a patient is transplanted (5).

That is why in our collection there is one article (Azevedo et al.) dedicated to the analysis of the complex relationship between sarcopenia and NAFLD. The authors discuss the key mechanisms linking NAFLD to sarcopenia and their clinical importance: the impact of body composition phenotypes on muscle morphology, the concept of sarcopenic obesity, the relationship between sarcopenia and the severity of the liver damage, and the future directions and existing gaps in the knowledge.

Vitamin D deficiency is among the well-known factors that influence the interplay between the muscle and liver together with insulin resistance, obesity, chronic low-grade inflammation, physical inactivity, aging, unhealthy diet composition, different hormonal changes, and oxidative stress. However, vitamin C deficiency could be a risk factor for NASH patients and its role as therapy in NAFLD is investigated in an article by Xie et al..

On the other hand, weight loss and lifestyle changes have a central role in the management of NAFLD. One mini review of our collection (Berkovic et al.) addresses the importance of physical activity in prevention, treatment, and its extrahepatic benefits in NAFLD.

De novo or recurrent NASH is increasingly reported following LT and up to one third of patients may develop recurrent bridging fibrosis/cirrhosis. Knowing the genetic and lifestyle risk factors for recurrent NAFLD (Iacob et al.) and trying to actively modify some of them is of utmost importance to prevent associated complications and retransplantation.

In conclusion, our e-book brings evidence to the fact that sarcopenia should be a component of initial evaluation of all cirrhotic patients regardless of severity or etiology of cirrhosis and should be regularly monitored and treated appropriately in order to ameliorate prognosis on the waiting list and after LT.

The continuous rise in the global prevalence of NAFLD, its progressive course, and associated consequences of sarcopenic obesity leads to the need of screening and fast implementation of preventive measures and treatment of NASH patients before and following LT.

AUTHOR CONTRIBUTIONS

SI, SB, DD, and LG wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Effect of Body Mass Index on the Prognosis of Liver Cirrhosis

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Objective: At present, the association of body mass index (BMI) with the prognosis of liver cirrhosis is controversial. Our retrospective study aimed to evaluate the impact of BMI on the outcome of liver cirrhosis.

Methods: In the first part, long-term death was evaluated in 436 patients with cirrhosis and without malignancy from our prospectively established single-center database. In the second part, in-hospital death was evaluated in 379 patients with cirrhosis and with acute gastrointestinal bleeding (AGIB) from our retrospective multicenter study. BMI was calculated and categorized as underweight (BMI <18.5 kg/m^2), normal weight ($18.5 \leq$ BMI < 23.0 kg/m^2), and overweight/obese (BMI ≥ 23.0 kg/m^2).

Results: In the first part, Kaplan–Meier curve analyses demonstrated a significantly higher cumulative survival rate in the overweight/obese group than the normal weight group ($p = 0.047$). Cox regression analyses demonstrated that overweight/obesity was significantly associated with decreased long-term mortality compared with the normal weight group [hazard ratio (HR) = 0.635; 95% CI: 0.405–0.998; $p = 0.049$] but not an independent predictor after adjusting for age, gender, and Child–Pugh score (HR = 0.758; 95%CI: 0.479–1.199; $p = 0.236$). In the second part, Kaplan–Meier curve analyses demonstrated no significant difference in the cumulative survival rate between the overweight/obese and the normal weight groups ($p = 0.094$). Cox regression analyses also demonstrated that overweight/obesity was not significantly associated with in-hospital mortality compared with normal weight group (HR = 0.349; 95%CI: 0.096-1.269; $p = 0.110$). In both of the two parts, the Kaplan–Meier curve analyses demonstrated no significant difference in the cumulative survival rate between underweight and normal weight groups.

Conclusion: Overweight/obesity is modestly associated with long-term survival in patients with cirrhosis but not an independent prognostic predictor. There is little effect of overweight/obesity on the short-term survival of patients with cirrhosis and with AGIB.

Keywords: body mass index, liver cirrhosis, obesity, prognosis, outcome

INTRODUCTION

Overweight/obesity, which is defined as excessive body fat accumulation, is a common public health problem (1). The global age-standardized prevalence of obesity defined by high body mass index (BMI) is increased from 3.2 to 10.8% in men and from 6.4 to 14.9% in women between 1975 and 2014 (2). Overweight/obesity is considered a risk factor for liver diseases (3, 4). Increased adipose tissues lead to triglyceride deposition in the liver, produce various transduction signals that alter lipid and glucose metabolisms, and then cause insulin resistance and increased release of free fatty acids, which are the causes of hepatic steatosis (5). Hepatic steatosis can contribute to lipid peroxidation and hepatic stellate cell activation, which further induce cellular injury and inflammation and accelerate the progression of liver fibrosis and cirrhosis (6, 7). However, the impact of BMI on outcomes in liver cirrhosis is still controversial. Some studies demonstrated that obesity was an independent risk factor for cirrhosis-related death or hospitalization (8). Other studies supported that the patients with cirrhosis and with obesity had a lower mortality than those without (9). Considering the controversy of the existing evidence, this study aimed to examine the effect of BMI on the prognosis of patients with liver cirrhosis.

METHODS

Study Design

This retrospective study was carried out following the rules of the 1975 Declaration of Helsinki and approved by the Medical Ethical Committee of the General Hospital of Northern Theater Command with an approval number of Y (2021) 023. It was divided into two major parts. In both of the two parts, if the patients are lacking height and weight, they were excluded from the current study.

In the first part, we retrospectively selected patients with cirrhosis and without malignancy from our prospectively established database (10). Eligible patients should be consecutively admitted to our department and underwent an endoscopy and contrast-enhanced CT or MRI scans between December 2014 and December 2020. They were regularly followed *via* telephone or through outpatient visits and/or by reviewing medical records until February 2021. Death and the patients with liver transplantation during follow-up were recorded. Liver transplantation-free survival was the major endpoint of the first part. Patients who underwent liver transplantation were followed until the time point when the liver transplantation was performed.

In the second part, we retrospectively selected patients with cirrhosis and with acute gastrointestinal bleeding (AGIB) who received terlipressin and/or somatostatin/octreotide from our multicenter study (registration number: NCT03846180), which has been further updated after some publications (11–14). Notably, in this part, the eligible patients were consecutively admitted to 13 centers from 8 provinces or municipalities in China between January 2010 and December 2018, and patients who underwent transjugular intrahepatic portosystemic shunt, splenectomy, surgical shunt, or liver transplantation were excluded from the study. In-hospital death was the major endpoint of the second part.

Diagnosis and Definitions

Liver cirrhosis was diagnosed based on clinical manifestations, laboratory tests, radiological examinations, and/or histological data. AGIB was defined as hematemesis, melena, and/or hematochezia within 5 days before admission (15).

BMI was calculated by dividing weight in kilograms by the square of height in meters (16). According to the WHO classification for Asian populations, all patients were categorized as underweight (BMI < 18.5 kg/m^2), normal weight ($18.5 \leq$ BMI < 23.0 kg/m^2), and overweight/obese (BMI ≥ 23.0 kg/m^2) (17).

Statistical Analyses

First, continuous variables were described as mean ± SD and median (range), and categorical variables were described as frequency (percentage). The difference was compared using Mann–Whitney *U* test and Chi-squared test or Fisher's exact test. Second, survival probability curves were calculated by the Kaplan–Meier curve analyses and compared by the log-rank test. Third, univariate Cox regression analyses were performed to explore the association of BMI with mortality, and multivariable Cox regression analyses were performed by adjusting for age, gender, and Child–Pugh scores to identify whether BMI was an independent predictor of death. Hazard ratios (HRs) with 95% CIs were calculated. Fourth, time-dependent receiver operating characteristic (T-ROC) curve analyses were used to evaluate the performance of BMI for predicting death, and area under the curve (AUC) and concordance index (C-index) were calculated. A two-tailed $p < 0.05$ was considered statistically significant. All statistical analyses were performed by using SPSS version 26.0 (IBM Corp, Armonk, New York, USA) and R version 4.0.3 with packages

Abbreviations: BMI, body mass index; AGIB, acute gastrointestinal bleeding; HR, hazard ratio; T-ROC, time-dependent receiver operating characteristic; AUC, area under the curve; C-index, concordance index; HCV, hepatitis C virus; INR, international normalized ratio; PLT, platelet count; HBV, hepatitis B virus; ALT, alanine aminotransferase; SCr, serum creatinine; SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system.

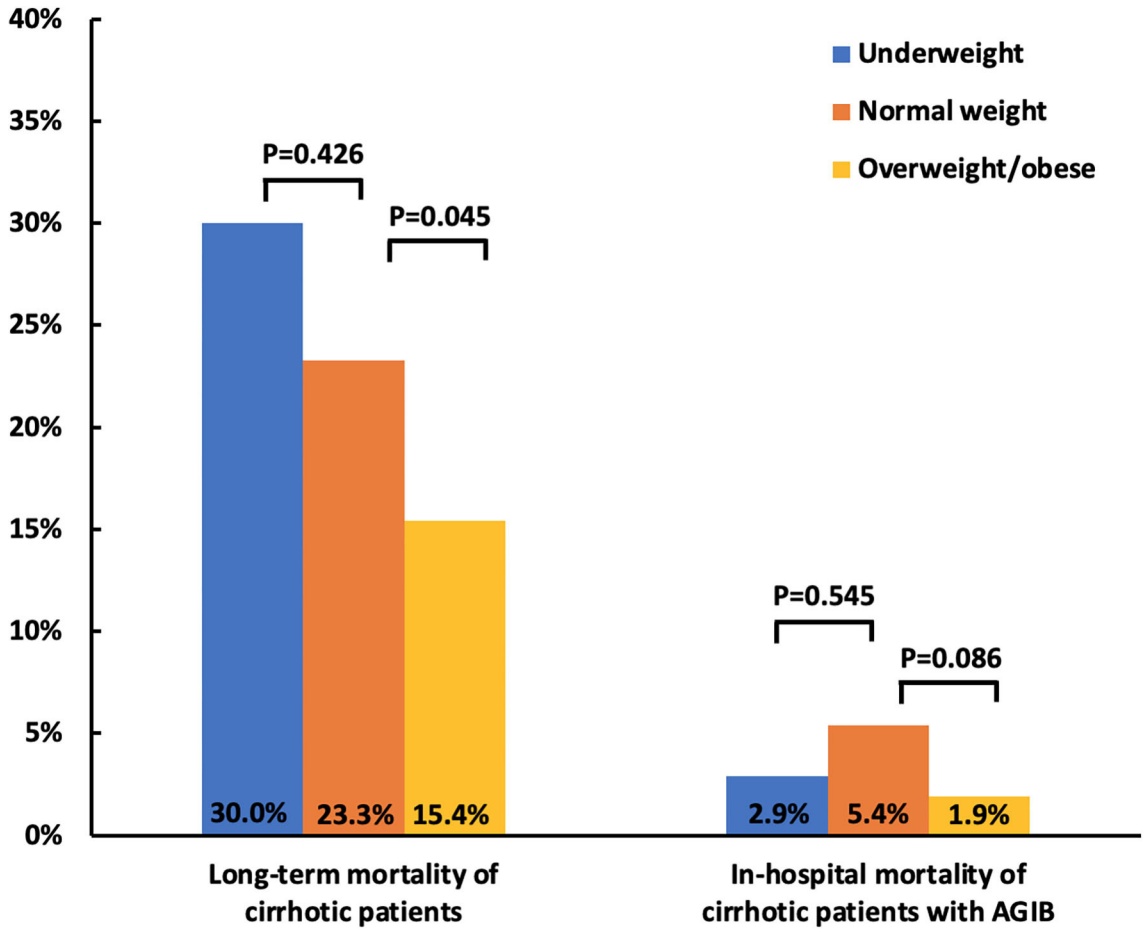

FIGURE 1 | Bar charts showing the association of BMI with mortality in patients with cirrhosis. BMI, body mass index; AGIB, acute gastrointestinal bleeding.

survival, survminer, and timeROC (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

First Part: Long-Term Outcomes of Patients With Cirrhosis

Overall, 436 of 527 patients with cirrhosis registered in our prospective database had BMI data at their admissions and were included in the present study. Among them, 30 (6.9%) were underweight and 234 (53.7%) overweight/obese. The median BMI was 17.34 (range: 14.82–18.42 kg/m^2) in underweight group, 21.02 (range: 18.52–22.95 kg/m^2) in normal weight group, and 25.47 (range: 23.01–37.37 kg/m^2) in overweight/obese group. During a median follow-up period of 2.28 (range: 0.03–5.59 years), 1 patient was lost to follow-up, 6 underwent liver transplantation, and 85 died. Among them, 62 (72.9%) patients died of liver diseases, 14 (16.5%) non-liver diseases, and 9 (10.6%) unknown causes. The mortality was 30% (9/30) in underweight group, 23.3% (40/172) in normal weight group, and 15.4% (36/234) in overweight/obese group (**Figure 1**).

Compared with normal weight group, underweight group had significantly lower proportions of hepatitis C virus (HCV) (0 vs. 11.6%; $p = 0.034$) and international normalized ratio (INR) (1.31 $\pm$ 0.40 vs. 1.34 $\pm$ 0.26; $p = 0.026$) and higher platelet count (PLT) (163.10 $\pm$ 137.51 vs. 97.65 $\pm$ 66.90; $p = 0.004$) (**Table 1**). The Kaplan–Meier curve analyses demonstrated no significant difference in the cumulative survival rate between normal weight and underweight groups ($p = 0.190$) (**Figure 2A**). Univariate Cox regression analyses also demonstrated that underweight was not significantly associated with long-term mortality (HR = 1.617; 95%CI: 0.783–3.338; $p = 0.194$) (**Supplementary Table 1**).

Compared with normal weight group, overweight/obese group had significantly higher proportions of hepatitis B virus (HBV) (44.0 vs. 34.3%; $p = 0.048$) and alcohol abuse (49.1 vs. 35.5%; $p = 0.006$) (**Table 1**) and lower mortality (15.4 vs. 23.3%; $p = 0.045$) (**Figure 1**). The Kaplan-Meier curve analyses demonstrated that overweight/obese group had a significantly higher cumulative survival rate than normal weight group ($p = 0.047$) (**Figure 2A**). Univariate

TABLE 1 | Baseline characteristics of patients with cirrhosis in the first part.

Variables	No. pts	Normal weight group	No. pts	Underweight group	*P*-value*	No. pts	Overweight/obese group	*P*-value#
Demographics								
Age (years)	172	54.69 ± 10.51 55.51 (30.21–78.36)	30	56.95 ± 13.88 57.08 (20.57–81.09)	0.376	234	55.35 ± 10.39 55.13 (20.58–88.73)	0.407
Gender (male)	172	117 (68.0%)	30	15 (50.0%)	0.056	234	175 (74.8%)	0.134
BMI (kg/m^2)	172	20.96 ± 1.24 21.02 (18.52–22.95)	30	17.31 ± 0.85 17.34 (14.82–18.42)	**<0.0001**	234	25.84 ± 2.36 25.47 (23.01–37.37)	**<0.0001**
Comorbidities								
Diabetes	172	24 (14.0%)	30	4 (13.3%)	0.928	234	34 (14.5%)	0.870
Hypertension	172	22 (12.8%)	30	2 (6.7%)	0.339	234	43 (18.4%)	0.129
Etiology of liver cirrhosis								
HBV	172	59 (34.3%)	30	11 (36.7%)	0.802	234	103 (44.0%)	**0.048**
HCV	172	20 (11.6%)	30	0	**0.034**	234	16 (6.8%)	0.093
Alcohol abuse	172	61 (35.5%)	30	11 (36.7%)	0.899	234	115 (49.1%)	**0.006**
Laboratory tests								
RBC (10^{12}/L)	172	3.26 ± 0.84 3.24 (1.45–5.20)	30	3.17 ± 0.69 3.09 (1.78–4.56)	0.517	234	3.41 ± 0.86 3.43 (1.43–5.62)	0.148
WBC (10^9/L)	172	4.46 ± 3.48 3.6 (0.7–23.1)	30	5.54 ± 3.92 4.35 (0.8–19.6)	0.085	234	4.11 ± 2.86 3.45 (1.0–30.4)	0.649
PLT (10^9/L)	172	97.65 ± 66.90 77.5 (18–377)	30	163.10 ± 137.51 116 (39–646)	**0.004**	234	96.27 ± 59.60 81.5 (23–457)	0.577
TBIL (μmol/L)	172	27.34 ± 25.41 20.05 (5.2–172.1)	30	43.9 ± 52.9 15.5 (6.6–216.5)	0.870	234	29.56 ± 27.87 21.05 (4.9–215.3)	0.182
ALB (g/L)	171	31.94 ± 5.96 32.1 (17.2–46.0)	30	31.06 ± 5.44 31.15 (21.8–40.6)	0.450	233	32.99 ± 6.34 32.6 (14.2–50.6)	0.125
ALT (U/L)	172	33.45 ± 49.75 21.85 (6.58–590.00)	30	37.55 ± 58.50 21.38 (7.53–332.5)	0.887	234	41.98 ± 103.09 25.33 (4.23–1465.5)	0.090
GGT (U/L)	172	82.76 ± 140.76 35.57 (7.54–1081.31)	30	131.60 ± 232.46 58.64 (11.9–1283.02)	0.135	234	91.43 ± 183.08 41.88 (8.23–1779.18)	0.104
SCr (μmol/L)	170	67.37 ± 26.30 63.23 (14.80–267.63)	30	61.17 ± 19.36 57.45 (32.65–117.66)	0.173	230	65.38 ± 16.98 63.24 (16.50–131.91)	0.987
Na (mmol/L)	170	139.10 ± 2.83 139.0 (127.2–145.5)	30	136.90 ± 5.72 137.6 (118.0–146.7)	0.058	234	138.96 ± 2.95 139 (127–151)	0.727
INR	169	1.34 ± 0.26 1.29 (0.89–2.77)	30	1.31 ± 0.40 1.16 (0.93–2.43)	**0.026**	232	1.33 ± 0.27 1.25 (0.91–2.55)	0.334
Child-Pugh score	169	7.28 ± 1.80 7 (5–13)	30	8.0 ± 2.12 7 (5–12)	0.092	231	7.05 ± 1.88 7 (5–13)	0.124
Child-Pugh class A/B+C	169	68 (40.2%)/101 (59.8%)	30	9 (30.0%)/21 (70.0%)	0.289	231	106 (45.9%)/125 (54.1%)	0.260
MELD score	169	11.43 ± 3.57 10.50 (6.54–27.84)	30	12.45 ± 6.76 9.68 (6.43–30.03)	0.293	229	11.33 ± 4.09 10.04 (6.43–28.91)	0.232
Decompensated events								
Ascites	172	107 (62.2%)	30	22 (73.3%)	0.242	234	123 (52.6%)	0.053
HE	172	6 (3.5%)	30	0	0.376	234	5 (2.1 %)	0.407
AGIB	172	60 (34.9%)	30	9 (30.0%)	0.603	234	65 (27.8%)	0.125

**P-value was compared between underweight and normal weight groups; #p-value was compared between overweight/obese and normal weight groups. Bold numerals showed statistically significant.*

No. Pts., numbers of patients; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; RBC, red blood cell; WBC, white blood cell; PLT, platelet count; TBIL, total bilirubin; ALB, albumin; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; SCr, serum creatinine; Na, sodium; INR, international normalized ratio; MELD, model for end-stage liver disease; HE, hepatic encephalopathy; AGIB, acute gastrointestinal bleeding.

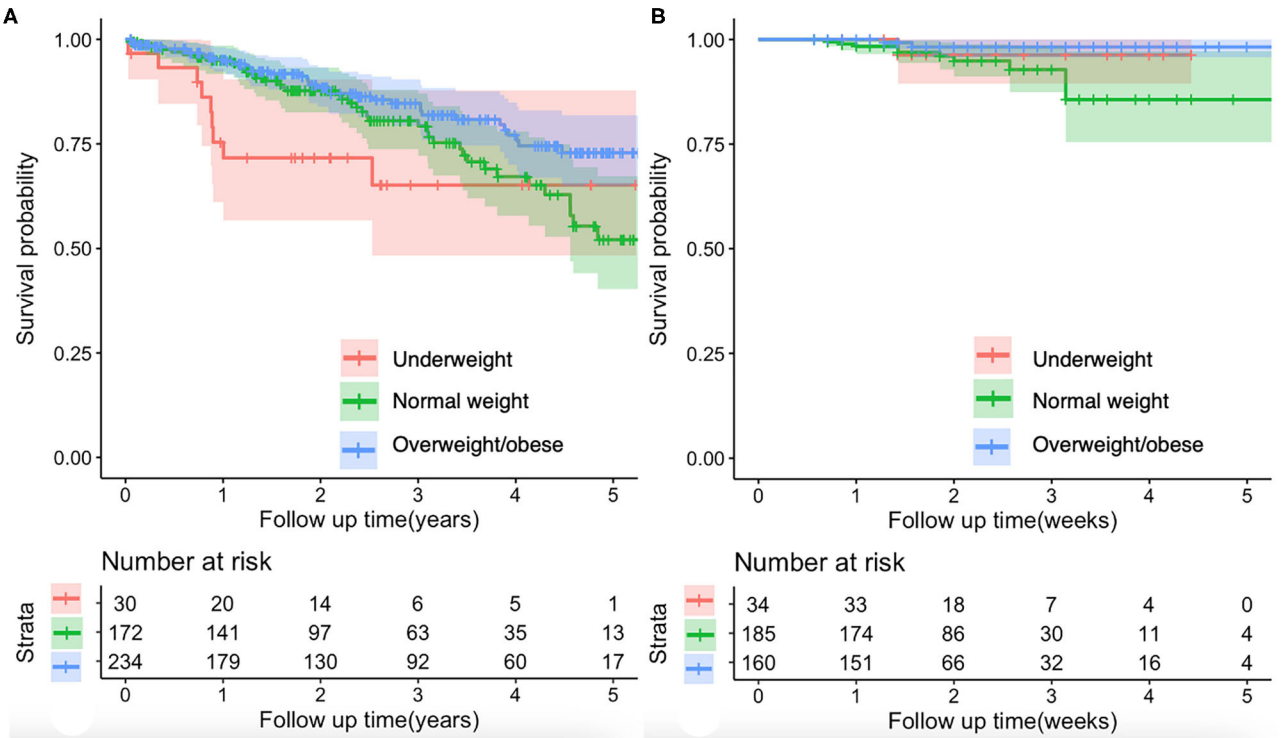

FIGURE 2 | Kaplan–Meier curves showing the effect of BMI on the mortality of patients with cirrhosis. **(A)** Long-term mortality of patients with cirrhosis. There was no significant difference in the cumulative survival rate between normal weight and underweight groups (log-rank test, $p = 0.190$). The overweight/obese group had a significantly higher cumulative survival rate than the normal weight group (log-rank test, $p = 0.047$). **(B)** In-hospital mortality of patients with cirrhosis and with AGIB. There was no significant difference in the cumulative survival rate between normal weight and underweight groups (log-rank test, $p = 0.491$) or between normal weight and overweight/obese groups (log-rank test, $p = 0.094$). AGIB, acute gastrointestinal bleeding.

Cox regression analyses demonstrated that overweight/obesity was significantly associated with decreased long-term mortality (HR = 0.635; 95%CI: 0.405–0.998; $p = 0.049$). After adjusting for age, gender, and Child–Pugh score, overweight/obesity was not an independent predictor of decreased long-term mortality (HR = 0.758; 95%CI: 0.479–1.199; $p = 0.236$) (**Supplementary Table 1**).

Time-dependent receiver operating characteristic analyses of BMI for predicting long-term mortality of patients with cirrhosis are shown in **Figure 3A**. The AUCs at 1-, 2-, and 3-year during follow up were 0.613 (95%CI: 0.487–0.740), 0.566 (95%CI: 0.472–0.659), and 0.585 (95%CI: 0.495–0.675), respectively. C-index was 0.568 (95% CI: 0.497–0.639).

Second Part: In-hospital Outcomes of Patients With Cirrhosis and With AGIB

Overall, 379 of 1,582 patients with cirrhosis and with AGIB recorded in our multicenter study had BMI data at their admissions and were included in the present study. Among them, 34 (9.0%) were underweight and 160 (42.2%) overweight/obese. The median BMI was 17.70 (range: 16.33–18.49 kg/m^2)in underweight group, 21.09 (range: 18.56–22.99 kg/m^2) in normal weight group, and 25.15 (range: 23.03–43.94 kg/m^2) in overweight/obese group. During a median hospitalization period of 13 (range: 4–48) days, 14 patients died. Among them 11 (78.6%) patients died of liver diseases and 3 (21.4%) died of non-liver diseases. In-hospital mortality was 2.9% (1/34) in underweight group, 5.4% (10/185) in normal weight group, and 1.9% (3/160) in overweight/obese group (**Figure 1**).

Compared with normal weight group, underweight group had significantly lower proportion of men (47.1 vs. 75.1%; $p = 0.001$) and higher PLT (113.32 ± 81.32 vs. 86.69 ± 65.13; $p = 0.004$) (**Table 2**). The Kaplan–Meier curve analyses demonstrated no significant difference in the cumulative survival rate between normal weight and underweight groups ($p = 0.491$) (**Figure 2B**). Univariate Cox regression analyses also demonstrated that underweight was not significantly associated with in-hospital mortality (HR = 0.494; 95%CI: 0.063–3.866; $p = 0.502$) (**Supplementary Table 1**).

Compared with normal weight group, overweight/obese group had significantly lower alanine aminotransferase (ALT) (60.49 ± 141.16 vs. 67.77 ± 238.96; $p = 0.039$) and higher serum creatinine (SCr) (75.36 ± 27.03 vs. 70.05 ± 22.73; $p = 0.044$) (**Table 2**). The Kaplan–Meier curve analyses demonstrated no significant difference in the cumulative survival rate between normal weight and overweight/obese groups ($p = 0.094$) (**Figure 2B**). Univariate Cox regression analyses

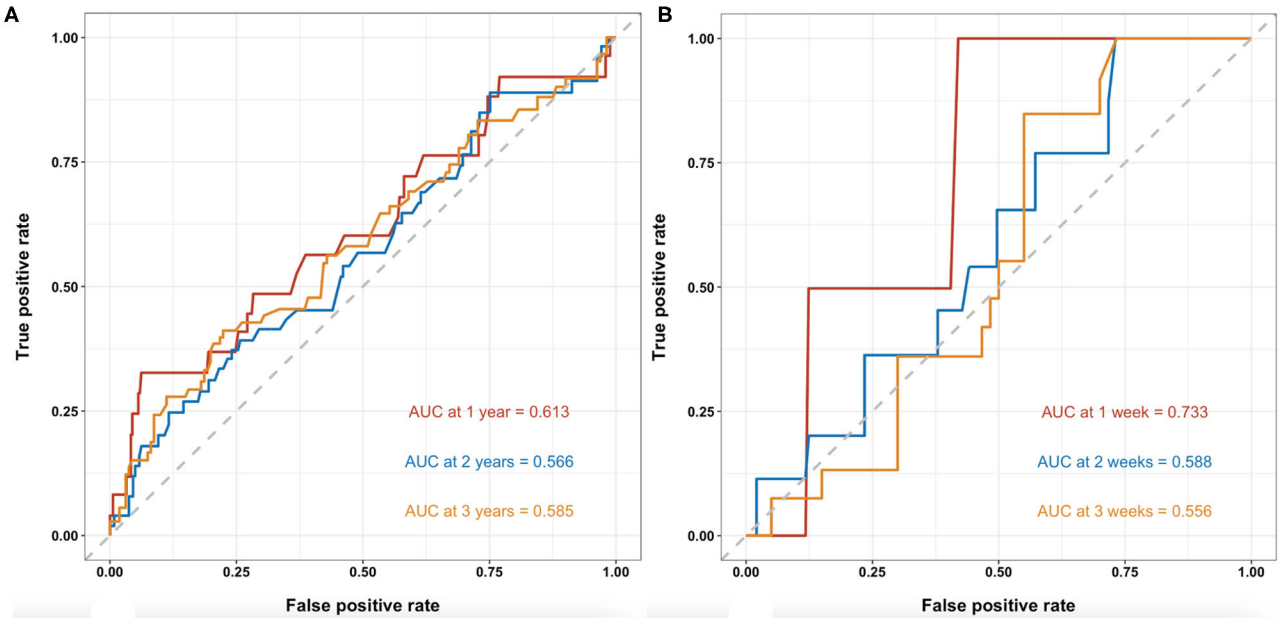

FIGURE 3 | T-ROC curves of BMI for the predictability of mortality in patients with cirrhosis. **(A).** Long-term mortality of patients with cirrhosis at 1-, 2-, and 3-year during follow-up. The AUCs were 0.613 (95%CI: 0.487–0.740), 0.566 (95%CI: 0.472–0.659), and 0.585 (95%CI: 0.495–0.675), respectively. **(B)** In-hospital mortality of patients with cirrhosis with AGIB at 1-, 2-, and 3-week during hospitalization. The AUCs were 0.733 (95%CI: 0.527–0.938), 0.588 (95%CI: 0.422–0.755), and 0.556 (95%CI: 0.410–0.702), respectively. T-ROC, time-dependent receiver operating characteristic; BMI, body mass index; AUC, area under the curve.

also demonstrated that overweight/obesity was not significantly associated with in-hospital mortality (HR = 0.349; 95%CI: 0.096–1.269; $p = 0.110$) (**Supplementary Table 1**).

Time-dependent receiver operating characteristic analyses of BMI for predicting in-hospital mortality of patients with cirrhosis and with AGIB are shown in **Figure 3B**. The AUCs at 1-, 2-, and 3-week during hospitalizations were 0.733 (95%CI: 0.527–0.938), 0.588 (95%CI: 0.422–0.755), and 0.556 (95%CI: 0.410–0.702), respectively. C-index was 0.610 (95% CI: 0.477–0.742).

DISCUSSION

The first objective of the present work was to explore the relationship of BMI with the long-term prognosis of patients with cirrhosis. We found that overweight/obesity was inversely associated with long-term mortality of patients with liver cirrhosis. This finding supported the "obesity paradox" that overweight/obese patients could have superior survival. It was first proposed by Fleischmann et al. (18) in patients undergoing hemodialysis (18) and further validated in subjects with chronic diseases, such as cardiovascular diseases, hypertension, and diabetes (19–22).

The pathophysiology of the "obesity paradox" remains to be elucidated, and there are some underlying explanations (**Supplementary Figure 1**). First, fat storage in overweight/obese patients may protect the balance of muscle protein catabolism in chronic wasting diseases (23). Body protein is crucial for survival because it can maintain cell function and support cell architecture (24). Muscle protein metabolism is preserved in patients with obesity and with chronic cardiac failure, indicating better outcomes but increased in patients without obesity (25). Similarly, sarcopenia, which is mainly caused by increased muscle protein metabolism (26, 27), is associated with lower BMI in patients with liver cirrhosis (28), and further leads to higher mortality (29). Second, adipose tissue, which has been recognized as an endocrine organ, can secrete diverse adipokines (30, 31). Adiponectin, an anti-inflammatory adipokine, can inhibit the proliferation and activation of hepatic stellate cells, which produce extracellular matrix proteins in the case of liver injury and promote the occurrence of liver fibrosis (32). Leptin, another adipokine, can prevent ectopic lipid accumulation in non-adipose tissues, augment immune response, and improve bacterial clearance and survival in animal models (33, 34). Both of which are increased in overweight/obese patients with liver cirrhosis, probably improving the outcomes of the patients (35). Third, patients with cirrhosis and with hepatic edema have systemic vasodilation and underfilled arteries, decreasing effective circulatory blood volume and activating cardiac sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) which can stimulate sodium and water retention. Prolonged sodium and water retention will cause hyponatremia and pulmonary edema and increase cardiac afterload (36, 37). Overweight/obesity can alleviate the activities of cardiac SNS and RAAS, thereby inhibiting hyperdynamic circulation and conferring survival benefits (38). Fourth, overweight/obese patients are more likely to

TABLE 2 | Baseline characteristics of patients with cirrhosis and with AGIB in the second part.

Variables	No. Pts	Normal weight group	No. Pts	Underweight group	*P*-value*	No. Pts	Overweight/obese group	*P*-value#
Demographics								
Age (years)	185	55.19 ± 12.46 54.62 (22.98–82.01)	34	57.35 ± 15.78 55.64 (23–88.21)	0.458	160	55.42 ± 12.53 54.26 (25.44–86.39)	0.851
Gender (male)	185	139 (75.1%)	34	16 (47.1%)	**0.001**	160	131 (81.9%)	0.130
BMI (kg/m^2)	185	21.05 ± 1.34 21.09 (18.56–22.99)	34	17.59 ± 0.60 17.70 (16.33–18.49)	**<0.0001**	160	26.03 ± 2.93 25.16 (23.03–43.94)	**<0.0001**
Comorbidities								
Diabetes	185	27 (14.6%)	34	3 (8.8%)	0.368	160	33 (20.6%)	0.141
Hypertension	185	23 (12.4%)	34	5 (14.7%)	0.715	160	22 (13.8%)	0.717
Etiology of liver cirrhosis								
HBV	185	116 (62.7%)	34	17 (50.0%)	0.163	160	95 (59.4%)	0.527
HCV	185	7 (3.8%)	34	2 (5.9%)	0.571	160	9 (5.6%)	0.417
Alcohol abuse	185	43 (23.2%)	34	4 (11.8%)	0.134	160	40 (25%)	0.703
Laboratory tests								
RBC (10^{12}/L)	185	2.92 ± 0.86 2.86 (1.15–5.14)	34	2.92 ± 0.83 2.82 (1.33–4.68)	0.986	160	2.98 ± 0.76 2.85 (1.34–5.26)	0.422
WBC (10^9/L)	185	6.15 ± 4.03 5.39 (1.15–23.4)	34	6.52 ± 3.68 6.51 (1.3–17.1)	0.328	160	6.76 ± 4.59 5.89 (1.4–31.04)	0.087
PLT (10^9/L)	185	86.69 ± 65.13 68 (2–476)	34	113.32 ± 81.32 97.5 (21–473)	**0.004**	160	85.04 ± 42.19 78 (5–201)	0.197
TBIL (μmol/L)	185	50.02 ± 73.01 24.73 (3.9–518)	34	33.60 ± 40.46 25.4 (4–244.6)	0.434	160	43.36 ± 76.41 24.2 (6.4–720.9)	0.509
ALB (g/L)	185	30.51 ± 5.92 31 (11.0–46.8)	34	30.52 ± 6.27 30.45 (10.1–44.6)	0.893	160	29.98 ± 6.12 30 (14.6–49.8)	0.313
ALT (U/L)	185	67.77 ± 238.96 30.0 (6.3–2651)	34	41.84 ± 33.60 31.2 (8.62–140)	0.611	160	60.49 ± 141.16 33.15 (6.42–1575)	**0.039**
GGT (U/L)	185	124.26 ± 223.83 46.9 (6–1958)	34	117.26 ± 135.69 52.5 (6–531)	0.829	160	113.92 ± 221.86 45.2 (7–2145)	0.688
SCr (μmol/L)	185	70.05 ± 22.73 66.7 (25–202.2)	34	72.6 ± 46.17 64.2 (27–305)	0.338	160	75.36 ± 27.03 70.05 (24.9–223.3)	**0.044**
Na (mmol/L)	185	137.51 ± 5.10 138.0 (115.6–157)	34	136.61 ± 5.71 137.3 (121.8–148)	0.401	159	137.49 ± 4.39 138 (119.7–147.5)	0.965
INR	182	1.39 ± 0.31 1.33 (0.92–3.04)	34	1.33 ± 0.26 1.27 (1.07–2.48)	0.189	159	1.40 ± 0.29 1.32 (0.98–2.77)	0.684
Child-Pugh score	182	7.79 ± 1.77 8 (5–12)	34	7.82 ± 1.62 7.5 (5–12)	0.917	159	7.64 ± 1.96 7 (5–15)	0.235
Child-Pugh class A/B+C	182	49 (26.9%)/133 (73.1%)	34	7 (20.6%)/27 (79.4%)	0.439	159	48 (30.2%)/111 (69.8%)	0.505
MELD score	182	13.86 ± 5.71 12.04 (6.43–32.59)	34	13.54 ± 5.51 11.73 (7.5–28.72)	0.867	159	13.61 ± 5.55 12.08 (6.9–37.59)	0.816
Decompensated events								
Ascites	185	114 (61.6%)	34	23 (67.6%)	0.505	160	89 (55.6%)	0.259
HE	185	10 (5.4%)	34	3 (8.8%)	0.438	160	6 (3.8%)	0.466

**P-value was compared between underweight and normal weight groups; #p-value was compared between overweight/obese and normal weight groups. Bold numerals showed statistically significant. AGIB, acute gastrointestinal bleeding; No. Pts., numbers of patients; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; RBC, red blood cell; WBC, white blood cell; PLT, platelet count; TBIL, total bilirubin; ALB, albumin; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; SCr, serum creatinine; Na, sodium; INR, international normalized ratio; MELD, a model for end-stage liver disease; HE, hepatic encephalopathy.*

receive medical interventions, including antihypertensive drugs for decreasing systolic blood pressure, which can make short-term hemodynamic status more stable (39, 40), and statins for treating hyperlipidemia (41), which can have a favorable impact on outcomes of cirrhosis and portal hypertension (42).

Age, gender, and body function may interact with the relationship between BMI and prognosis (43, 44). Accordingly, this study adjusted some potential confounding factors, including age, gender, and Child–Pugh score, in multivariable Cox regression analysis. By comparison, the previous study by Karagozian et al. (9) selected patients with cirrhosis from the National Inpatient Sample database, which was lacking laboratory data, such as hepatic function (9). Thus, these results should be more reliable. This study found that BMI was not an independent risk factor of decreased long-term mortality, indicating that the prognostic impact of BMI might not be as strong as Child–Pugh score. This finding can be explained by the hypothesis of "reverse causation" that overweight/obesity may not be a cause for a better outcome, but a consequence (45–47). Another explanation is that BMI is convenient but unable to comprehensively measure body composition (2, 48), such as muscle and subcutaneous and visceral adipose tissue and their specific distributions in the body (49). Besides, body weight may be masked by fluid retention resulting from ascites in patients with cirrhosis, inaccurately or falsely evaluating the prognostic impact of BMI.

This study demonstrated that BMI was not significantly associated with in-hospital outcomes of patients with cirrhosis and with AGIB, which is consistent with the previous study regarding the association of obesity with in-hospital mortality of patients with non-variceal gastrointestinal bleeding (50). This may be explained by the complexity of evaluating the outcomes of acute injuries, which should not be attributed to the effect of body weight alone. Fatal injuries brought by decompensated events are far beyond the potential benefits of overweight/obesity.

There were some limitations in this study. First, we did not obtain the dynamic changes of BMI during follow-up. Second, we did not have an external validation cohort to verify the present findings. Third, BMI data were missing in a proportion of our AGIB patients, probably producing a selection bias. Fourth, the interventions used during the hospitalization and follow-up, which might be beneficial for the outcomes, were not available. Fifth, we did not evaluate the specific values of muscle mass or the distribution of adipose tissue due to the absence of dual-energy X-ray absorptiometry, waist circumference, and waist to hip ratio.

In conclusion, there is a modest association of overweight/obesity with decreased long-term mortality of patients with cirrhosis. However, BMI cannot act as an independent prognostic predictor of liver cirrhosis. In the future, prospective large-scale studies should be attempted to combine BMI with other indicators involved in measuring muscle mass and adipose tissue to more precisely predict the clinical outcomes of liver cirrhosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Committee of the General Hospital of Northern Theater Command. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XQ: conceptualization and supervision. YYi and XQ: methodology, formal analysis, visualization, and writing—original draft. YL, LS, SY, BL, SL, YYa, ST, FM, YW, YC, BL, QZ, and XQ: resource. YYi, YL, LS, SY, BL, SL, YYa, ST, FM, YW, YC, BL, QZ, and XQ: data curation and writing—review and editing. All authors have made an intellectual contribution to the manuscript and approved the submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.700132/full#supplementary-material

Supplementary Figure 1 | Schematic diagram showing the underlying explanations of obesity paradox. SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system.

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Serum Albumin Before CRRT Was Associated With the 28- and 90-Day Mortality of Critically Ill Patients With Acute Kidney Injury and Treated With Continuous Renal Replacement Therapy

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Introduction: Although low serum albumin (ALB) may worsen acute kidney injury (AKI), additional study is needed to establish the connection between ALB and the prognosis of critically ill patients with AKI and treated with continuous renal replacement therapy (CRRT).

Methods: A secondary analysis of a bi-center, retrospective, and observational study, such as critically ill patients with AKI and treated with CRRT from January 2009 to September 2016. The univariate analysis, multi-factor regression analysis, sensitivity analysis, and curve-fitting analysis were applied to explore the association of ALB with the 28 and 90 days mortality of critically ill patients with AKI and treated with CRRT, and the removal efficiency of serum phosphorus.

Results: From January 2009 to September 2016, 1,132 cases with AKI and treated with CRRT met the inclusion criteria and enrolled in this study. We found that the higher ALB before CRRT, the lower the 28- and 90-day mortality of patients with AKI and treated with CRRT, the higher removal efficiency of serum phosphorus, the adjusted hazard ratio (HR) value for 28-day mortality in the four models were separately 0.92 (0.90, 0.95), 0.91 (0.89, 0.94), 0.92 (0.89, 0.95), and 0.92 (0.89, 0.95); the adjusted HR value for 90 day mortality in the four models were 0.91 (0.89, 0.94), 0.92 (0.89, 0.95), 0.92 (0.89, 0.95), and 0.92 (0.89, 0.96); the adjusted OR value for the removal efficiency of serum phosphorus in the four models were separately −0.04 (−0.07, −0.01), −0.05 (−0.08, −0.01), −0.04 (−0.08, −0.01), and −0.04 (−0.08, −0.01). The sensitivity analysis and curve-fitting analysis also showed that ALB before CRRT was correlated with the 28 and 90 days mortality of critically ill patients with AKI and treated with CRRT and the removal efficiency of serum phosphorus.

Conclusion: The higher the serum ALB before CRRT, the lower the mortality of critically ill patients with AKI and treated with CRRT, and the higher the clearance efficiency of serum phosphorus.

Keywords: serum albumin, acute kidney infusion, 28- and 90-day mortality, critically ill patients, continuous renal replacement therapy

INTRODUCTION

Acute kidney injury (AKI) is a frequent complication of critically ill patients. Approximately 30–50% of critically ill patients develop AKI, and the mortality for individuals with AKI may reach 50% (1). Approximately 40% of the AKI patients may advance to life-threatening renal dysfunction (1, 2), such as hyperkalemia, severe acidosis, severe azotemia, oliguria, or continuous anuria, and will need renal replacement treatment, among which continuous renal replacement therapy (CRRT) is the most commonly used renal replacement therapy in the intensive care unit (ICU) (1). The efficiency of CRRT clearance is closely related to the prognosis of patients (3). According to various research studies, the serum phosphorus clearance of CRRT is linked to the serum creatinine and urea nitrogen clearance (4–6). Simultaneously, serum phosphorus clearance of CRRT is linked to the prognosis of critically ill patients (7, 8). ALB is one of the most critical proteins in the human plasma because it may maintain plasma colloid osmotic pressure, engage in material transport in blood circulation, and facilitate communication among the intracellular fluid, extracellular fluid, and tissue fluid (9). Hypoalbuminemia is widespread in patients with critical illnesses, and it is widely recognized as being linked to patient deterioration and higher death (10, 11). The incidence of hypoproteinemia in hospitalized patients is ∼21% (12), but its incidence in acute illness is more than 50% (13).

In addition, ALB is closely related to the occurrence and progression of AKI. According to David R Williamson's study, ALB administration was linked with a dose-dependent risk of AKI associated with colloids after heart surgery (14). Low serum ALB was shown to be an independent risk factor for AKI in the meta-analysis of Michael Joannidis, which comprised 43 retrospective observational cohort studies that include 68,000 patients (15).

However, fewer studies have been conducted to investigate the relationship between serum ALB and the prognosis of critically ill patients with AKI and treated with CRRT, and it is unclear whether serum ALB affects the clearance efficiency of serum phosphorus. Therefore, this research assumes that the higher the serum ALB before CRRT, the lower the mortality of severe AKI patients and the higher the serum phosphorus clearance efficiency.

Abbreviations: CRRT, continuous renal replacement therapy; ALB, albumin; AKI, acute kidney injury; AKIN, acute kidney injury network; CKD, chronic kidney disease; ICU, intensive care unit; BMI, body mass index; MAP, mean arterial pressure; CCI score, Charlson comorbidity index; COPD. chronic obstructive pulmonary disease; WBC, white blood cell; HB, hemoglobin; BUN, blood urea nitrogen; Cr, creatinine; CRP, C-reactive protein; SOFA, sequential organ failure assessment; CVVH, continuous venovenous Hemofiltration.

METHODS

Study Design

A secondary analysis of a bi-center, retrospective, and observational study including critically ill patients with acute kidney injury (AKI) and treated with CRRT from January 2009 to September 2016 at Yonsei University Health System Severance Hospital and National Health Insurance Service Medical Center Ilsan hospital, Republic of Korea.

Objective

The study aimed to explore the relationship of ALB with the 28- and 90-day mortality of patients with AKI and treated with CRRT and with the removal efficiency of serum phosphorus.

Ethics Approval and Consent to Participate

New ethics permission and consent to participate were not applicable since the original author had received ethical approval while performing this research, and our study was a retrospective analysis of data reuse.

Data Source

The data used in this study were shared by Seung Hyeok Han, which were stored in the Dryad database (https://datadryad.org//resource/doi:10.5061/dryad.6v0j9) (7). The database is a public data repository, which contains data uploaded by the authors to make their research data discoverable, freely reusable, and citable.

Inclusion Criteria

(1) Patients were complicated with AKI and treated with CRRT in the intensive care unit (ICU); (2) the stage of AKI was two or more according to the acute kidney injury network (AKIN) criteria, in which serum creatinine and urine outputs were taken into account.

Exclusion Criteria

Patients with the following situation were excluded in this study: (1) age < 18 years; (2) pregnant or lactating women; (3) with postrenal obstruction; (4) with Stage 5 chronic kidney disease (CKD), kidney transplantation, dialysis, or CRRT; (5) the value of ALB was missing or outliers.

Participants

From January 2009 to September 2016, 2,110 patients were presented with AKIN stage 2 or more and treated with CRRT, of which 978 patients were excluded in the ICU at Yonsei University Health System Severance Hospital and National Health Insurance Service Medical Center Ilsan hospital: (1) age < 18 years ($n = 42$); (2) pregnant or lactating women ($n = 12$); (3) with postrenal obstruction ($n = 263$); (4) with the history of

stage 5 CKD, kidney transplantation, dialysis, or CRRT ($n = 585$); (5) the value of ALB was missing ($n = 9$) or outliers ($n = 3$), the ALB of two patients were 0 g/L, and the another was 5.9 g/L). Finally, a total of 1,132 patients with AKI and treated with CRRT were included in the current study (as shown in **Figure 1**).

Grouping

According to ALB before CRRT, patients were divided into three groups: ALB $<$ 25 g/L ($n = 436$), 25 g/L $\leq$ ALB $<$ 30 g/L ($n = 401$), and 30 g/L $\leq$ ALB.

The Outcome Indicators

(1) 28 and 90 days mortality; (2) the removal efficiency of serum phosphorus, delta phosphate = phosphate (24 h)-phosphate (0 h).

Collection of Clinical and Biochemical Data

The following variables were included in the current study, such as age, sex, body mass index (BMI), mean arterial pressure (MAP), complications (myocardial infarction, congestive heart failure, cerebrovascular disease, peripheral vascular disease, dementia, diabetes mellitus, hypertension, and chronic obstructive pulmonary disease), Charlson comorbidity index (CCI) score, biochemical laboratory tests, e.g., K^+, $HCO3^-$, phosphate (0 h), phosphate (24 h), delta phosphate, white blood cell (WBC), hemoglobin (HB), blood urea nitrogen (BUN), creatinine (Cr) and C-reactive protein (CRP), and sequential organ failure assessment (SOFA) score. Age, gender, BMI, and CCI scores were collected at the time of admission; and the other variables were collected at 0 h of CRRT.

CRRT Protocol

The nephrologist assessed whether the patients should be treated with CRRT based on the development of AKI in the ICU patients. The CRRT machines were the multiFiltrate (Fresenius Medical Care, Bad Homburg, Germany) or the Prismaflex (Baxter International Inc. Lundia AB, Sweden). The applied dialyzers had a surface area of 1.0–1.4 m^2 with a sieving coefficient for albumin and ß2-microglobulin of 0.001 and 0.58–0.65, respectively. The parameters of CRRT were the following: (1) model: Continuous venovenous hemofiltration (CVVH) through the internal jugular, subclavian, or femoral vein; (2) blood flow: the start was at 100 ml/min and up to 150 ml/min; (3) The total dialysis and replacement dose were targeted to deliver $\geq$ 35 ml/kg/h in all the patients.

Statistical Analysis

(1) Statistical description: Mean $\pm$ SD ($x \pm s$) was used for the continuous variables of baseline data in the groups, and counts numerical values and percentages were shown in the data. The data were compared using the *t*-test, if continuous data had a normal distribution and homogeneity of variance. Mann–Whitney's *U*-test was performed if the continuous data did not meet the normal distribution or homogeneity of the variance. For categorical data, the χ^2-test was utilized. (2) Analyze the relationship between ALB and 28- and 90-day mortality of patients, and delta phosphate by univariate and multivariate analysis. A multivariate Cox regression was performed for 28- and 90-day mortality, and multivariate logistic regression was performed for delta phosphate. (3) To further understand the relationship between ALB and 28- and 90-day mortality of patients, subgroup analyses were performed on the age, MAP, congestive heart failure, hypertension, AKIN stage,

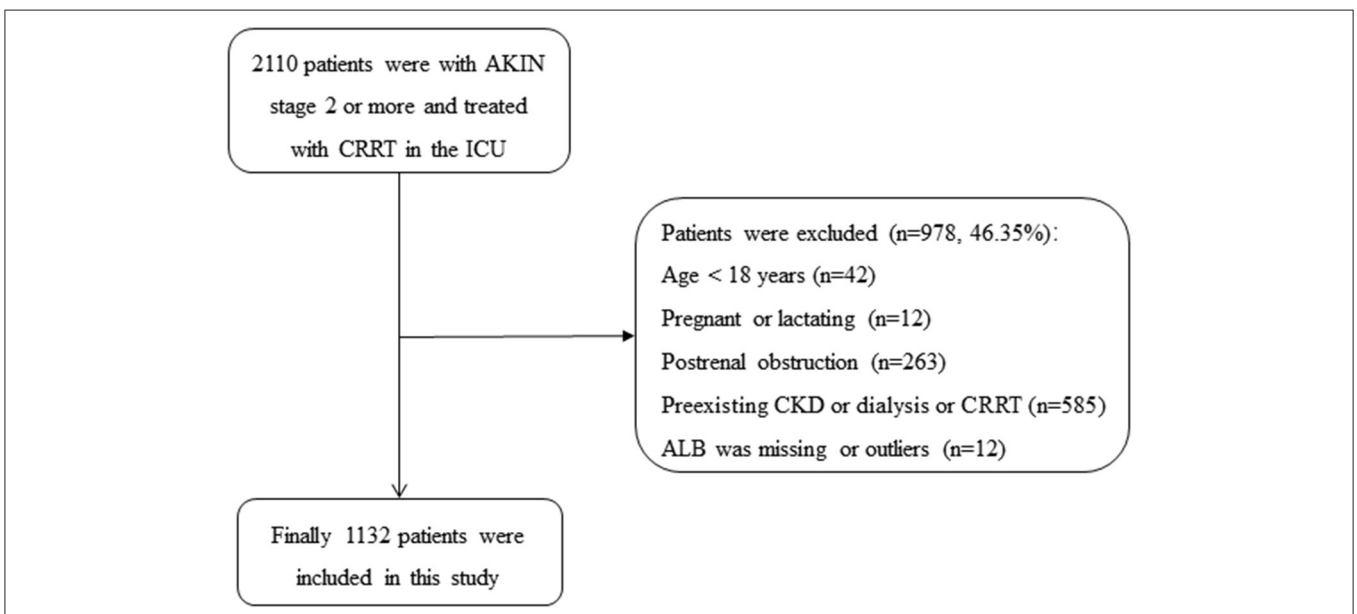

FIGURE 1 | Flowchart of patient selection. AKIN, acute kidney injury network; ALB, albumin; CKD, chronic kidney diseases; CRRT, continuous renal replacement therapy; ICU, intensive care unit.

mechanical ventilation, SOFA score, AKI causes, and CRRT causes. (4) The selection of adjustment variables were by the following: if the confounders influenced the effective estimate of ALB by more than 10% and identified with the literature, we would adjust it. (5) Curve fitting analysis was used to investigate the connection among ALB, 28- and 90-day mortality, and delta phosphate. All statistical analyses were carried out by EmpowerStats 2.0 (Copyright 2009 X&Y Solutions, Inc.) and R software (version 3.4.3). The value of $P < 0.05$ was statistically significant.

TABLE 1 | The clinical characteristics of patients.

Variables	Alb < 25 (*n* = 436)	25 ≤ Alb < 30 (*n* = 401)	30 ≤ Alb (*n* = 295)	*P*-value
Age, year	63.31 ± 14.21	64.27 ± 14.09	61.82 ± 14.89	0.091
Sex (M/F)	280/156	241/160	178/117	0.399
BMI, kg/m^2	23.52 ± 4.72	23.75 ± 4.49	24.24 ± 4.48	0.034
Myocardial infarction, *n* (%)	31 (7.11%)	39 (9.73%)	39 (13.22%)	0.023
Congestive heart failure, *n* (%)	54 (12.39%)	68 (16.96%)	63 (21.36%)	0.005
Cerebrovascular disease, *n* (%)	47 (10.83%)	44 (11.03%)	22 (7.46%)	0.233
Peripheral vascular disease, *n* (%)	19 (4.36%)	16 (3.99%)	10 (3.39%)	0.806
Dementia, *n* (%)	21 (4.82%)	17 (4.24%)	4 (1.36%)	0.041
Diabetes mellitus, *n* (%)	146 (33.49%)	145 (36.25%)	104 (35.25%)	0.698
Hypertension, *n* (%)	241 (55.28%)	211 (52.62%)	142 (48.14%)	0.165
COPD, *n* (%)	29 (6.65%)	29 (7.23%)	22 (7.46%)	0.905
CCI score	3.17 ± 2.40	3.19 ± 2.05	3.09 ± 2.25	0.418
K^+, mmol/L	4.74 ± 1.11	4.58 ± 1.05	4.81 ± 1.13	0.016
$HCO3^-$, mmol/L	15.76 ± 5.41	17.72 ± 5.82	17.46 ± 5.86	< 0.001
Phosphate (0 h), mg/dL	5.79 ± 2.57	5.49 ± 2.05	6.03 ± 2.58	0.061
Phosphate (24 h), mg/dL	4.85 ± 2.56	4.24 ± 1.83	4.64 ± 2.52	0.025
Delta phosphate	−0.96 ± 2.40	−1.23 ± 1.81	−1.41 ± 2.62	0.043
MAP, mmHg	76.43 ± 14.51	78.13 ± 14.33	77.30 ± 14.70	0.533
Hb, g/dL	9.28 ± 2.12	9.66 ± 2.09	10.08 ± 2.45	< 0.001
BUN, mg/dL	57.78 ± 31.84	56.18 ± 27.68	52.52 ± 29.91	0.020
Cr, mg/dL	2.70 ± 1.62	2.67 ± 1.39	2.84 ± 1.88	0.794
CRP, mg/dL	121.51 ± 108.85	120.20 ± 116.13	82.83 ± 91.19	< 0.001
WBC, 10^9/L	13.76 ± 12.768	16.04 ± 14.82	13.75 ± 8.74	0.038
SOFA score	12.33 ± 3.38	12.41 ± 3.40	11.40 ± 3.83	< 0.001
2 h urine output before CRRT, ml	65.13 ± 99.67	72.94 ± 99.04	79.40 ± 111.15	0.087
AKI cause				< 0.001
Sepsis, *n* (%)	324 (74.31%)	279 (69.58%)	189 (64.07%)	
Nephrotoxin, *n* (%)	14 (3.21%)	14 (3.49%)	8 (2.71%)	
Ischemia, *n* (%)	46 (10.55%)	31 (7.73%)	20 (6.78%)	
Surgery, *n* (%)	24 (5.50%)	37 (9.23%)	31 (10.51%)	
Others, *n* (%)	28 (6.42%)	40 (9.98%)	47 (15.93%)	
AKIN stages				0.866
2, *n* (%)	113 (25.92%)	102 (25.44%)	81 (27.46%)	
3, *n* (%)	323 (74.08%)	299 (74.56%)	214 (72.54%)	
Mechanical ventilation, *n* (%)	361 (82.80%)	318 (79.30%)	212 (72.11%)	0.002
CRRT cause				0.190
Volume overload, *n* (%)	48 (11.01%)	55 (13.72%)	55 (18.64%)	
Metabolic acidosis, *n* (%)	94 (21.56%)	86 (21.45%)	60 (20.34%)	
Hyperkalemia, *n* (%)	25 (5.73%)	14 (3.49%)	18 (6.10%)	
Uremia, *n* (%)	44 (10.09%)	44 (10.97%)	26 (8.81%)	
Oliguria, *n* (%)	110 (25.23%)	109 (27.18%)	72 (24.41%)	
Other, *n* (%)	115 (26.38%)	93 (23.19%)	64 (21.69%)	
CRRT does, ml/kg	36.87 ± 4.83	36.66 ± 4.99	36.27 ± 4.53	0.317
28-day death, *n* (%)	307 (70.41%)	257 (64.09%)	141 (47.80%)	< 0.001
90-day death, *n* (%)	350 (80.28%)	294 (73.32%)	171 (57.97%)	< 0.001

RESULTS

Baseline Characteristics

In this research, 1,132 patients with AKI and treated with CRRT were included. The ages of three groups included were as follows: ALB $<$ 25 g/L ($n = 436$), 25 g/L $\leq$ ALB $<$ 30 g/L ($n = 401$), and 30 g/L $\leq$ ALB were 63.31 $\pm$ 14.21, 64.27 $\pm$ 14.09, and 61.82 $\pm$ 14.89 years, respectively, with no significant difference ($P = 0.091$). The male-to-female ratio among the three groups was 280/156 (1.79), 241/160 (1.51), and 178/117 (1.52), with no statistical difference ($P = 0.399$). The BMI among the three groups was 23.52 $\pm$ 4.72, 23.75 $\pm$ 4.49, and 24.24 $\pm$ 4.48 kg/m^2, respectively, with statistical significance ($P = 0.034$). The 28-day mortality among the three groups was 307 (70.41%), 257 (64.09%), and 141 (47.80%), respectively, with statistical significance ($P < 0.001$). The 90-day mortality among the three groups was 350 (80.28%), 294 (73.32%), and 171 (57.97%), respectively, with statistical significance ($P < 0.001$). There was a significant difference between myocardial infarction, congestive heart failure, and dementia ($P < 0.05$). There was a significant difference in HCO^{3-}, phosphate (24 h), delta phosphate, HB, CRP, WBC, SOFA score, AKI cause, and CRRT cause across the three groups ($P < 0.05$). There was no significant difference in the other variables between the groups ($P > 0.05$) (as shown in **Table 1**).

ALB Was Associated With the 28- and 90-day Mortality of Patients With AKI and Treated With CRRT

When ALB was used as a continuous variable, the higher the serum ALB before CRRT, the lower the mortality of critically ill patients with AKI and treated with CRRT. The adjusted hazard ratio (HR) value for 28-day mortality in the four models were separately 0.92 (0.90, 0.95), 0.91 (0.89, 0.94), 0.92 (0.89, 0.95), and 0.92 (0.89, 0.95). The adjusted HR value for 90-day mortality in the four models were 0.91 (0.89, 0.94), 0.92 (0.89, 0.95), 0.92 (0.89, 0.95), and 0.92 (0.89, 0.96). When ALB was used as a classification variable and ALB $<$ 25 g/L as a reference, it was discovered that when 25 g/L $\leq$ ALB $<$ 30 g/L and 30 g/L $\leq$ ALB, the 28- and 90-day mortality of patients with AKI and treated with CRRT were substantially decreased (as shown in **Table 2**).

The Sensitivity Analysis Was Used to Detect the Relationship Between the ALB and the 28- and 90-day Mortality of Patients With AKI and Treated With CRRT

Age, MAP, myocardial infarction, congestive heart failure, diabetes mellitus, hypertension, AKIN stage, mechanical ventilation, SOFA score, AKI causes, and CRRT causes were

TABLE 2 | Multivariate logistic regression analysis for 28- and 90-day mortality.

Exposure	28-day mortality (Adjusted HR 95%CI)	*P*-value	90-day mortality (Adjusted HR 95%CI)	*P*-value
Model 1				
Alb, g/L	0.92 (0.90, 0.95)	< 0.001	0.91 (0.89, 0.94)	< 0.001
Alb, g/L				
<25	1.00 (Reference)		1.00 (Reference)	
25≤ and <30	0.71 (0.53, 0.96)	0.027	0.62 (0.45, 0.87)	0.006
30≤	0.37 (0.27, 0.51)	< 0.001	0.32 (0.23, 0.45)	< 0.001
Model 2				
Alb, g/L	0.91 (0.89, 0.94)	< 0.001	0.92 (0.89, 0.95)	< 0.001
Alb, g/L				
<25	1.00 (Reference)		1.00 (Reference)	
25≤ and <30	0.54 (0.36, 0.80)	0.002	0.53 (0.35, 0.81)	0.003
30≤	0.35 (0.23, 0.54)	< 0.001	0.37 (0.24, 0.59)	< 0.001
Model 3				
Alb, g/L	0.92 (0.89, 0.95)	< 0.001	0.92 (0.89, 0.95)	< 0.001
Alb, g/L				
<25	1.00 (Reference)		1.00 (Reference)	
25≤ and <30	0.57 (0.38, 0.87)	0.009	0.56 (0.36, 0.88)	0.011
30≤	0.35 (0.22, 0.56)	< 0.001	0.39 (0.24, 0.63)	< 0.001
Model 4				
Alb, g/L	0.92 (0.89, 0.95)	< 0.001	0.92 (0.89, 0.96)	< 0.001
Alb, g/L				
<25	1.00 (Reference)		1.00 (Reference)	
25≤ and <30	0.58 (0.37, 0.91)	0.017	0.59 (0.37, 0.94)	0.027
30≤	0.37 (0.22, 0.60)	< 0.001	0.41 (0.24, 0.68)	< 0.001

Model 1: Adjusted for age; sex; body mass index (BMI); Charlson comorbidity index (CCI score). Model 2: Adjusted for model 1 plus C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), phosphate (0 h), K^+, HCO3-. Model 3: Adjusted for model 2 plus acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, (acute kidney injury network (AKIN) stages, CRRT does, 2 h urine output before CRRT initiation. Model 4: Adjusted for model 3 plus sequential organ failure assessment (SOFA) score.

all subjected to sensitivity analysis. ALB was associated with 28-day mortality in the sensitivity analysis, except in individuals with myocardial infarction, AKIN stage 2, or CRRT cause (hyperkalemia, uremia, and oliguria). Additional sensitivity analysis for 90-day mortality revealed that ALB was linked with 90-day death in all the patients except those with myocardial infarction, congestive heart failure, AKIN stage 2, or CRRT cause (hyperkalemia, uremia, and oliguria; as shown in **Table 3**).

ALB Was Associated With the Removal Efficiency of Phosphate

When ALB was used as a continuous variable, the higher the serum ALB before CRRT treatment, the higher the clearance efficiency of serum phosphorus. The adjusted OR values for delta phosphate in the four models were −0.04 (−0.07, −0.01), −0.05 (−0.08, −0.01), −0.04 (−0.08, −0.01), and −0.04 (−0.08, −0.01). When ALB was used as a classification variable, it

TABLE 3 | The subgroup analysis of multivariate logistic regression analysis for 28- and 90-day mortality.

Exposure	28-day mortality (Adjusted HR 95%CI)	*P*-value	90-day mortality (Adjusted HR 95%CI)	*P*-value
Age, year				
<65	0.95 (0.92, 0.98)	0.001	0.97 (0.94, 1.00)	0.031
65≤	0.96 (0.93, 0.98)	< 0.001	0.94 (0.92, 0.97)	< 0.001
MAP, mmHg				
<65	0.91 (0.87, 0.95)	< 0.001	0.94 (0.91, 0.98)	0.006
65≤	0.96 (0.94, 0.98)	< 0.001	0.96 (0.94, 0.98)	< 0.001
Myocardial infarction				
Yes	1.05 (0.95, 1.17)	0.315	0.98 (0.88, 1.08)	0.638
No	0.96 (0.94, 0.98)	< 0.001	0.96 (0.94, 0.98)	< 0.001
Congestive heart failure				
Yes	0.94 (0.90, 0.99)	0.018	0.961 (0.919, 1.004)	0.077
No	0.96 (0.94, 0.98)	< 0.001	0.956 (0.938, 0.975)	< 0.001
Diabetes mellitus				
Yes	0.95 (0.92, 0.98)	< 0.001	0.94 (0.91, 0.97)	< 0.001
No	0.98 (0.96, 1.00)	0.111	0.97 (0.95, 0.99)	0.007
Hypertension				
Yes	0.96 (0.94, 0.99)	0.005	0.95 (0.92, 0.97)	< 0.001
No	0.95 (0.92, 0.97)	< 0.001	0.96 (0.93, 0.98)	< 0.001
AKIN stage				
2	0.97 (0.93, 1.00)	0.081	0.97 (0.93, 1.00)	0.062
3	0.95 (0.93, 0.97)	< 0.001	0.95 (0.93, 0.97)	< 0.001
Mechanical ventilation				
Yes	0.97 (0.95, 0.99)	0.001	0.96 (0.94, 0.98)	< 0.001
No	0.95 (0.91, 1.00)	0.036	0.95 (0.91, 0.99)	0.027
SOFA score				
<8	0.85 (0.74, 0.98)	0.026	0.86 (0.77, 0.96)	0.007
8≤ and <12	0.95 (0.92, 0.99)	0.006	0.95 (0.92, 0.98)	0.001
12≤	0.97 (0.94, 0.99)	0.011	0.97 (0.95, 0.99)	0.012
AKI causes				
Sepsis	0.96 (0.94, 0.98)	< 0.001	0.95 (0.93, 0.97)	< 0.001
Non-sepsis	0.94 (0.90, 0.98)	0.004	0.95 (0.91, 0.98)	0.004
CRRT causes				
Volume overload	0.89 (0.84, 0.94)	< 0.001	0.91 (0.86, 0.95)	< 0.001
Metabolic acidosis	0.95 (0.91, 0.99)	0.015	0.94 (0.90, 0.98)	0.002
Hyperkalemia	1.01 (0.86, 1.17)	0.935	1.03 (0.91, 1.18)	0.617
Uremia	1.02 (0.92, 1.13)	0.667	0.97 (0.88, 1.07)	0.549
Oliguria	1.00 (0.97, 1.05)	0.820	0.98 (0.95, 1.02)	0.292
Other	0.94 (0.91, 0.98)	0.005	0.96 (0.92, 0.99)	0.022

Adjusted variables (without the subgroup analysis variables themselves): age; sex; body mass index (BMI); Charlson comorbidity index (CCI score), C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), phosphate (0 h), K+, HCO3-, acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, acute kidney injury network (AKIN) stages, CRRT does, 2 h urine output before CRRT initiation, sequential organ failure assessment (SOFA) score.

was also found that the higher the ALB of patients, the higher the removal efficiency of serum phosphorus (as shown in **Table 4**).

The Relationship Among ALB and 28-, 90-day Mortality, and the Removal Efficiency of Serum Phosphorus Explored by Curve Fitting Analysis

In this study, we discovered that the higher the ALB, the lower the 28- and 90-day mortality of patients with AKI treated with CRRT, and the higher the delta phosphate in the curve fitting analysis. The following variables were adjusted in curve fitting analysis: age, sex, BMI, CCI, CRP, WBC, HB, phosphate (0 h) (except for delta phosphate), k^{+}, HCO^{3-}, AKI cause, CRRT cause, AKIN stages, CRRT dose, 2 h urine output before CRRT, and SOFA score (as shown in **Figures 2–4**).

DISCUSSION

According to this study, the higher the ALB before CRRT, the lower the 28- and 90-day mortality of patients with AKI treated with CRRT. It was also discovered that the higher the ALB before CRRT, the greater the serum phosphorus clearance efficiency.

Hypoalbuminemia had been demonstrated in certain studies to exacerbate AKI and to deteriorate the prognosis of individuals with AKI. According to Michael Joannidis's study, hypoalbuminemia accelerated the progression of AKI, increased the need for CRRT therapy for AKI patients, and was a significant predictor of AKI incidence (OR: 2.96, 95% *CI*: 2.05–4.26) and mortality (OR: 2.47, 95% *CI*: 1.51–4.05) (16). A retrospective cohort study of 381 critically ill patients by Praveen Kolumam Parameswaran also found that serum hypoproteinemia was an independent risk factor for AKI (OR: 1.810, 95% *CI*: 1.102–2.992) in critically ill patients (17). It also promoted the progress of AKI to CKD (17). In addition, we constructed a prediction model about AKI, and they found that ALB was an independent predictor of 28-day mortality of AKI patients (18). The possible mechanisms are as follows: (1) Plasma colloid osmotic pressure was critical in controlling the exchange of water between the inside and outside of blood vessels and in maintaining blood volume, while albumin was the primary molecule responsible for sustaining plasma colloid osmotic pressure; (2) Hypoalbuminemia, when accompanied by blood volume reduction causes the liquid in blood vessels to leak out, further reducing the volume of blood vessels and aggravating renal perfusion, along with worsening acute kidney damage (7, 8, 18–20).

TABLE 4 | Multivariate logistic regression analysis for delta phosphate.

Exposure	delta phosphate (Unadjusted OR 95%CI)	*P*-value	delta phosphate (Adjusted OR 95%CI)	*P*-value
Model 1				
Alb, g/L	−0.04 (−0.07, −0.01)	0.004	−0.04 (−0.07, −0.01)	0.003
Alb, g/L				
<25	Reference		Reference	
25≤ and <30	−0.27 (−0.60, 0.06)	0.111	−0.24 (−0.58, 0.09)	0.153
30≤	−0.44 (−0.81, −0.07)	0.019	−0.48 (−0.85, −0.10)	0.012
Model 2				
Alb, g/L	−0.04 (−0.07, −0.01)	0.004	−0.05 (−0.08, −0.01)	0.006
Alb, g/L				
<25	Reference		Reference	
25≤ and <30	−0.27 (−0.60, 0.06)	0.111	−0.33 (−0.76, 0.10)	0.137
30≤	−0.44 (−0.81, −0.07)	0.019	−0.61 (−1.09, −0.13)	0.013
Model 3				
Alb, g/L	−0.04 (−0.07, −0.01)	0.004	−0.04 (−0.08, −0.01)	0.010
Alb, g/L				
<25	Reference		Reference	
25≤ and <30	−0.27 (−0.60, 0.06)	0.111	−0.24 (−0.68, 0.19)	0.271
30≤	−0.44 (−0.81, −0.07)	0.019	−0.60 (−1.08, −0.11)	0.016
Model 4				
Alb, g/L	−0.04 (−0.07, −0.01)	0.004	−0.04 (−0.08, −0.01)	0.014
Alb, g/L				
<25	Reference		Reference	
25≤ and <30	0.27 (−0.60, 0.06)	0.111	−0.23 (−0.67, 0.20)	0.293
30≤	−0.44 (−0.81, −0.07)	0.019	−0.58 (−1.06, −0.09)	0.020

Model 1: Adjusted for age; sex; body mass index (BMI); Charlson comorbidity index (CCI score). Model 2: Adjusted for model 1 plus C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), K+, HCO3-. Model 3: Adjusted for model 2 plus acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, acute kidney injury network (AKIN) stages, CRRT does, 2 h urine output before CRRT initiation. Model 4: Adjusted for model 3 plus sequential organ failure assessment (SO-0+FA) score.

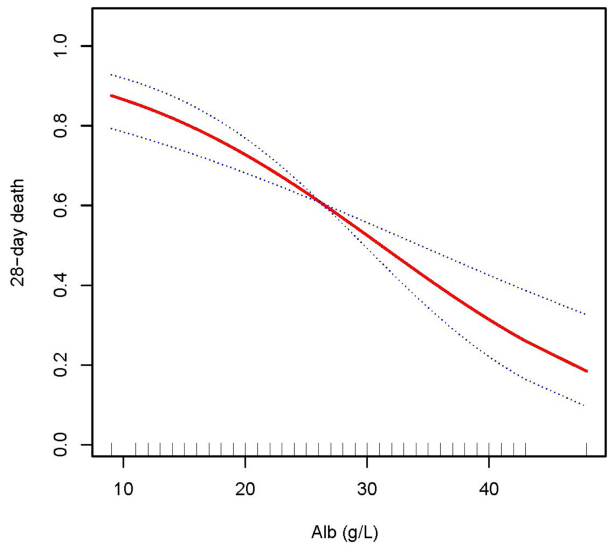

FIGURE 2 | Adjusted smoothing function of ALB for 28-day mortality. After adjusting for age, gender, body mass index (BMI), Charlson comorbidity index (CCI score), C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), phosphate (0 h), K^+, HCO^{3-}, acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, (acute kidney injury network (AKIN) stages, CRRT dose, 2 h urine output before CRRT initiation, and sequential organ failure assessment (SOFA) score, curve fitting analysis showed that the 28-day mortality rate decreased as the ALB increased.

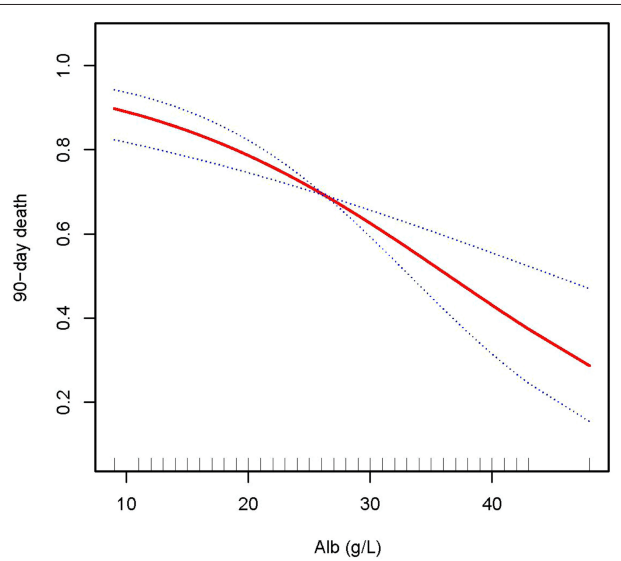

FIGURE 3 | Adjusted smoothing function of ALB for 90-day mortality. After adjusting for age, gender, body mass index (BMI), Charlson comorbidity index (CCI score), C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), phosphate (0h), K^+, HCO^{3-}, acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, (acute kidney injury network (AKIN) stages, CRRT dose, 2 h urine output before CRRT initiation, andsequential organ failure assessment (SOFA) score, curve fitting analysis showed that the 90-day mortality rate decreased as the ALB increased.

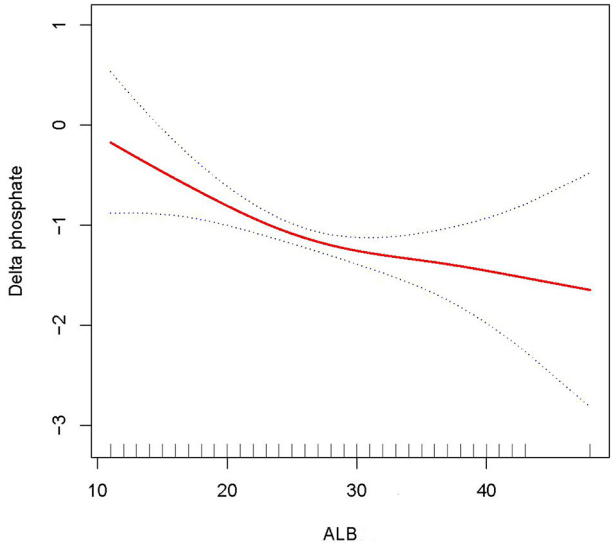

FIGURE 4 | Adjusted smoothing function of ALB for delta phosphate. After adjusting for age, gender, body mass index (BMI), Charlson comorbidity index (CCI score), C-reactive protein (CRP), white blood cell (WBC), hemoglobin (HB), K^+, HCO^{3-}, acute kidney injury (AKI) cause, continuous renal replacement therapy (CRRT) cause, (acute kidney injury network (AKIN) stages, CRRT dose, 2 h urine output before CRRT initiation, and sequential organ failure assessment (SOFA) score, curve fitting analysis showed that delta phosphate increased as the ALB increased.

Continuous renal replacement therapy is often utilized in renal replacement therapy for critically ill patients, especially for those with hemodynamic instability (21). CRRT treatment for critically ill patients removes excess water and certain potentially toxic macromolecular compounds (22). Several studies had shown that insufficient CRRT treatment would result in adverse outcomes for patients. Simultaneously, it raised the risk of re-CRRT therapy, thus increasing the medical risk, treatment cost, and length of stay (23). According to studies, serum phosphorus clearance in CRRT was positively associated with and reasonably near to the serum creatinine and urea nitrogen clearance in CRRT (4–6). Meanwhile, investigations had indicated that phosphate (0 h), phosphate (24 h), and delta phosphate were all linked to a higher risk of death in critically ill patients with septic AKI undergoing CRRT (7, 8, 18, 19). This study found that the higher serum ALB, the more serum phosphorus decreased after CRRT. However, since there were no indications of creatinine or urea nitrogen following CRRT therapy in this research, the connection between albumin and the clearance efficiency of CRRT treatment was not yet established and needed to be further investigated and validated.

Hypotension often occurs during the early stages of CRRT. The primary cause of hypotension in the early stages of CRRT is insufficient blood vessel content (24). Serum ALB is required to maintain enough blood vessel content. The higher the serum ALB, the more abundant the blood vessel content, so the lower the risk of hypotension during CRRT. According to studies,

hypotension 1 h after the start of CRRT increased the hospital mortality and was an independent predictor of hospital mortality (25). To summarize, it was considered that the higher serum ALB might enhance the prognosis of critically ill patients with AKI and treated with CRRT: (1) The higher serum ALB, the higher the clearance efficiency of serum phosphorus; (2) The higher the serum ALB, the more the blood vessel content, and the lower risk of hypotension during CRRT.

Age, MAP, myocardial infarction, congestive heart failure, diabetes mellitus, hypertension, AKIN stage, mechanical ventilation, SOFA score, AKI causes, and CRRT causes were all subjected to the sensitivity analysis. It was found that ALB was associated with the 28-day mortality, except for patients with myocardial infarction, AKIN stage 2, and CRRT cause (hyperkalemia, uremia, and oliguria). The potential explanation was that the risk of mortality from myocardial infarction was very high, masking the effect of serum ALB; several studies indicated that patients with AKIN stage 2 who had CRRT could not improve their prognosis (1, 26), which may be the reason why serum ALB did not affect the prognosis of patients with myocardial infarction and AKIN stage 2 undergoing CRRT in this study. Studies had also shown that hyperkalemia, oliguria, or anuria were not related to patient mortality, which was similar to the findings of this research (27, 28).

Strength of the Study

(1) Through multivariate analysis, sensitivity analysis, and adjusting the potential confounding factors, this study got a more consistent conclusion: The higher the serum ALB, the better the prognosis of patients with AKI and treated with CRRT; (2) This study also made it clear that increasing the serum ALB might improve the clearance efficiency of serum phosphorus, and then improve the prognosis of critically ill patients with AKI and treated with CRRT.

Limitations of the Study

(1) This research belongs to a bi-center, retrospective, and observational cohort study and lacked important data such as length of the sessions, how many sessions throughout the ICU stay, and methods for coagulation. This lead to a certain possible danger of bias in this research, so its conclusion needed to be verified by a prospective study. (2) The AKIN standard was used to diagnose acute renal injury in this research; however, Kidney Disease Improving Global Outcomes (KDIGO) may be a superior AKI diagnostic standard. (3) Due to the lack of data on creatinine and urea nitrogen after CRRT in this study, only the relationship between ALB and serum phosphorus clearance was obtained in this study, while the relationship between ALB and CRRT clearance efficiency was needed to be further studied. (4) Albumin will rely on the condition of extracellular hydration and blood volume, although hydration quantity, nutritional status of the patients, and exogenous protein supplements might influence albumin measurements, and lead to a certain potential risk of bias. (5) In addition, some patients lost to follow-up, which increased the possibility of bias in the results.

CONCLUSION

The higher the serum ALB before CRRT, the lower the mortality of critically ill patients with AKI and treated with CRRT, and the higher the clearance efficiency of serum phosphorus.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://datadryad.org//resource/doi:10.5061/dryad.6v0j9.

ETHICS STATEMENT

Agreement to participate in the study was not required because our review was a retrospective study of data reuse, and the patients' data was anonymous. Ethical approval was not provided for this study on human participants. The original author had obtained ethical approval when conducting his study. The ethics committee waived the requirement of written informed consent for participation in this study.

AUTHOR CONTRIBUTIONS

JL and HW participated in the research design, the revision of the manuscript, and data analysis. BS participated in the data analysis and writing of the paper. YG and ZZ participated in improving and revising the paper. HP provided substantial advice in designing the study and assisting in the division of labor, writing, and revising the paper. All authors contributed to the article and approved the submitted version.

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Relationship Between Sleep–Wake Disturbance and Risk of Malnutrition in Hospitalized Patients With Cirrhosis

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Both sleep–wake disturbance and malnutrition are common in cirrhosis and might be associated with similar adverse outcomes, such as impaired health-related quality of life, hepatic encephalopathy, and sarcopenia, but there is no study investigating the relationship between these two. We aimed to explore the relationship between sleep–wake disturbance [estimated by the Pittsburgh Sleep Quality Index (PSQI)] and malnutrition risk [estimated by the Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT)]. About 150 patients with cirrhosis were prospectively recruited. The nutritional risk is classified as low (0 points), moderate (1 point), and high (2–7 points) according to the RFH-NPT score. A global PSQI >5 indicated poor sleepers. Furthermore, multivariate linear regression analyses were performed to determine the relationship between *sleep–wake* disturbance and malnutrition. The median PSQI was seven, and RFH-NPT was two in the entire cohort, with 60.67 and 56.67% rated as poor sleep quality and high malnutrition risk, respectively. Patients with cirrhosis with poor sleep quality had significantly higher RFH-NPT score (3 vs. 1, $P = 0.007$). Our multivariate analyses indicated that male patients ($\beta = 0.279$, $P < 0.001$), ascites ($\beta = 0.210$, $P = 0.016$), and PSQI ($\beta = 0.262$, $P = 0.001$) were independent predictors of malnutrition. In addition, the differences regarding PSQI score were more significant in male patients, as well as those >65 years or with Child-Turcotte-Pugh class A/B (CTP-A/B) or the median model for end-stage liver disease (MELD) <15. Taken together, the sleep–wake disturbance is strongly correlated with high malnutrition risk in patients with cirrhosis. Given sleep–wake disturbance is remediable, it is tempting to incorporate therapies to reverse poor sleep quality for improving nutritional status in patients with cirrhosis.

Keywords: malnutrition, PSQI, RFH-NPT, sleep-wake disturbance, liver cirrhosis

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INTRODUCTION

Sleep–wake disturbance is a common feature of cirrhosis and advanced chronic liver diseases. It has been estimated that approximately 60% or more of patients with cirrhosis regarded themselves as poor sleepers, which was determined by the Pittsburgh Sleep Quality Index (PSQI) (1). The causality between deteriorating sleep and other predisposing factors in patients with cirrhosis is still under extensive investigation with inconsistent data. Montagnese et al. showed sleep deterioration assessed by PSQI is irrelevant to the presence/degree of hepatic encephalopathy (HE) (2). Conversely, another study implicated that sleep quality, with 24-h polysomnography (PSG), improves in parallel with the amelioration of HE (3). Furthermore, sleep–wake disturbance could negatively and independently affect health-related quality of life (HRQoL) and increase the risk of developing hepatic malignancies (4, 5).

Malnutrition is another cirrhosis-associated complication, which could result in an increased risk of liver failure, infection, higher prevalence of complications due to portal hypertension, and prolonged hospitalized stays (6). The prevalence of malnutrition dramatically increased in correspondence with aggravated liver function, while more than half the patients with cirrhosis represent malnourished and concomitant decompensated insults (7). More recently, several studies have investigated the relationships between sleep disorders and malnutrition risk in distinct pathological entities. Notably, Soysal et al. implicated a close association between moderate/severe insomnia and the presence of malnutrition as well as high malnutrition risk in elders (8). Another study found that sleep disorders are significantly correlated with malnutrition risk in older adults (9). Given these two complications might share multiple converging mechanisms and lead to similar outcomes, we speculate that poor sleep quality might be associated with a high risk of malnutrition in cirrhotics.

As far as we can determine, there is a paucity of data exploring the association between sleep–wake disturbance and nutritional state among hospitalized cirrhotics. Collectively, the present study aimed to (1) analyze the association between sleep quality and nutritional state; and (2) clarify the differences regarding PSQI scores in terms of malnutrition risk across various subgroups.

MATERIALS AND METHODS

Study Cohort

Patients with cirrhosis aged ≥18 years, who were hospitalized between December 2019 and January 2021, were prospectively enrolled for the current study. Those with concomitant malignancies, severe HE (*via* the time to finish a numbers connection test of >120 s), and presence with acute-on-chronic liver failure were excluded (10, 11). In concert with previous work conducted by Ghabril et al., we did not exclude patients with active alcohol since they contribute to a significant subset of patients with cirrhosis (4). In our center, HE was detected from the time to complete the number connection test performed upon hospitalization and categorized as present if ≥60 s needed to complete the test (10, 12, 13). The diagnosis of liver cirrhosis was based on medical history, laboratory examinations, imaging results, endoscopic data, and/or liver biopsy.

Sleep Quality

We evaluated the sleep quality using a Chinese version of the PSQI for screening sleep–wake disturbance (14). The reliability and validity of PSQI have gained broad acceptance worldwide. It comprises a total of 10 questions ranging from seven components of sleep patterns, namely, subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, sleep medication use, and daytime dysfunction. Each component is scored on 0–3 points. The sum of the scores of all seven categories composes the total PSQI score. A higher score unravels poorer sleep quality, where a global PSQI score >5 has been verified to discriminate between poor from good sleeper (15).

Royal Free Hospital-Nutritional Prioritizing Tool

The RFH-NPT scores were calculated according to our previous depiction (16). Briefly, the risk of malnutrition was categorized into low (0 points), moderate (1 point), and high (2–7 points) in terms of RFH-NPT scores. Initially, we inquired and recorded the presence of tube feeding or acute alcoholic hepatitis, because these medical issues might predispose subjects to highly malnourished conditions. Next, we clarified between the groups of patients present with or without edema/ascites. At last, the total scores were summarized, and individuals were demarcated to the corresponding risk groups.

Clinical and Laboratory Metrics

Details in relation to clinical and laboratory results have been explicitly introduced in our previous publication (17). Because a large number of patients with cirrhosis presented with fluid retention, it is more reasonable to calculate dry weight for assessing body mass index (BMI). We calculated the dry weight by subtracting 5% for mild ascites, 10% for moderate ascites, and 15% for bulky ascites for subjects with edema and ascites, and 5% of body weight was subtracted for patients with peripheral edema (18).

Statistical Analyses

Descriptive statistics were presented as mean ± SD, median [interquartile range (IQR)], proportions, or simple frequencies as appropriate. Continuous data were compared by an independent Student's *t*-test or Mann–Whitney *U* test in cases without normal distribution. Multiple comparisons were performed by using the one-way ANOVA or the Kruskal–Wallis test with Dunn's post-*hoc* test. The univariate analysis accounted for the correlation that exists between demographic/laboratory parameters, PSQI scores, and RFH-NPT scores. Multivariate linear regression analysis was implemented to figure out the independent factors associated with the risk of malnutrition as measured by RFH-NPT. We regarded $P < 0.05$ as statistically significant. All statistical analyses were carried out by using SPSS 21.0 (IBM, New York, NY, USA) and Graphpad Prism 8.0.1 (La Jolla, CA, USA).

RESULTS

Table 1 describes the baseline characteristics of the study population separated by PSQI. A total of 150 patients with cirrhosis (male patients: $n = 70$, 46.67%) with a mean age of 61.24 ± 10.31 years were recruited to the investigation. The etiologies of cirrhosis were due to chronic viral infection in 38 (25.33%), alcohol in 35 (23.33%), autoimmune/cholestatic liver disease in 46 (30.67%), and cryptogenic/non-alcoholic fatty liver disease (NAFLD) in 31 participants (20.67%). Ninety-three patients (62.00%) presented with ascites upon admission, while this number was 13 with HE (8.67%). Among the overall subjects, 48 (32.00%) were classified as Child-Turcotte-Pugh (CTP) class A, 82 (54.67%) as CTP-B, and 20 as CTP-C (13.33%). The median model for end-stage liver disease (MELD) score was 9.6 (IQR, 7.2–12.2). When stratified by PSQI as good and poor sleepers, a total of 91 cirrhotics (60.67%) were classified as poor sleepers with PSQI of <5 points. Intriguingly, our results indicated that the poor sleepers have lower BMI (22.89 vs. 24.69 kg/m^2, $P = 0.003$), more ascites (69.23 vs. 50.85%, $P = 0.024$), and higher RFH-NPT scores (3 vs. 1 points, $P = 0.007$).

Table 2 shows the results of linear regression analyses of the sleep–wake disturbance associated with malnutrition risk estimated by RFH-NPT. Our univariable analyses showed age (β coefficient = 0.187, $P = 0.024$), male (β coefficient = 0.280, $P = 0.001$), ascites (β coefficient = 0.356, $P < 0.001$), PSQI (β coefficient = 0.350, $P < 0.001$), CTP score (β coefficient = 0.268, $P = 0.001$), albumin (β coefficient = −0.165, $P = 0.049$), and sodium (β coefficient = −0.186, $P = 0.027$) were factors associated with original RFH-NPT score with a $P < 0.05$. Further multivariate linear regression implicated that male (β coefficient = 0.279, $P < 0.001$), ascites (β coefficient = 0.210, $P = 0.016$), and PSQI (β coefficient = 0.262, $P = 0.001$) were independent factors for malnutrition risk as indicated by RFH-NPT.

The median PSQI score in the study population was seven (IQR, 4–10). As shown in **Table 3**, the patients at high malnutrition risk exhibited the highest PSQI score in comparison with other groups (7 vs. 6 vs. 5, $P = 0.008$). As shown in **Figure 1A**, the median PSQI score in the high malnutrition risk group was significantly higher than that in the low/moderate malnutrition risk groups (7 vs. 5, $P = 0.0017$). Additionally, the median PSQI score in the high malnutrition risk group was significantly higher than that in the low/moderate malnutrition risk group in male cirrhotics (7 vs. 4, $P = 0.0010$; **Figure 1B**). Conversely, no significant difference was observed regarding PSQI score between these two groups in female patients (7 vs. 6, $P = 0.1219$; **Figure 1C**).

As shown in **Figures 1D,E**, when stratified by age we showed that the median PSQI score in the high malnutrition risk group was markedly higher than that in the low/moderate malnutrition risk group in patients with cirrhosis <65 years (7 vs. 6, $P = 0.0121$). On the other hand, the median PSQI score in the high malnutrition risk group had a tendency toward significance in comparison with that in the low/moderate malnutrition risk group in patients with cirrhosis aged ≥65 years (7 vs. 5, $P = 0.0553$). Notably, the median PSQI scores in the high malnutrition risk group were significantly higher than those in the low/moderate malnutrition risk group rated as CTP-A (7 vs. 5, $P = 0.0151$), CTP-B (7 vs. 6, $P = 0.0442$), and with MELD <15 points (7 vs. 5, $P = 0.0071$; **Figure 2**).

DISCUSSION

Our present study explored the relationship between sleep–wake disturbance and malnutrition risk in hospitalized patients with cirrhosis, and the RFH-NPT score was observed to be significantly higher in poor sleepers. Moreover, PSQI has been demonstrated to be an independent risk factor positively correlated with RFH-NPT, namely, a high risk of malnutrition. In addition, the differences with respect to PSQI scores were markedly pronounced in male patients as well as those who were <65 years or with less deteriorating liver function.

Mounting evidence has addressed that both sleep–wake disturbance and malnutrition appear to be prevalent in patients with cirrhosis (19, 20). From the clinical perspective, sleep–wake disturbance might negatively impact HRQoL, depression, and psychological distress (4, 21). Notably, PSQI increased in parallel with HE, and patients with cirrhosis with higher PSQI scores suffered from worse HRQoL (22). More recently, a systemic review of 109 studies intended to comprehensively summarize the factors relevant to poor HRQoL in patients with cirrhosis (23). Their findings showed that malnutrition, as a modifiable issue, is among the top factors, which are associated with impairment in HRQoL in most studies. Collectively, since sleep–wake disturbance and malnutrition can lead to similarly adverse outcomes, and both are prevalent in cirrhotics; it is imperative to explore the interaction between these two complications.

In fact, some pioneering scholars in the field of geriatrics have already corroborated a close relationship between various sleep–wake abnormalities and malnutrition in older adults. Tuna et al. reported a negative correlation between the Simplified Nutritional Assessment Questionnaire score (perception of appetite, taste of food, portion of a meal enough for subjects to feel full, and number of daily meals) and the global PSQI score, which unravels the elderly with poor sleep quality exhibit a higher risk of weight loss (24). Another study showed that insomnia is significantly correlated with malnourished status and associated with a low Mini Nutritional Assessment score (8). A study recruiting 6,792 community-dwelling older adults in West China indicated that poor sleepers determined by PSQI >5 are associated with 162% higher risk of malnutrition (odds ratio [OR]: 1.62, 95% CI, 1.44–1.82) compared with good sleepers (9). However, there are scant data regarding the relationship between sleep disturbance and malnutrition risk in cirrhotics.

An important finding of the current investigation was that sleep–wake disturbance represents an independent risk factor for RFH-NPT, which refers to the risk of malnutrition, after adjusting for confounding variables. The standardized coefficient is noted with the strongest value ($\beta = 0.262$, $P = 0.001$) in comparison with other modifiable covariates (ascites: $\beta = 0.210$, $P = 0.016$). Therefore, it is tempting to effectively reverse disturbed sleep to improve malnourishment. As a matter of fact, a randomized, placebo-controlled trial conducted by

TABLE 1 | Baseline characteristics of cirrhotic patients classified by PSQI score.

	Total ($n = 150$)	Good sleepers ($n = 59$)	Poor sleepers ($n = 91$)	*P*
Age (years)	61.24 ± 10.31	59.83 ± 12.43	62.19 ± 8.56	0.628
Gender, *n* (%)				0.246
Male	70 (46.67)	31 (52.54)	39 (42.86)	
Female	80 (53.33)	28 (47.46)	52 (57.14)	
BMI (kg/m^2)	23.44 (20.29, 26.17)	24.69 (22.19, 27.43)	22.89 (19.37, 25.39)	0.003
Etiology, *n* (%)				0.945
Viral infection	38 (25.33)	18 (30.51)	20 (21.98)	
Alcohol	35 (23.33)	11 (18.64)	24 (26.37)	
AILD/Cholestatic	46 (30.67)	17 (28.81)	29 (31.87)	
Cryptogenic/NAFLD	31 (20.67)	13 (22.04)	18 (19.78)	
Ascites, *n* (%)	93 (62.00)	30 (50.85)	63 (69.23)	0.024
Hepatic encephalopathy, *n* (%)	13 (8.67)	6 (10.17)	7 (7.69)	0.768
CTP score	7 (6,9)	7 (6,9)	7 (6,9)	0.339
CTP class, *n* (%)				0.336
A	48 (32.00)	23 (38.98)	25 (27.47)	
B	82 (54.67)	29 (49.15)	53 (58.24)	
C	20 (13.33)	7 (11.86)	13 (14.29)	
RFH-NPT	2 (0, 6)	1 (0, 4)	3 (1,6)	0.007
MELD	9.6 (7.2, 12.2)	9.9 (7.8, 11.9)	9.5 (5.8, 12.6)	0.610
Na (mmol/L)	140 (137, 142)	140 (138, 142)	140 (137, 142)	0.621
K (mmol/L)	3.9 (3.5, 4.1)	3.8 (3.5, 4.1)	3.9 (3.5, 4.1)	0.867
Albumin (g/L)	28 (25,32)	28 (26,33)	28 (24,32)	0.311
PT-INR	1.28 (1.18, 1.44)	1.3 (1.19, 1.48)	1.26 (1.17, 1.42)	0.238
Hemoglobin (g/L)	86 (70, 109)	88 (73, 114.00)	85 (66, 109)	0.291
ALT (U/L)	22 (15,36)	23 (15,33)	21 (15,37)	0.703
Total bilirubin (μmol/L)	21.60 (13.20, 37.50)	19.35 (11.85,42.13)	21.00 (14.00, 37.40)	0.479

Data were expressed as mean ± standard deviation, median (interquartile range), proportions or simple frequencies as appropriate.
We classify all cirrhotic patients into good sleepers (PSQI ≤ 5) and poor sleepers (PSQI > 5).
PSQI, Pittsburgh Sleep Quality Index; BMI, body mass index; AILD, autoimmune liver disease; NAFLD, non-alcoholic fatty liver disease; CTP, Child-Turcotte-Pugh; RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; MELD, model for end-stage liver disease; PT-INR, prothrombin-international normalized ratio; ALT, alanine aminotransferase.

TABLE 2 | A multivariate linear regression analysis to assess association between covariates and RFH-NPT.

	Coefficients for the associations with RFH-NPT					
	Simple regression			Multiple regression		
Variable	β	95% CI	*P*	β	95% CI	*P*
Age (years)	0.187	0.025, 0.348	0.024	0.115	−0.034, 0.264	0.130
Gender (Male)	0.280	−0.437, −0.123	0.001	0.279	0.423,−0.134	<0.001
BMI (kg/m^2)	−0.122	−0.289, 0.043	0.146			
Ascites	0.356	0.203, 0.508	<0.001	0.210	0.039, 0.377	0.016
PSQI	0.350	0.196, 0.502	<0.001	0.262	0.114, 0.404	0.001
CTP score	0.268	0.108, 0.431	0.001	0.099	−0.082, 0.280	0.281
Hemoglobin (g/L)	0.052	−0.115, 0.219	0.538			
Hepatic encephalopathy	−0.032	−0.195, 0.130	0.695			
MELD score	−0.051	−0.217, 0.116	0.550			
Albumin (g/L)	−0.165	−0.329, 0.000	0.049	−0.009	−0.174, 0.156	0.916
ALT(U/L)	−0.092	−0.257, 0.075	0.278			
Na (mmol/L)	−0.186	−0.349, −0.022	0.027	−0.096	−0.246,−0.054	0.210

RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; CTP, Child-Turcotte-Pugh; MELD, model for end-stage liver disease; ALT, alanine aminotransferase; CI, confidence interval.

TABLE 3 | Characteristics of cirrhotic patients classified by RFH-NPT scores.

	Low risk ($n = 46$)	Moderate risk ($n = 19$)	High risk ($n = 85$)	*P*
Age (years)	59.62 ± 11.10	58.21 ± 11.56	62.99 ± 9.33	0.157
Gender, *n* (%)				0.062
Male	15 (32.61)	9 (47.37)	46 (54.12)	
Female	31 (67.39)	10 (52.63)	39 (45.88)	
BMI (kg/m^2)	25.13 ± 4.31	23.70 ± 4.67	22.85 ± 4.31	0.023
Etiology, *n* (%)				<0.001
Viral infection	15 (32.61)	9 (47.37)	14 (16.47)	
Alcohol	2 (4.35)	2 (10.53)	31 (36.47)	
AILD/cholestatic	15 (32.61)	7 (36.84)	24 (28.24)	
Cryptogenic/NAFLD	14(30.43)	1 (5.26)	16 (18.82)	
Ascites, *n* (%)	15 (32.61)	13 (68.42)	65 (76.47)	<0.001
Hepatic encephalopathy, *n* (%)	4 (8.70)	2 (10.53)	7 (8.24)	0.950
CTP score	7 (6, 8)	7 (6, 9)	8 (7, 9)	0.006
CTP class, *n* (%)				
A	22 (47.83)	7 (36.84)	19 (22.35)	0.039
B	21 (45.65)	9 (47.37)	52 (61.18)	
C	3 (6.52)	3 (15.79)	14 (16.47)	
PSQI	5 (3, 8)	6 (3, 10)	7 (5, 11)	0.008
MELD	9.9 (8.4, 12.4)	10.1 (8.2, 11.3)	9.2 (5.2, 12.3)	0.536
Na (mmol/L)	141 (139, 142)	140 (137, 141)	139 (137, 142)	0.058
Albumin (g/L)	29 (26, 32)	28 (24, 31)	28 (24, 32)	0.255
ALT (U/L)	21 (13, 30)	22 (16, 41)	23 (15, 37)	0.693
Total bilirubin (μmol/L)	16.85 (12.05, 31.03)	22.70 (11.40, 36.90)	22.60 (14.10, 49.03)	0.103
PT-INR	1.23 (1.18, 1.38)	1.26 (1.17, 1.37)	1.3 (1.18, 1.49)	0.566

Data were expressed as mean ± standard deviation, median (interquartile range), proportions or simple frequencies as appropriate.
The risk of malnutrition was categorized into low (0 points), moderate (1 point) or high (2–7 points) in terms of RFH-NPT scores.
RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; BMI, body mass index; AILD, autoimmune liver disease; NAFLD, non-alcoholic fatty liver disease; CTP, Child-Turcotte-Pugh; PSQI, Pittsburgh Sleep Quality Index; MELD, model for end-stage liver disease; PT-INR, prothrombin-international normalized ratio; ALT, alanine aminotransferase.

Sharma et al. clearly showed that 5 mg/day zolpidem for 4 weeks in CTP-A/B patients with cirrhosis and insomnia results in significant increases in total sleep time, sleep efficiency, and improvement in polysomnographic parameters of sleep initiation and maintenance (25).

How might impaired sleep quality lead to high malnutrition risk? We offer several possible mechanisms for this pathway. First, chronic inflammation has been widely proved to be related to sleep–wake disturbance and elevated inflammatory cytokines might regulate and modulate sleep–waking behavior among patients with cirrhosis (26). For instance, Tsai et al. found serum interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels are remarkably elevated in poor sleepers (PSQI $>$ 5), and IL-6 appears to be an independent predictor of poor sleep quality (27). On the other hand, extensive and persistent inflammatory milieu also predisposes individuals to malnourished conditions *via* increased muscle catabolism and resting energy expenditure (28). In a word, inflammation might serve as an upstream factor influencing both sleep–wake disturbance and malnutrition synergistically. Second, malnourished decompensated patients with cirrhosis are recommended to consume small and frequent snacks and to include a carbohydrate-based late-evening snack in the dietary regimen necessary to spare hepatic glycogen depletion (7, 29–31). However, late food timing has been described to induce decreased energy expenditure, impaired glucose tolerance, and body temperature (32). Mistimed food and sleeps also result in changes in inflammatory markers and plasma proteins in human beings (33). Third, decreased dietary nutrient intake and impaired global protein synthesis have been demonstrated to contribute to sarcopenia in cirrhosis (7). Sarcopenia is a major component of malnutrition. Intriguingly, Nishikawa et al. showed that sleep–wake disturbance is closely associated with sarcopenia especially in cirrhotics (34). Last, it is suggested that cirrhotics have increased daytime levels of melatonin and delayed onset of melatonin peak at night (35, 36). Indeed, disrupted melatonin rhythm might give risk to a biological clock phase-shift, and impaired circadian rhythm might contribute to the pathogenesis of sleep–wake disturbance in cirrhosis (37). Patients with cirrhosis at risk of malnutrition might be deficient in tryptophan, which negatively impacts the biosynthesis of melatonin (38).

Our subgroup analyses indicated that the differences regarding PSQI scores are more significant between low/moderate and high malnutrition risk groups in male patients with cirrhosis, <65 years or with relatively preserved liver function (CTP-A/B or MELD $<$ 15). The reasons for these

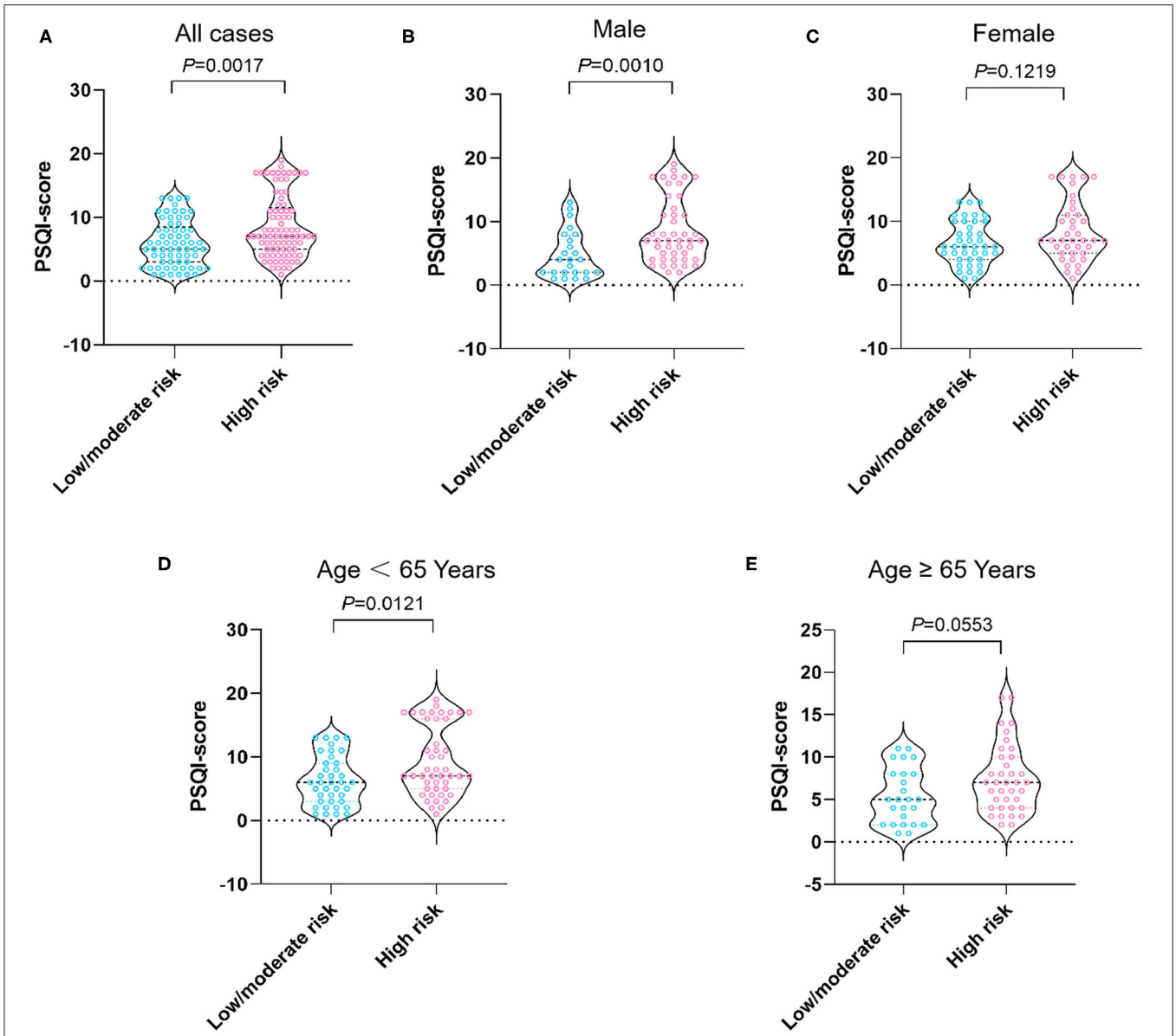

FIGURE 1 | PSQI score stratified by nutritional status in all cases **(A),** male patients **(B)**, female patients **(C)**, patients aged <65 years **(D)**, and aged 65 years and over **(E)**. The risk of malnutrition was categorized into low (0 points), moderate (1 point), and high (2–7 points) in terms of RFH-NPT scores. PSQI, Pittsburgh Sleep Quality Index; RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool.

remain elusive, and further studies will be necessary to confirm our results. In view of these results, we provide useful clues and shed light on the targeted population who will be beneficial from the management of sleep difficulties. For instance, zolpidem, a high-affinity positive modulator of ω1 GABAA receptors, might be effective and safe in CTP-A/B patients in improving PSQI score (25).

We acknowledge the following limitations to this study. First, we could not establish causal relationships between sleep–wake disturbance and malnutrition risk. Second, we implemented a self-reported sleep questionnaire rather than objective methods, such as PSG or actigraphy, allowing more precise measurement. However, it should be emphasized that correlations between subjective and objective sleep–wake disturbances are moderate (19). Third, we excluded subjects with severe HE due to lacking reliability in self-reported scale, which might lead to selection bias. Fourth, the sample size was relatively small in the current study. As a matter of fact, the vast majority of previous studies regarding clinical relevance or implication of sleep disorders in patients with advanced chronic liver diseases is based on a small cohort (ranging from 12 to 193 subjects) (4, 27, 39, 40). However, we believe our single-center findings appear to be the first step to instigate a further multi-center investigation. Last, it has been documented that other sleep–wake abnormalities, such

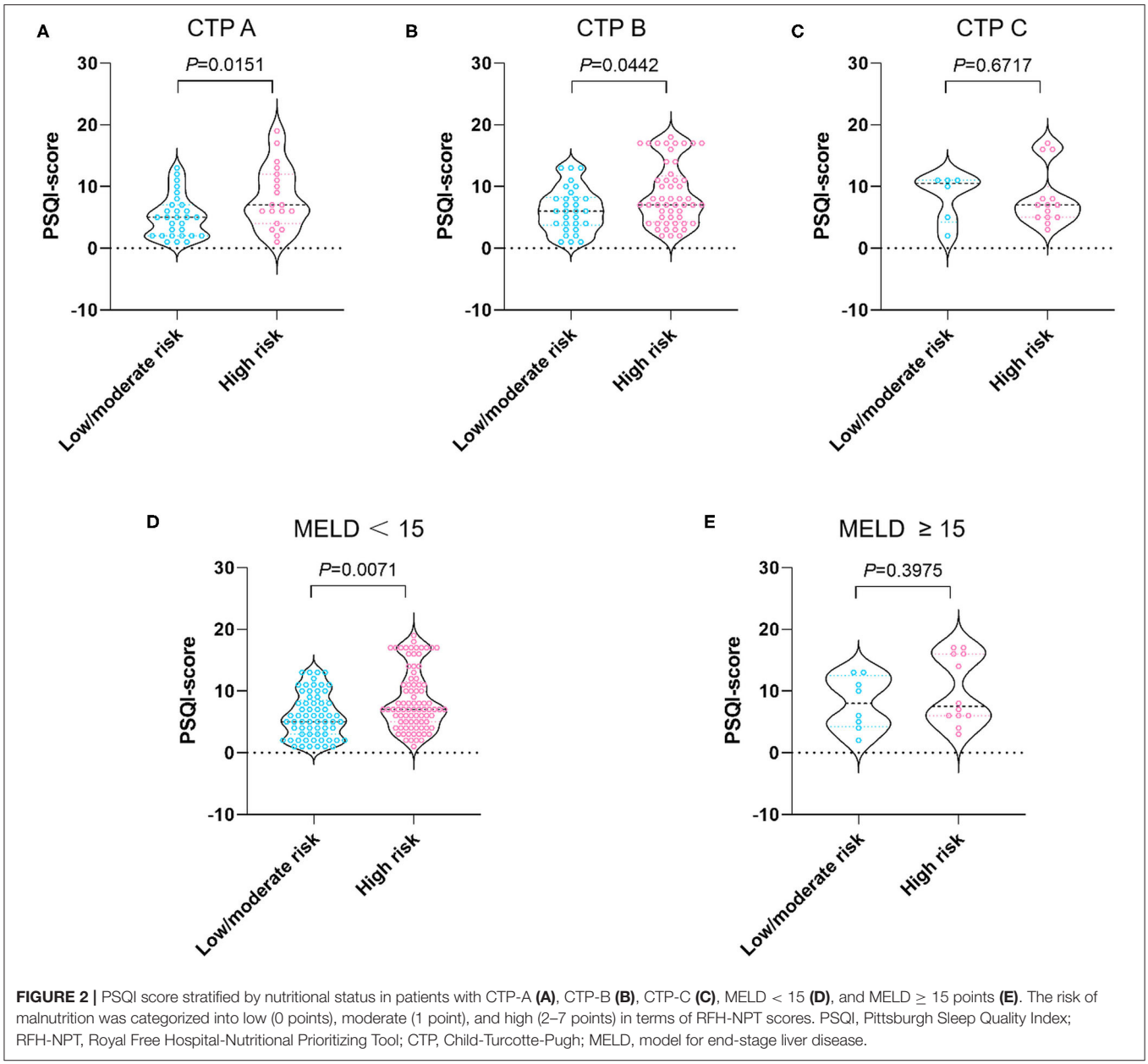

FIGURE 2 | PSQI score stratified by nutritional status in patients with CTP-A **(A)**, CTP-B **(B)**, CTP-C **(C)**, MELD < 15 **(D)**, and MELD ≥ 15 points **(E)**. The risk of malnutrition was categorized into low (0 points), moderate (1 point), and high (2–7 points) in terms of RFH-NPT scores. PSQI, Pittsburgh Sleep Quality Index; RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; CTP, Child-Turcotte-Pugh; MELD, model for end-stage liver disease.

as insomnia, excessive daytime sleepiness, and impaired sleep duration, might be correlated with nutritional status. Actually, our research group is now conducting seminal investigations with respect to the clinical implications of these pathologic entities in cirrhotics.

CONCLUSION

In conclusion, poor sleep quality is strongly correlated with high malnutrition risk in patients with cirrhosis. Considering sleep–wake disturbance as a remediable complication, it is tempting to incorporate therapies to reverse poor sleep quality aiming at improving nutritional status in cirrhosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tianjin Medical University General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YH, XW, ZY, BW, and CS designed the study, analyzed the data, and prepared the original draft. HF, CL, and LM conducted the study and edited the manuscript. XF and LL analyzed the data and reviewed the manuscript. BC, XC, and LS collected the data and conducted statistical analysis. BW and CS designed and monitored the study and made critical revisions to the manuscript. All authors have approved the final draft submitted.

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NAFLD and Physical Exercise: Ready, Steady, Go!

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Along with the increase in obesity and type 2 diabetes, the non-alcoholic fatty liver disease (NAFLD) incidence is escalating, thus becoming a leading cause of liver cirrhosis and a significant burden of liver-related outcomes. Since there is no pharmacotherapy available to address the NAFLD, the most effective solutions seem to be lifestyle changes centered on physical activity. Exercise could mediate its beneficial effects directly on the liver and indirectly via extrahepatic pathways, forming a dose-response relationship with NAFLD in terms of prevalence and disease severity. Health-enhancing physical activity (HEPA) levels are mainly needed to exert beneficial effects in obese subjects, while even a small amount of exercise can be beneficial for lean individuals to prevent NAFLD. This mini-review addresses three major points regarding physical activity and NAFLD: prevention, treatment, and extrahepatic benefits, offering recommendations on type and intensity of exercise in liver disease.

Keywords: non-alcoholic fatty liver disease, sedentary activities, physical activity, aerobic exercise, high-intensity interval training, strength training

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term inclosing a spectrum of clinical and pathological fatty liver disease entities which may lead to cirrhosis and hepatocellular carcinoma (HCC) (1). The prevalence of NALFD is increasing worldwide and is estimated at around 25% (2). However, the true prevalence of NALFD seems to be much higher, given the global rise of metabolic syndrome due to changes in eating habits and inclination toward a sedentary lifestyle.

Metabolic syndrome has become a growing morbidity cluster epidemic resulting in a sharp rise in obesity, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia. Its liver manifestation—NAFLD has become the most common cause of the chronic liver disease (2). Moreover, the prevalence of NAFLD among patients with T2DM is even higher, 56%, while the overall prevalence of non-alcoholic steatohepatitis (NASH), a progressive form of NAFLD, reaches 37% (3). Finally, the incidence of NAFLD-related HCC, accompanied by life-threatening complications, is continuously increasing (4). Furthermore, lean individuals with NAFLD share the same severe histological phenotype as obese subjects and are associated with metabolic syndrome and an increased risk of all-cause mortality (5).

A recent meta-analysis assessing RCTs with dietary interventions but without any added physical activity tried to establish the effect of different dietary modifications on intrahepatic lipid content (IHL), liver fibrosis, and liver function in patients with NAFLD. The study showed Mediterranean

diet without energy restriction leads to significant reduction of IHL. However, it is important to note that the diet without exercise did not lead to significant changes in liver enzymes, lipid profile, fasting glucose or insulin, or homeostatic assessment for insulin resistance. On the other hand, hypocaloric diet with foods high in unsaturated fatty acids significantly decreases ALT and AST, but its effects on steatosis remain to be established (6).

NAFLD development in obese and non-obese individuals is closely related to a sedentary lifestyle and a western diet (7). Physical activity, especially structured exercise, has been shown to improve hepatic steatosis and is the core treatment during the whole NAFLD disease spectrum. Physical activity has an essential role in weight reduction and maintenance, influences healthier body composition, reduces hepatic steatosis and NAFLD-associated cardiovascular and malignant burden (8). Importantly, modest weight gain in lean individuals has deleterious effects on metabolic disturbances primarily through increased visceral adipose tissue (9, 10). Bodyweight and waist circumference reduction achieved through lifestyle intervention are independent predictors of NAFLD resolution in lean patients (11).

PHYSICAL ACITIVTY IN CONTEXT OF LIVER DISEASE

Health-enhancing physical activity defined as either vigorous activity at least 3 days/week and accumulating at least 1,500 metabolic equivalents (METs)-minutes per week (MET-min/week) or seven or more days/week of any combination of walking, moderate, or vigorous activities accumulating at least 3,000 MET-min/week has been recently independently (after adjusted for confounders such as diet and obesity) associated with a lower risk of both NAFLD and lean NAFLD in the Asian population, while the risk of lean NAFLD was significantly lower even in minimally active lean individuals compared inactive lean individuals (adjusted OR, 0.8; 95% CI, 0.6–0.98) (12). Skeletal muscle as an endocrine organ secretes cytokines and myokines, through which, while working/contracting, it communicates with liver and adipose tissue, among others, and is involved in an anti-inflammatory response (13). In addition, physical activity (1,500 MET-min/week of vigorous or 3,000 MET-min/week of intermediate activity) significantly lowers the ALT levels and improves the hepatocellular injury in individuals with NAFLD (8). Although research regarding the effects of exercise on NAFLD is relatively recent, both experimental and clinical data support its importance, especially that of vigorous intensity, which effectively decreases intrahepatic lipid content and slows down the progression to NASH (14). In a small RCT including 24 individuals with biopsy-proven NASH, the benefit was seen from 12-week cycling and resistance training to decrease hepatic triglyceride content, plasma triglyceride levels, and visceral fat. However, no effects were reported concerning BMI, liver enzymes, or inflammation and fibrosis, suggesting weight managing strategies should be incorporated in NASH treatment (15). In patients with cirrhosis, exercise can acutely increase portal pressure, but it has positive health effects in the long term. Moreover, physical activity can improve the aerobic capacity, which is decreased in patients with advanced cirrhosis and adds to anyhow high mortality burden (16). In addition, by increasing skeletal muscle mass physical activity improves sarcopenia and reduces the risk of encephalopathy (17–20). Evidence regarding the effects of exercise on HCC risk is still scarce, but epidemiological studies suggest a lower risk in patients who regularly and vigorously exercise (21).

EXERCISE AND NAFLD: WHAT IS KNOWN ON THE MECHANISM(S)

In many of the published studies, the effect of exercise on improvement of liver fat content was seen even in patients who did not achieve the weight loss therefore suggesting the direct effects on liver (22, 23). Although this direct relation is still largely elusive, the available evidence implies different metabolic and molecular pathways which are involved in the reduction of hepatic fat induced by exercise.

One of the most prominent and studied mechanisms is certainly related to insulin resistance (IR). Mechanistically, IR in peripheral tissues such as adipose tissue results in an incomplete suppression of lipase, leading to enhanced lipolysis and release of free fatty acids (FFAs), which are taken up by the liver (24). Therefore, an improvement in IR might reduce the FFA flux to the liver. Moreover, IR in skeletal muscle causes the glucose transport to the liver, which is the fuel for FFA *de novo* synthesis (25). The main transcription factor controlling liver fatty acid metabolism, sterol regulatory element-binding protein 1 (SREBP-1), which is elevated in the NASH can be decreased by either 12-week aerobic exercise of high intensity or resistance training through the increase of AMPK, leading to reduction of *de novo* lipogenesis in hepatocytes (26, 27). Moreover, exercise might also induce epigenetic mechanisms such as reduction of DNA hypermethylation which positively effects *de novo* lipogenesis (28).

In addition, exercise might also influence liver fatty acid metabolism by increasing expression of peroxisome proliferator-activated receptor-gama (PPAR-gama), in a similar way as the thiazolidinediones (29). Besides, animal models and small scale studies suggest exercise impacts liver mitochondrial function, and can influence inflammation through up-regulation of antioxidant enzymes and anti-inflammatory markers (24, 30).

THE ROLE OF PHYSICAL ACTIVITY IN THE NAFLD PREVENTION

Sitting for $\geq$3 h per day has been associated with increased all-cause mortality (relative risk 1.30; 95% CI 1.06–1.56), and sedentary behavior, in general, was reported higher in people predisposed to develop obesity T2DM, NAFLD, and metabolic syndrome (31). There is a strong association between increased hepatic triglyceride content and each hour spent sedentary during a day, while prospective cohort studies identified sedentary behavior as an independent risk factor for NAFLD development and potentially progression (32, 33). A large prospective randomized Da Quing study including 110,660 men and women with glucose impairment showed exercise

was associated with 46% ($P < 0.0005$) reduction of risk in developing diabetes, irrespective of baseline glucose levels and body mass index (BMI), suggesting a vital role of physical activity in the prevention of metabolic disorders associated with insulin resistance (34). The results of the HELENA study suggest that high cardiorespiratory fitness (CRF) might have protective effects on liver enzyme levels in adolescents with high waist circumference and that the exercise focusing on increasing CRF and decreasing abdominal fat might be a good tool in the prevention and treatment of NAFLD during adolescence (35). A study by Sung and co-workers following 169,347 men and women by ultrasound for 5 years provided the first longitudinal epidemiological data supporting the role of exercise in both the prevention and treatment of NAFLD. During follow-up, out of 126,811 adults without NAFLD at baseline, 23% developed NAFLD at follow-up. On the other hand, of the 42,536 individuals with NAFLD at baseline, 34% of cases resolved. After adjusting for potential confounders, any moderate to vigorous exercise level was associated with a reduced risk of new NAFLD and resolution of already present NAFLD. The most significant benefits were seen while exercising 5 days per week and in the case of increasing the frequency of exercise bouts over time (36). Similar results were confirmed by another more recent longitudinal follow-up study where people who were already active or became physically active during the course of follow-up were less likely to develop NAFLD compared with those that remained inactive (OR = 0.75, $p = 0.03$ and 0.75, $p = 0.04$, respectively), irrespective of BMI (37).

THE ROLE OF PHYSICAL ACTIVITY IN THE NAFLD TREATMENT

Growing evidence highlights the need for physical activity in reducing the body weight (at best >10%) in order to improve liver histology and reduce fibrosis in NAFLD patients (38, 39). Weight loss achieved through physical activity improves hepatic and peripheral insulin sensitivity, but physical activity, regardless of the effects on body mass, also directly decreases the pro-inflammatory and oxidative stress markers and improves liver enzymes. According to the data from a recently published systemic review encompassing 24 exercise-only studies in

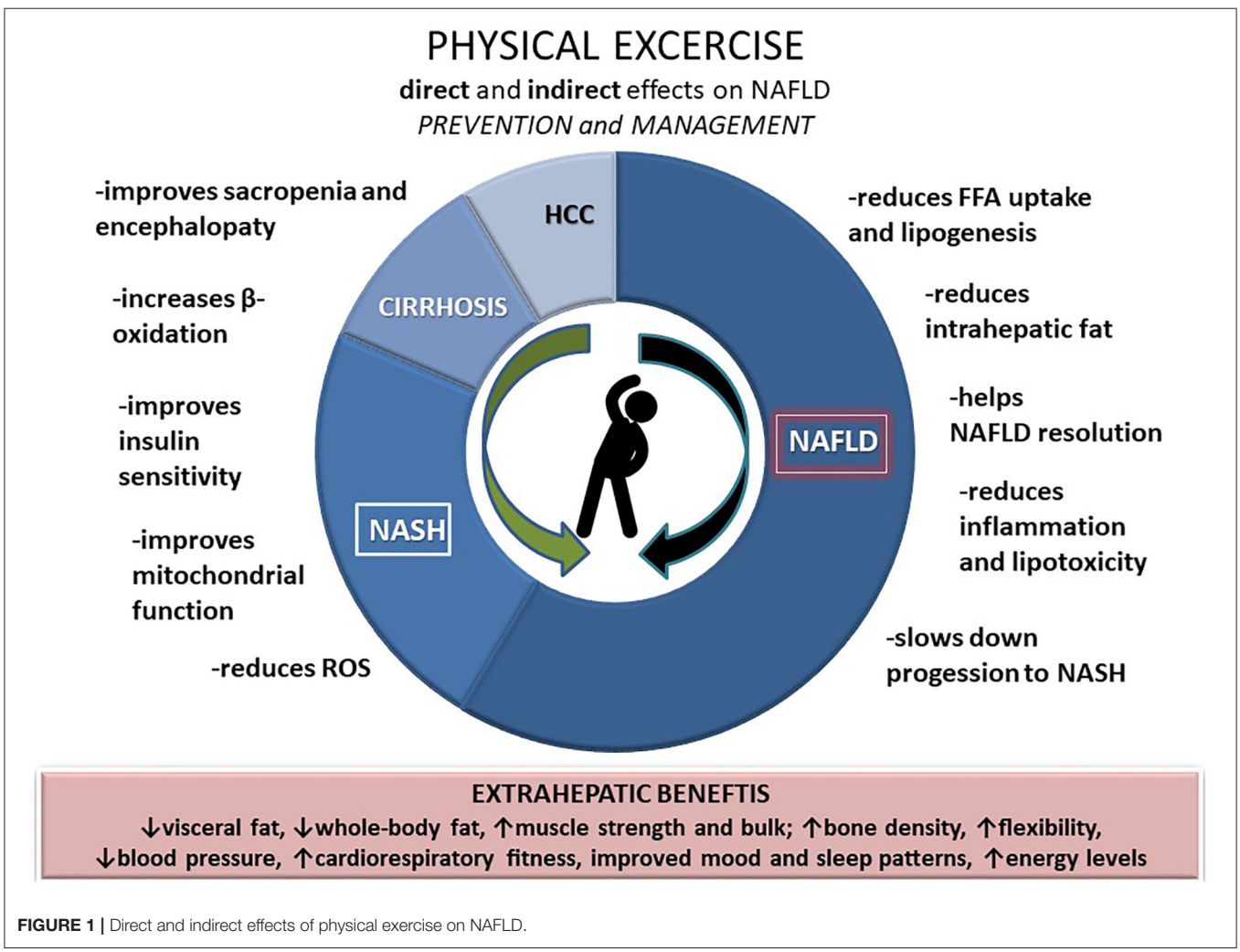

FIGURE 1 | Direct and indirect effects of physical exercise on NAFLD.

NAFLD, structured exercise leads to a 20–30% relative reduction in hepatic steatosis, independent of weight loss (40). In addition, exercise might also affect the gut microbiota and modulate the liver inflammatory response and NASH progression (41) (**Figure 1**).

There is currently a gap in knowledge of the type, duration, and/or intensity of physical activity that would bring the best results for patients with NAFLD. It seems that both aerobic and anaerobic training for at least 4 months decrease to the same extent the overall adipose tissue, hepatic fat, and BMI, while no data exists on their potentially differential effects on liver histology (22). On the other hand, liver histology tends to depend on the exercise intensity and, according to some data, improves more with high-intensity activity (22, 42).

EXTRAHEPATIC BENEFITS OF PHYSICAL ACTIVITY AND HOW IT AFFECTS NAFLD PROGNOSIS

In a randomized control trial recruiting NAFLD patients, exercise was associated with improvement of endothelial function, evaluated by flow-mediated dilatation of the brachial artery (43). Mentioned NO-dependent vascular dilatation is an important protective mechanism for cardiovascular health. Moreover, with its effect on muscle mass, physical activity reduces the risk of sarcopenia and improves cardiorespiratory fitness, which is low, especially in patients with advanced liver disease such as cirrhosis (19). Physical activity improves insulin sensitivity on the peripheral tissues and the liver and improves glucose metabolism (or glycemic control in clinically manifest diabetes), slowing down NAFLD progression and reducing overall cardiovascular risk. Moreover, it reduces systemic inflammation, lowers arterial blood pressure, and improves dyslipidemia (44). The established beneficial effect of physical activity on cardiovascular health reported in the general population is also applicable for the NAFLD patients, and given that cardiovascular disease remains the leading cause of death in NAFLD patients, encouraging regular exercise should be advocated and prescribed to all NAFLD patients, and during the entire disease course (45).

RECOMMENDATIONS FOR PHYSICAL ACTIVITY IN THE NAFLD PATIENTS

Physical activity and specially structured exercises offer benefits independent of weight loss and represent the core treatments for NAFLD patients. Both aerobic and resistance training effectively reduce hepatic steatosis and reduce the NAFLD-associated cardiovascular risk (46). The exercise program should be tailored to a patient's preference and capacity, depending on physical fitness level, stage of the liver disease, and other comorbidities. High-intensity interval training (HIIT) is an attractive exercise modality for treating patients with NAFLD, especially those who lack time to exercise, while it reduces visceral adipose tissue, intrahepatic fat, and fibrosis (47). General recommendations include 150 min of weekly accumulated moderate-intensity aerobic exercise, accompanied by strength and endurance training at least two to three times weekly, avoiding consecutive days and including 8–10 exercises using the major muscle groups, with 10–15 repetitions in a moderate to high intensity. In addition, just reducing or breaking up sedentary time by few minutes of walking should also be a therapeutic target for patients who cannot attend the structured exercise programs. To assure the therapeutic effects, attention should be paid to patients' compliance to exercise and attain exercise goals (48, 49).

As majority of NAFLD patients are obese, special attention must be put on an exercise program which would be doable and also lead to a meaningful weight loss (10%) and improvements in cardiorespiratory fitness to provide health benefits (39). Current literature supports the evidence that both aerobic and anaerobic exercise with a duration of 20–60 min per session when performed in moderate intensity and practiced 4–7 days weekly for at least 6 months (with and without diet restriction) can lead to improvements in liver histology and therefore reversal of liver damage in NASH patients (50), while recently published study on overweight and obese patients supports the beneficial role of aerobic exercise regardless of dose and intensity (low-intensity/ high-volume, high-intensity/low-volume, low-intensity/low-volume) on the reduction of liver fat content (42). Therefore, recent guidelines emphasize the importance of exercise but leave the choice of training to be individually tailored according to patients' preferences and likelihood of adherence to exercise program in the long term (51).

AUTHOR CONTRIBUTIONS

MC: drafted and wrote and reviewed the manuscript. IB-C and AM: collected data and wrote and reviewed the manuscript. VC: critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Impact of Sarcopenia on Survival and Clinical Outcomes in Patients With Liver Cirrhosis

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Background: Sarcopenia is now recognized more and more as a biomarker with poor outcomes in cirrhotic patients.

Aims: The purpose of this study was to investigate the prevalence of sarcopenia in patients with liver cirrhosis and prospectively investigate the association between sarcopenia and different complications and its impact on survival.

Material and Methods: This prospective study included patients with liver cirrhosis admitted to our department from 2018 to 2020. Sarcopenia was assessed according to EWGSOP2 criteria, incorporating low Handgrip strength (<27 kg for men and <16 kg for women) with low skeletal muscle index evaluated by CT (<50 for men and <39 for women). Associations between sarcopenia and portal hypertension-related complications, infectious complications, and risk of hepatocellular carcinoma, the number of in-hospital days, 30-day readmission, and survival over the next 6 and 12 months were analyzed.

Results: A total of 201 patients were enrolled in the study, 63.2% male, mean age 61.65 ± 9.49 years, 79.6% Child-Pugh class B and C. The primary etiology of liver cirrhosis was alcohol consumption (55.2%). The prevalence of sarcopenia was 57.2 %, with no significant differences between the male and female groups. Significant associations were found between sarcopenia and portal hypertension-related complications, infectious complications, and risk of hepatocellular carcinoma. In multivariate analysis, sarcopenia was assessed as a risk factor alone, increasing the risk for ascites 3.78 times, hepatocellular carcinoma by 9.23 times, urinary tract infection by 4.83 times, and spontaneous peritonitis 2.49 times. Sarcopenia was associated with more extended hospital stay and higher 30 days readmission. Six months and 1-year survival were reduced in the sarcopenia group than in the non-sarcopenia group ($p < 0.0001$).

Conclusion: Sarcopenia is a common complication of liver cirrhosis and associates with adverse health-related outcomes and poor survival rates.

Keywords: sarcopenia, liver cirrhosis, clinical outcomes, survival, handgrip strength, skeletal muscle index

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INTRODUCTION

During the last years, various scientific groups attempted to develop different definitions for sarcopenia. The European Working Group on Sarcopenia in Older People (EWGSOP2) (1) updated in 2019 their previous definition of sarcopenia, which is now defined as a muscle disease (low muscle quantity and quality) associated with low muscle strength. Liver cirrhosis is one of the most representative chronic diseases, which can be complicated by sarcopenia. Clinical practice guidelines of the European Association for the study of the Liver (EASL) (2) and the European Society for Clinical Nutrition and Metabolism (ESPEN) (3) recommend screening for sarcopenia as its early recognition is a critical aspect of the care of these patients.

The prevalence of sarcopenia in Liver Cirrhosis is around 23–60% (4), but this percentage depends on the severity of the underlying liver disease and the diagnostic tools and criteria utilized.

Previous studies have evaluated the association between sarcopenia and higher rates of other cirrhosis complications, infections, hospital admissions, and reduced survival (5–8), but few of those studies applied the new EWGSOP2 criteria to define sarcopenia. Data are lacking, whether the 2010 or 2019 diagnosis criteria better predict complications and poor prognosis.

Therefore, the present study aimed to evaluate the prevalence of sarcopenia in patients with liver cirrhosis using the 2019 sarcopenia consensus definition of EWGSOP2 and prospectively investigate the association between sarcopenia and a higher rate of complication and poor survival.

MATERIALS AND METHODS

Study Design and Population Selection

This is a prospective, observational study, carried out in a tertiary Department of Gastroenterology and Hepatology, from January 2018 to December 2020 on 201 patients with liver cirrhosis.

Liver cirrhosis diagnosis was based on physical examination, abdominal ultrasound, laboratory tests, ultrasound-based elastography, upper endoscopy, and radiological evidence. Child Pugh's score and the Model for End-Stage Liver Disease (MELD) score were used for liver function assessment.

The present study includes 201 patients. Based on the following inclusion criteria: patients with liver cirrhosis older than 18 years and availability of a diagnostic reference standard method (Contrast-enhanced Computer Tomograph). The exclusion criteria were: patients with any factors that could independently influence sarcopenia such as Human Immunodeficiency Virus, tuberculosis, obstructive pulmonary disease, chronic renal failure, congestive heart failure, neuromuscular disorders, inflammatory bowel disease, other malignancies than hepatocellular carcinoma.

Data collected from medical charts included: age, gender, etiology, albumin, INR, Sodium, Thrombocytes, Child-Pugh score, MELD score, presence of ascites, presence of esophageal varices, upper gastrointestinal bleeding (upper GI bleed), urinary tract infection (UTI), Pneumonia, spontaneous bacterial peritonitis (PBS), hepatic encephalopathy (HE), hepatorenal syndrome (SHR), Hepatocellular carcinoma (HCC), 30-day readmission, length of hospitalization, 6 months and 1-year mortality.

The study protocol was approved by the local Ethical Committee and was performed in accordance with the Helsinki Declaration of 1975, after informed consent to participate in the study was obtained from every patient.

Anthropometric Measurements

Handgrip Strength (HGS)

Dominant handgrip strength was measured using a Jamar dynamometer. The patient was examined while sitting down with the elbow flexed at 90° and the arm along the body or in dorsal position with the elbow supported and the head at 30°. Each patient used the dominant hand and performed the test three times with a pause of 10–30 s between the tests. The highest record value was used. All values were recorded in kilograms.

Skeletal Muscle Index (SMI)

Computer Tomography (CT) images for cross-sectional skeletal muscle mass assessment were analyzed at the level of lumbar 3 by a single observer, using National Institutes of Health ImageJ software. For muscle tissue, standard attenuation values ranged from 29 to 150 Hounsfield units. The cross-sectional areas achieved were normalized for patient height, obtaining the skeletal muscle index, which is expressed as *a cross-sectional muscle area/height*^{2}. The measurements were done by an experienced radiologist.

Diagnosis of Sarcopenia

Sarcopenia was defined based on the EWGSOP2 criteria using the combination of low SMI and low HGS with stratification of gender and age-specific cut-off values. **Table 1** outlines the cut-offs used for SMI and HGS (1).

Statistical Analysis

The statistical analysis was performed using MedCald software for windows (MedCalc Software, version 19.3.1, Ostend, Belgium). Categorical data were described as number and percentage, and continuous data were described as mean and standard deviation. Skewed data were described as median and interquartile line. For correlation analysis of categorical data Spearman's rho and Kendall's tau-b were used. A 5% significance level was considered. Predictors for sarcopenia were assessed using regression analysis. A risk analysis was made.

TABLE 1 | Cut-offs values used to define sarcopenia.

	HGS	SMI
Male	<27 kg	<50 cm^2/m^2
Female	<16 kg	<39 cm^2/m^2

TABLE 2 | Baseline characteristics of the study population.

Parameter	Values
Age [years] (mean ± SD)	61.65 ± 9.49
• <40 years	• 1 (0.5%)
• 40–60 years	• 114 (56.7%)
• >60 years	• 86 (42.7%)
Gender–Males *n* (%)	96 (61.5%)
Child-Pugh classification	
• A	• 41 (20.4%)
• B	• 82 (40.8%)
• C	• 78 (38.8%)
Mean Child Pugh score (points)	8.79 ± 2.24
Mean MELD score (points)	16.56 ± 7.59
Ascites *n* (%)	
• Absent	• 44 (21.8%)
• Present	• 157 (78.8%)
Etiology of cirrhosis *n* (%)	
• Hepatitis B	• 18 (9%)
• Hepatitis C	• 50 (24.9%)
• Alcohol abuse	• 111 (55.2%)
• Other	• 22 (10.9%)

RESULTS

Patients Characteristics

Two hundred and one patients fulfilled the inclusion criteria and were included in the analysis, mean age 61.65 ± 9.49 years. The male gender was predominant 63.2%. Regarding etiology, more than half (55.2%) had alcoholic cirrhosis, 24.8% hepatitis C virus (HCV) cirrhosis, 8.9% hepatitis B virus (HBV) cirrhosis, 10.9% other etiologies. According to the Child-Pugh Classification: 20.4% were A class, 40.8% were B, and 38.81% were C. **Table 2** shows the baseline characteristics of the study population.

Prevalence of Sarcopenia

According to the EWGSOP2 criteria, the prevalence of sarcopenia in our overall cohort was 57.2% ($p < 0.0001$). 108/160 patients (67.5%) in the decompensated group had sarcopenia, while only 7/41 patients (17.07%) were sarcopenic in the compensated group.

There were no differences between gender concerning the prevalence of sarcopenia, 76 male patients with sarcopenia vs. 39 female patients with sarcopenia, $p = 0.37$.

Sarcopenia and Clinical Outcomes and Survival Rates

When comparing the two study groups, we found significant differences between the sarcopenic group vs. the non-sarcopenic group regarding albumin level, MELD score, Child-Pugh score, sodium level, INR level, and hospitalization days ($p < 0.05$). We also found differences in proportions between the two groups regarding hepatic encephalopathy rate, ascites rate, HCC rate, urinary tract infection rate, hepato-renal syndrome rate, esophageal varices, and 6 months and 1-year mortality ($p < 0.05$) (**Table 3**).

TABLE 3 | Comparation between sarcopenic and non-sarcopenic patient's characteristics.

Parameter	Overall	Sarcopenia	Normal weight	*p*-value
Age	61.65 ± 9.49	61.33 ± 9.34	62.09 ± 9.78	0.57
Gender (male)	127 (63.2%)	76 (59.8%)	51 (40.2%)	0.37
MELD score	16.56 ± 7.59	18.69 ± 7.74	13.72 ± 6.39	<0.0001
Child Pugh score	8.79 ± 2.24	9.71 ± 1.90	4.56 ± 2.07	<0.0001
SMI	44.44 ± 6.76	42.38 ± 6.21	47.33 ± 6.53	<0.0001
HGS	22.10 ± 8.55	17.84 ± 5.48	28.04 ± 8.47	<0.0001
Albumin	2.53 ± 0.72	2.27 ± 0.61	2.89 ± 0.71	<0.0001
Sodium	136.3 ± 5.38	134.95 ± 6.00	138.17 ± 3.74	<0.0001
INR	1.47 ± 0.38	1.54 ± 0.41	1.38 ± 0.31	0.002
Thrombocytes	124 (22-552)	129.6 (22-156)	148 (47-552)	0.35
Hepatic Encephalopathy	61 (30.3%)	50 (81.9%)	11 (18.9%)	<0.001
Ascites	157 (78.1%)	109 (69.4%)	48 (30.6%)	<0.0001
Esophageal varices	157 (78.1%)	97 (61.7%)	60 (38.3%)	0.01
Hepato-renal syndrome	7 (3.4%)	7 (100%)	0	0.04
Upper GI bleeding	76 (37.8%)	49 (64.4%)	27 (35.6%)	0.10
Hepatocellular carcinoma	65 (32.3%)	54 (83.0%)	11 (17.0%)	<0.0001
Urinary tract infection	61 (30.1%)	46 (75.4%)	15 (24.6%)	0.0005
Spontaneous peritonitis	40 (19.9%)	35 (87.5%)	5 (12.5%)	<0.0001
Pulmonary infections	34 (16.9%)	24 (70.5%)	10 (29.5%)	0.07
Hospitalization days	10.19 ± 6.03	13.36 ± 5.39	5.98 ± 3.90	<0.0001
30 days readmission	75 (37.5%)	64 (85.3%)	11 (14.7%)	<0.0001
6 months mortality	62 (30.8%)	56 (90.3%)	6 (9.7%)	<0.0001
1 year mortality	122 (60.6%)	96 (78.6%)	26 (21.4%)	<0.0001

While not statistically significant, a larger percentage of sarcopenic patients presented upper gastrointestinal bleeding and pulmonary infections vs. non-sarcopenic patients (64.4 vs. 35.6%, 70.5 vs. 29.5%, respectively).

As shown in **Table 4**, a correlation analysis of different factors was made, and various associations with sarcopenia were found. For example, regarding albumin and sodium levels, if albumin or sodium level decreases, sarcopenia chances increase, $p < 0.0001$. For MELD score, Child-Pugh Score, INR level, and length of hospitalization days, if the values are increasing, the chance of patients being sarcopenic is increasing as well.

Other factors associated with sarcopenia were hepatic encephalopathy, ascites, 30 days readmission rate, hepatocellular carcinoma, spontaneous peritonitis, urinary tract infection, hepato-renal syndrome, presence of esophageal varices, and 6 months and 1 year mortality ($p < 0.05$).

In multivariate analysis, sarcopenia was assessed as a risk factor alone, increasing the risk for ascites 3.78 times, hepatocellular carcinoma by 9.23 times, urinary tract infection

TABLE 4 | Regression and correlation analysis of factors involved in sarcopenia.

Parameter	Correlation coefficient	*p*-value
Albumin	−0.42	<0.0001
Encephalopathy	0.33	<0.0001
Hospitalization days	0.59	<0.0001
Ascites	0.46	<0.0001
30 days readmission	0.44	<0.0001
MELD score	0.32	<0.0001
Hepatocellular carcinoma	0.36	<0.0001
Upper gastrointestinal bleeding	0.11	0.10
INR	0.21	<0.0001
Urinary tract infection	0.24	0.0004
Sodium	−0.29	<0.0001
Spontaneous peritonitis	0.30	<0.0001
Hepato-renal syndrome	0.16	0.01
Age	−0.03	0.57
Pulmonary infections	0.12	0.07
Esophageal varices	0.17	<0.01
Child Pugh Score	0.47	<0.0001
6 months mortality	0.45	<0.0001
1 year mortality	0.53	<0.0001

TABLE 5 | Multivariate logistic regression analysis of factors associated with sarcopenia.

Parameter	Odds ratio	95% CI	*p*-value
1 year mortality	0.73	0.21 - 2.47	0.60
6 months mortality	1.37	0.40–4.73	0.60
30 days readmission	1.61	0.42–6.05	0.47
Ascites	3.78	0.85–16.86	0.04
Encephalopathy	0.68	0.20–2.33	0.05
Hepatocarcinoma	9.23	2.42–35.16	0.0001
Hospitalization days	1.35	1.15–1.58	0.60
INR	0.70	0.18–2.66	0.31
Urinary tract infections	4.83	1.77–13.22	0.002
MELD score	0.59	0.88–1.03	0.15
Sodium	0.92	0.83–1.03	0.18
Spontaneous peritonitis	2.49	0.63–9.77	0.03
Child Pugh score	1.01	0.69–1.76	0.93
Hepatorenal syndrome	5.30	0.75–6.80	0.42
Esophageal varices	2.30	0.80–6.57	0.11

by 4.83 times, and spontaneous peritonitis 2.49 times, as shown in **Table 5**.

DISCUSSION

Sarcopenia Assessment

Sarcopenia is a frequent complication of cirrhosis. There is a lack of consensus concerning which criteria to use to define sarcopenia in patients with liver cirrhosis. In 2019, EWGSOP2 (1) updated the definition of sarcopenia and provided consensus criteria in which low muscle mass and low muscle strength were required for the diagnosis. In an article published in 2020 by Traub et al. (9), a comparison between the 2010 and 2019 EWGSOP criteria was made, and the results showed that sarcopenia is less often diagnosed when using the 2019 criteria, but according to a study conducted by Anand et al. (10), the new definition of sarcopenia best predicts mortality and clinical outcomes. Son et al. concluded in a recent review (11) that further studies are required to determine which definition of sarcopenia is the most useful for predicting poor outcomes among patients with cirrhosis.

As only a few studies have used the combination of muscle mass and function to assess sarcopenia in patients with cirrhosis, we decided to apply this definition in our cohort of patients.

There are different diagnostic tools and tests available for the assessment of sarcopenia. In our study, to determine muscle mass, we used skeletal muscle index evaluated by Contrast-Enhanced CT, which is considered a gold standard for evaluating sarcopenia (2, 12). CT is frequently used in daily practice as a screening method for HCC, so it can also be used to assess sarcopenia. Although there are some limitations of this method regarding radiation exposure, costs, and the complexity of the measurement technique of SMI that requires radiological expertise and time, as well as a specialized software.

To assess muscle strength, we used HGS, a simple and inexpensive valuable tool in daily practice that can predict poor patients' outcomes and mortality (13).

Prevalence of Sarcopenia

In our cohort, by applying the EWGSOP2 (1) criteria and cut-offs, sarcopenia was diagnosed in 57.2% of the patients, like the results found in similar articles and reviews from the literature (4, 11, 14). Given that the majority of our patients were Child-Pugh B and C, and the most common etiology was alcohol abuse (55.2%), the high prevalence of malnutrition in our cohort can be explained.

Although studies from the literature (14, 15) say that sarcopenia is more prevalent in male patients with cirrhosis, in our study, there is no statistical difference regarding the prevalence of sarcopenia between males and females.

Sarcopenia and Survival

The prognosis of sarcopenic cirrhotic patients is significantly worse than that of non-sarcopenic patients, with a higher mortality rate (7, 15). In our study, there was a statistically significant difference between the sarcopenic and non-sarcopenic cirrhotic patients in terms of 6 months and 1-year mortality ($p < 0.0001$). The mortality rates at 6 months and 1 year of follow-up were significantly higher in sarcopenic cirrhotic patients (90.3 and 78.6%) than among non-sarcopenic cirrhotic patients (9.7 and 21.4%).

According to our analysis, sarcopenia is associated with more extended hospital stay and higher 30 days readmission, which is similar to the results found by Montano-Loza et al. (16). In the sarcopenic group, the average length of stay in the hospital was 13.36 ± 5.39 days, while the non-sarcopenia group had an average length of 5.98 ± 3.90 days, $p < 0.0001$.

Impact of Sarcopenia on Cirrhosis Complications

Complications regarding portal hypertension such as ascites, presence of esophageal varices, hepatic encephalopathy, hepatorenal syndrome are reported to have a strong correlation with the presence of sarcopenia (17, 18). Our results also showed similar findings, as a strong association between all the above complications and sarcopenia was found ($p < 0.0001$). According to our study, sarcopenia increases the risk for ascites by 3.78 times.

There were no statistical differences between the two groups concerning the risk of variceal bleeding.

In our study, we found a significant correlation between sarcopenia and HCC. Using multivariate logistic regression analysis, we found that sarcopenic patients have a 9.23 higher risk of developing hepatocellular carcinoma than non-sarcopenic patients. In a recently published article in Clinical Nutrition, Feng et al. (19) also found out that cirrhotic patients recorded to have sarcopenia at baseline assessment had a significantly increased risk of developing HCC during a median follow-up of 3.6 years. However, this finding was limited to male patients.

Published data showed that sarcopenia had been associated with an increased risk of infections (15, 20). A statistically significant association between sarcopenia and urinary tract infection and spontaneous bacterial peritonitis ($p < 0.0001$) was found in our research, as sarcopenia increases the risk for urinary tract infection by 4.83 times and spontaneous peritonitis by 2.49 times.

Sarcopenia and Other Implications

In the current study, a statistically significant difference was found between cirrhotic patients with and without sarcopenia in terms of MELD scoring; a higher MELD score was found in the sarcopenic group (mean MELD 18.69 $\pm$ 7.74 and 13.72 $\pm$ 6.39, respectively, $p < 0.0001$). We also found an association between sarcopenia and hypoalbuminemia, hyponatremia, thrombocytopenia, and higher INR levels. Similar differences were found in the study of Montano-Loza et al. (18).

Study Limitations

The limitations of the present study were: firstly, the single-center study design, so a more extensive multicenter study will be needed to confirm our findings. Secondly, the cut-off values used for SMI and HGS to define sarcopenia are from a different population sample because predefined values for sarcopenia in patients with cirrhosis are lacking. Thirdly, the lack of cohort homogeneity, as most of the patients were Child-Pugh B and C.

Despite the limitations, our study adds a notable contribution to the epidemiology of sarcopenia in cirrhotic patients and provides useful information regarding the prognostic value of sarcopenia in patients with liver cirrhosis using the EWGSOP2 criteria.

CONCLUSION

Sarcopenia is a highly prevalent complication of liver cirrhosis, and it is associated with a worsened clinical outcome, including increased hospitalization rates and reduced survival. A systematic evaluation of this common complication should be prioritized to increase the survival rate in these patients and decrease the hospitalization burden.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Medicine and Pharmacy Victor Babes Ethical Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RŞ, MD, and M-MT did the concept and design of the study and revised and completed the manuscript. M-MT performed the measurements, analyzed the data, drafted the manuscript, and revised and completed the manuscript. IS and AP revised and completed the manuscript. A-MG collected patient data and nutrition studies and revised and completed the manuscript. RL made statistical analyzed and revised and completed the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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Visceral Adiposity Associates With Malnutrition Risk Determined by Royal Free Hospital-Nutritional Prioritizing Tool in Cirrhosis

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Mounting evidence has suggested the clinical significance of body composition abnormalities in the context of cirrhosis. Herein, we aimed to investigate the association between visceral adiposity and malnutrition risk in 176 hospitalized patients with cirrhosis. The adiposity parameters were obtained by computed tomography (CT) as follows: total adipose tissue index (TATI), visceral adipose tissue index (VATI), subcutaneous adipose tissue index (SATI), and visceral to subcutaneous adipose tissue area ratio (VSR). Malnutrition risk was screened using Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT). Visceral adiposity was determined given a higher VSR based on our previously established cutoffs. Multivariate analysis implicated that male gender (OR = 2.884, 95% CI: 1.360–6.115, $p = 0.006$), BMI (OR = 0.879, 95% CI: 0.812–0.951, P = 0.001), albumin (OR = 0.934, 95% CI: 0.882–0.989, $P = 0.019$), and visceral adiposity (OR = 3.413, 95% CI: 1.344–8.670, $P = 0.010$) were independent risk factors of malnutrition risk. No significant difference was observed regarding TATI, SATI, and VATI among patients with low or moderate and high risk of malnutrition. In contrast, the proportion of male patients embracing visceral adiposity was higher in high malnutrition risk group compared with that in low or moderate group (47.27 vs. 17.86%, $p = 0.009$). Moreover, this disparity was of borderline statistical significance in women (19.05 vs. 5.88%, $p = 0.061$). Assessing adipose tissue distribution might potentiate the estimation of malnutrition risk in cirrhotics. It is pivotal to recognize visceral adiposity and develop targeted therapeutic strategies.

Keywords: visceral adiposity, cirrhosis, malnutrition, RFH-NPT, visceral to subcutaneous adipose tissue area ratio

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INTRODUCTION

Malnutrition is prevalent in patients with cirrhosis, which contributes to the increased risk of morbidity and mortality (1). It is of utmost importance to identify malnourished subjects and institute nutritional therapy with the purpose of reducing mortality, systemic inflammatory response, and infection (2, 3). The Royal Free Hospital-Nutritional Prioritizing Tool (RFH-NPT) is a cirrhosis-specific nutrition screening tool. In an established and validated cohort of 148 patients with chronic liver disease, the RFH-NPT represented a useful predictor of clinical deterioration and poor outcome (4). Our previous work also implicated that malnutrition risk estimated by RFH-NPT is dramatically associated with distorting immune function in the context of cirrhosis (5).

Evaluation of body composition, including muscle and adipose tissue, gives rise to an objective assessment of the patients' metabolic and nutritional status. Muscles are responsible for mechanical activity, whereas adipose tissue is involved in energy regulation and metabolic action (6). We and others have substantially clarified the prognostic utility of several abnormalities in body composition features for outcomes in patients with cirrhosis (7–9). More recently, Borges et al. found that sarcopenia (low muscle mass) serves as a predictor of malnourished condition and comorbidities in hospitalized patients with cancer (10). Furthermore, it has been documented that malnutrition determined by Patient-Generated Subjective Global Assessment (PG-SGA) is an indicator of sarcopenia in cirrhotics (11). However, the association between abnormal adiposity and malnutrition risk remains elusive in hospitalized patients with cirrhosis. The excessive depot of visceral adipose tissue might promote inflammation and metabolic dysregulation (12, 13). Likewise, failure to expand subcutaneous adipose tissue contributes to visceral fat deposition as well as insulin resistance (14, 15). Intriguingly, some investigations indicated that the distribution of adipose tissue rather than the absolute volume appears to be a major determinant for prognostication in various liver diseases (7, 16). Therefore, we aimed to investigate the association between visceral adiposity and malnutrition risk in hospitalized patients with cirrhosis.

METHODS

Patients

Among 243 adult patients aged not less than 18 years who were consecutively enrolled in Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital (TJMUGH) between 2019 and 2020, 12 with acute-on-chronic liver failure upon admission, 19 with concurrent cancers and 36 without CT scan during hospitalization were excluded from this work (**Figure 1**). Therefore, this retrospective cohort study explored and analyzed data from 176 patients {men, $n = 83$; women, $n = 93$; median age, 63 years [interquartile range (IQR), 56–68]}. Details of the diagnosis of liver cirrhosis, retrieval of laboratory results, and cirrhosis-associated complications have been comprehensively described elsewhere (17, 18). This work was conducted adherent to the Declaration of Helsinki, was approved by Ethics Committee of TJMUGH (2018-235), and was presented in accordance with the STROBE statement. Written informed consent was obtained from all participants.

Assessment of Computed Tomography Images

All CT images of the study cohort were achieved using a spectral CT scanner (Discovery 750 HD 64 row, General Electric Company, Boston, USA). Details in relation to image analyses of distinct adipose tissues have been explicitly depicted in our previous publication (7). In brief, body composition was quantified using an opensource software based on the MATLAB version R2010a (Mathworks Inc., Natick, Massachusetts, USA). The tissue-specific attenuation values from−190 to−30 Hounsfield unit (HU) were for subcutaneous or visceral adipose tissue. Acquired values were standardized for height in squared meters (cm^2/m^2). Accordingly, several parameters were obtained as follows: total adipose tissue index (TATI), visceral adipose tissue index (VATI), and subcutaneous adipose tissue index (SATI). Visceral adiposity was evaluated by visceral to subcutaneous adipose tissue area ratio (VSR) (9, 16).

Cutoff Values for VSR and Other Adiposity Parameters

The gender-specific cutoff values for body composition indices were generated separately in terms of our previous work by X-tile as follows: VSR (men, 1.47; women, 1.29), SATI (men, 29.10 cm^2/m^2; women, 26.75 cm^2/m^2), and VATI (men, 28.42 cm^2/m^2; women, 44.02 cm^2/m^2) (7). The X-tile project (Yale University School of Medicine, New Haven, Connecticut, USA) can attain a single, global estimation of each probable modality of dividing a cohort into low-level and high-level marker expressions.

Royal Free Hospital-Nutritional Prioritizing Tool

We demonstrated the RFH-NPT score in **Supplementary Figure S1**. Generally speaking, it takes approximate 3 min to complete this scale, which includes the components of alcoholic hepatitis, fluid overload and influence on dietary intake, body mass index (BMI), and unplanned weight loss. Taken together, the RFH-NPT discriminates patients with cirrhosis into low- (0 points), medium- (1 point), and high-risk (2–7 points) categories.

Statistical Analysis

Data were presented as mean ± standard deviation (SD), median (IQR), simple frequencies, or percentages (%) as appropriate. Continuous data were compared using an independent Student's *t*-test or the Mann–Whitney *U* test appropriately. Categorical variables were compared by χ^2 test or Fisher's exact test. Multivariate analysis performed by logistic regression analysis was used to figure out the independent risk factor of high risk of malnutrition. Odds ratio (OR) and 95% confidence interval (CI) were calculated. All *p*-values were two-sided, and we regarded $p < 0.05$ as statistical significance. The statistical analyses were carried out using MedCalc 15.2.2 (MedCalc, Mariakerke,

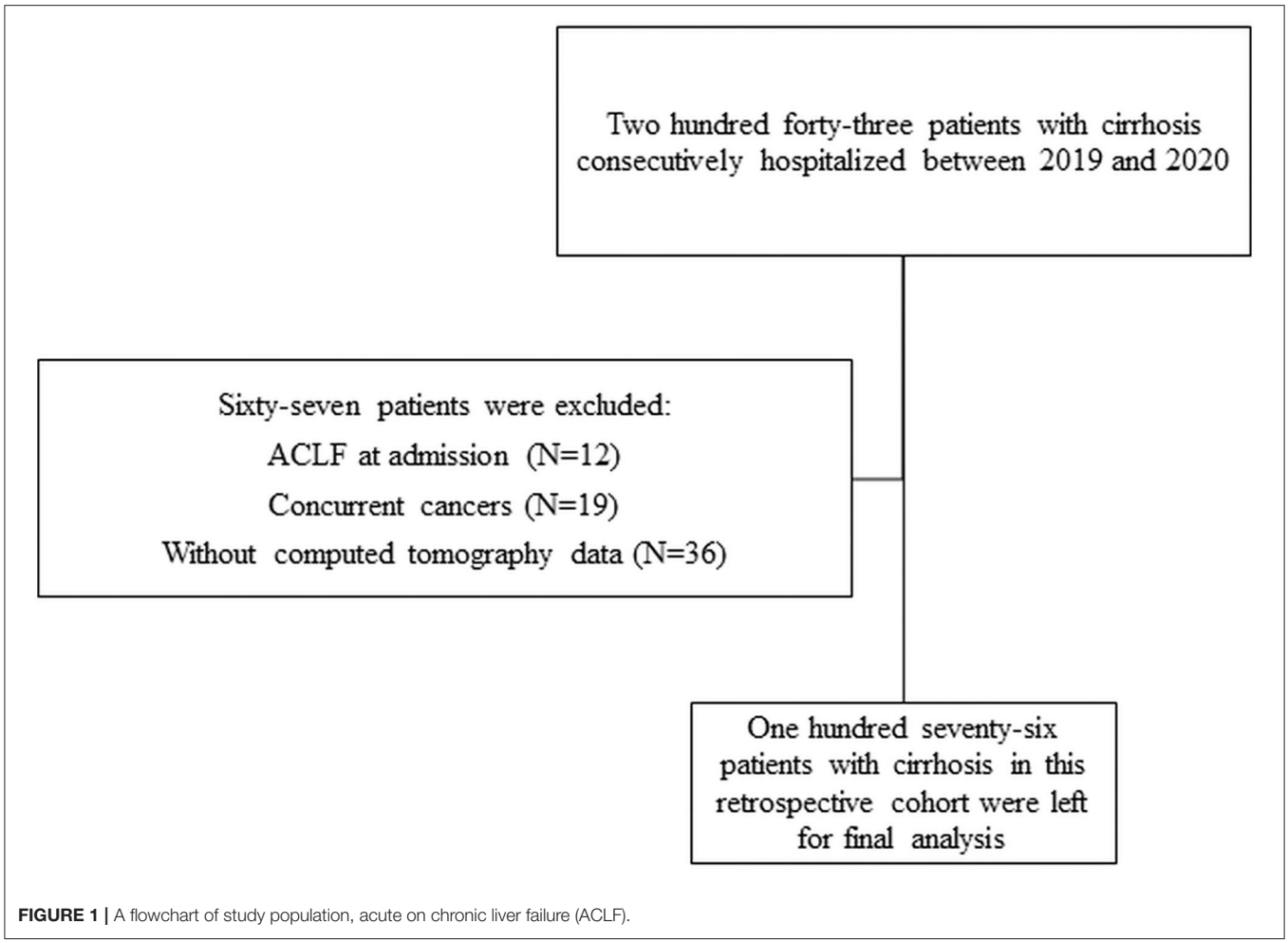

FIGURE 1 | A flowchart of study population, acute on chronic liver failure (ACLF).

Belgium) and Stata 14.0 (Stata Corporation, College Station, Texas, USA).

RESULTS

Table 1 shows the baseline features and laboratory data of the 176 patients. The etiology of cirrhosis was attributed to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection in 45 (25.57%), alcohol in 45 (25.57%), autoimmune liver disease in 41 (23.29%), and nonalcoholic fatty liver disease (NAFLD) and cryptogenic reasons in 45 (25.57%) subjects, respectively. The cirrhosis-associated complications consisted of ascites in 105 (59.66%), esophagogastric varices in 123 (69.89%), infection in 27 (15.34%), and hepatic encephalopathy in 16 (9.09%), respectively. Among the study population, 43 (24.43%) were categorized into CTP class A, 104 (59.09%) into CTP class B, and 29 (16.48%) into CTP class C. The median MELD-Na score upon hospitalization was 10 (IQR, 6–13.75).

Then, these patients were divided into two groups in terms of malnutrition risk determined by RFH-NPT score. Among them, 79 (44.89%) patients were in the low- or moderate-risk group and 97 (55.11%) were in the high-risk group. There were significant differences in gender, CTP class, etiology, the presence of ascites, BMI, the presence of sarcopenia, visceral adiposity, albumin, creatinine, and PT-INR. Patients with high risk of malnutrition were dominant in men, more CTP class B/C, more alcoholism, more ascites, lower BMI, more sarcopenia, more visceral adiposity, lower albumin, higher creatinine, and PT-INR.

Independent Risk Factor of High Risk of Malnutrition

The univariate and multivariate analyses of malnutrition risk are shown in **Table 2**. Univariate analysis revealed that age ($p = 0.041$), male gender ($p = 0.005$), alcoholism ($p < 0.001$), CTP class ($p = 0.011$), ascites ($p < 0.001$), BMI ($p = 0.001$), sarcopenia ($p = 0.007$), visceral adiposity ($p < 0.001$), and albumin ($p = 0.008$) were significantly associated with high risk of malnutrition. Taking into consideration that alcoholic liver disease that exhibits a large weight on original RFH-NPT score, we decided to construct two multivariate logistic regression model. In model 1, our results indicated that visceral

TABLE 1 | Baseline characteristics of cirrhotic patients stratified according to RFH-NPT risk classification.

	Total (*N* = 176)	RFH-NPT risk classification		*P*
		Low and Moderate (*N* = 79)	High (*N* = 97)	
Age (years)	63 (56–68)	62 (53.75–67)	64 (57–69)	0.106
Gender, *n* (%)				0.005
Male	83 (47.16)	28 (35.44)	55 (56.70)	
Female	93 (52.84)	51 (64.56)	42 (43.30)	
CTP, *n* (%)				0.009
A	43 (24.43)	27 (34.18)	16 (16.49)	
B	104 (59.09)	44 (55.70)	60 (61.86)	
C	29 (16.48)	8 (10.12)	21 (21.65)	
MELD-Na score	10 (6–13.75)	9(7–12)	10 (6-15)	0.394
Etiology, *n* (%)				<0.001
HBV/HCV	45 (25.57)	28 (35.44)	17 (17.53)	
Alcohol	45 (25.57)	4 (5.06)	41 (42.27)	
AILD	41 (23.29)	23 (29.11)	18 (18.56)	
NAFLD/Cryptogenic	45 (25.57)	24 (30.39)	21(21.64)	
Complications, *n* (%)				
Gastroesophageal varices	123 (69.89)	56 (70.89)	67 (69.07)	0.869
Hepatic encephalopathy	16 (9.09)	6 (7.59)	10 (10.31)	0.606
Ascites	105 (59.66)	33 (41.77)	72 (74.23)	<0.001
Infection	27 (15.34)	9 (11.39)	18 (18.56)	0.213
BMI (kg/m^2)	22.89 ± 4.72	24.28 ± 4.89	21.68 ± 4.24	<0.001
Waist circumference (cm)	93.79 ± 14.38	91.75 ± 12.37	95.52 ± 15.73	0.094
VSR	0.99 (0.3–1.42)	0.92 (0.67–1.08)	1.17 (0.86–1.66)	<0.001
Visceral adiposity	42 (23.86)	8 (10.13)	34 (35.05)	<0.001
High visceral adiposity	118 (67.05)	50 (63.29)	68 (70.10)	0.420
Low subcutaneous adiposity	40 (22.73)	13 (16.46)	27 (27.84)	0.103
Platelet (×10^9/L)	80 (55.25-114.80)	76 (47–113)	86 (60.50–119)	0.172
Albumin (g/L)	28 (24–32)	30 (25–34)	27 (23–30.50)	0.006
TBIL (μmol/L)	22.75 (14.40–38.90)	22.40 (13.60–36.14)	22.8 (14.9–44.7)	0.517
ALT (U/L)	23.50 (15–37)	25 (17–41)	23 (14–35.50)	0.072
AST (U/L)	31 (21.25–52.75)	31 (22-57)	31 (20.50–47.50)	0.657
Creatinine (μmol/L)	59 (49.25–73)	58 (47–66)	61 (51–82.50)	0.021
PT-INR	1.27 (1.17–1.46)	1.24 (1.17, 1.35)	1.31 (1.18–1.54)	0.023

Values are presented as the mean ± standard deviation, median (IQR), or number of patients (%).
RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; AILD, auto-immune liver disease; NAFLD, non-alcoholic fatty liver disease; CTP, Child-Turcotte-Pugh class; BMI, body mass index; VSR, visceral to subcutaneous ratio of adipose tissue area; MELD-Na, model for end-stage liver disease-sodium; TBIL, total bilirubin; PT-INR, prothrombin-international normalized ratio.

adiposity exhibits borderline significance (OR = 2.705, 95% CI: 0.968–7.557, $p = 0.058$). In model 2 excluding etiology, we found that male gender (OR = 2.884, 95% CI: 1.360–6.115, $p = 0.006$), BMI (OR = 0.879, 95% CI: 0.812–0.951, $p = 0.001$), albumin (OR = 0.934, 95% CI: 0.882–0.989, $p = 0.019$), and visceral adiposity (OR = 3.413, 95% CI: 1.344–8.670, $p = 0.010$) were independent risk factors of malnutrition risk determined by RFH-NPT in hospitalized patients with cirrhosis.

Gender-Stratified Analysis of Adipose Tissue and RFH-NPT

It has been suggested that CT quantification unravels significant variations regarding adipose tissue distribution pattern by gender (19, 20). Specially, men store higher levels of VATI, whereas women have higher levels of SATI in the context of cirrhosis. Therefore, we further investigated the association between distinct adipose depots and RFH-NPT-based malnutrition risk by gender. As shown in **Figure 2**, there was no significant

TABLE 2 | Univariate and multivariate analysis for malnutrition risk determined by RFH-NPT.

Variable	Univariate analysis			Multivariate analysis					
				Model 1*			Model 2*		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Age (years)	1.031	1.001, 1.062	0.041				1.033	0.999, 1.069	0.058
Gender							2.884	1.360, 6.115	**0.006**
Male	2.385	1.294, 4.396	0.005						
Female	Reference								
Etiology			<0.001	0.650		**<0.001**			
HBV/HCV	0.694	0.299, 1.608	0.394	0.650	0.255, 1.658	0.367			
Alcohol	11.714	3.592, 38.198	<0.001	9.994	2.846, 35.091	**<0.001**			
AILD	0.894	0.382, 2.094	0.797	0.804	0.306, 2.113	0.659			
NAFLD/Cryptogenic	Reference			Reference			Reference		
CTP			0.011						
A	Reference								
B	2.301	1.108, 4.778	0.025						
C	4.430	1.593, 12.315	0.004						
Ascites	4.015	2.121, 7.598	<0.001						
BMI	0.890	0.830, 0.593	0.001	0.866	0.796, 0.942	**0.001**	0.879	0.812, 0.951	**0.001**
Visceral adiposity	4.790	2.065, 11.112	<0.001	2.705	0.968, 7.557	0.058	3.413	1.344, 8.670	**0.010**
Albumin	0.933	0.887, 0.982	0.008	0.940	0.887, 0.996	**0.036**	0.934	0.882, 0.989	**0.019**

*Multivariate model 1: Age, gender, etiology, CTP class, BMI, ascites, visceral adiposity and albumin; *Final model presented.*
*Multivariate model 2: Age, gender, CTP class, BMI, ascites, visceral adiposity and albumin; *Final model presented.*
RFH-NPT, Royal Free Hospital-Nutritional Prioritizing Tool; OR, odds ratio; CI, confidence interval; AILD, auto-immune liver disease; NAFLD, non-alcoholic fatty liver disease; CTP, Child-Turcotte-Pugh class; BMI, body mass index.
The bold values indicate statistical significance.

difference with respect to TATI (men: 100.80 ± 43.54 vs. 92.19 ± 37.94 cm^2/m^2, $p = 0.355$; women: 111.70 ± 54.65 vs. 98.19 ± 49.92 cm^2/m^2, $p = 0.219$), SATI (men: 49.50 ± 25.16 vs. 36.39 ± 15.96 cm^2/m^2, $p = 0.058$; women: 62.23 ± 34.50 vs. 50.91 ± 27.35 cm^2/m^2, $p = 0.122$), and VATI (men: 51.09 ± 23.60 vs. 55.80 ± 25.88 cm^2/m^2, $p = 0.422$; women: 49.42 ±24.48 vs. 47.28 ± 26.05 cm^2/m^2, $p = 0.684$) in both genders among patients with low or moderate and high risk of malnutrition. In contrast, the proportion of male patients embracing visceral adiposity was higher in high risk of malnutrition group compared with that in low or moderate group (47.27 vs. 17.86%, $P = 0.009$). Moreover, this disparity was of borderline statistical significance in women (19.05 vs. 5.88%, $P = 0.061$).

DISCUSSION

As far as we can determine, this is the first work to explore the association between CT-defined abnormal adiposity and validated tool (RFH-NPT) for screening malnutrition risk in hospitalized patients with cirrhosis. Our results implicated that high VSR that corresponds to visceral adiposity is associated with higher risk of malnutrition independent of BMI. Furthermore, the distribution of adipose tissue might alter more profoundly in comparison with other adiposity parameters when stratified by malnutrition risk.

Mounting evidence has proved that malnutrition serves as a predictor of morbidity and mortality in patients with cirrhosis (21). It is tempting to apply targeted interventions with the purpose of ameliorating malnourished status and relevant complications. Given no generalized modality currently exists regarding malnutrition risk screening, a cirrhosis-specific approach, referring to RFH-NPT, has recently been developed, used, and recommended by several hepatology centers and scientific societies (4, 22–25). Notably, among eight screening tools for detecting the risk of malnutrition in cirrhosis, RFH-NPT represents the most accurate with a high sensitivity of 97.4% and a fair specificity of 73.3% (26). In addition, our previous work showed that malnourished status assessed by RFH-NPT is closely associated with immune dysfunction (5). Taken together, we preferentially adopt RFH-NPT in this work for identifying high risk of malnutrition in our retrospective cohort.

In our work, the hospitalized patients with cirrhosis and high risk of malnutrition were at lower levels of BMI and prone to embrace higher proportion of visceral adiposity. Although BMI has been a concern in most researches evaluating clinical implications or outcomes in cirrhosis, it seems an inaccurate measurement of body composition. The main drawbacks of BMI include its inability to discriminate between muscle and adipose tissue as along with confounding impact of fluid retention in cirrhosis (8, 27). In a word,

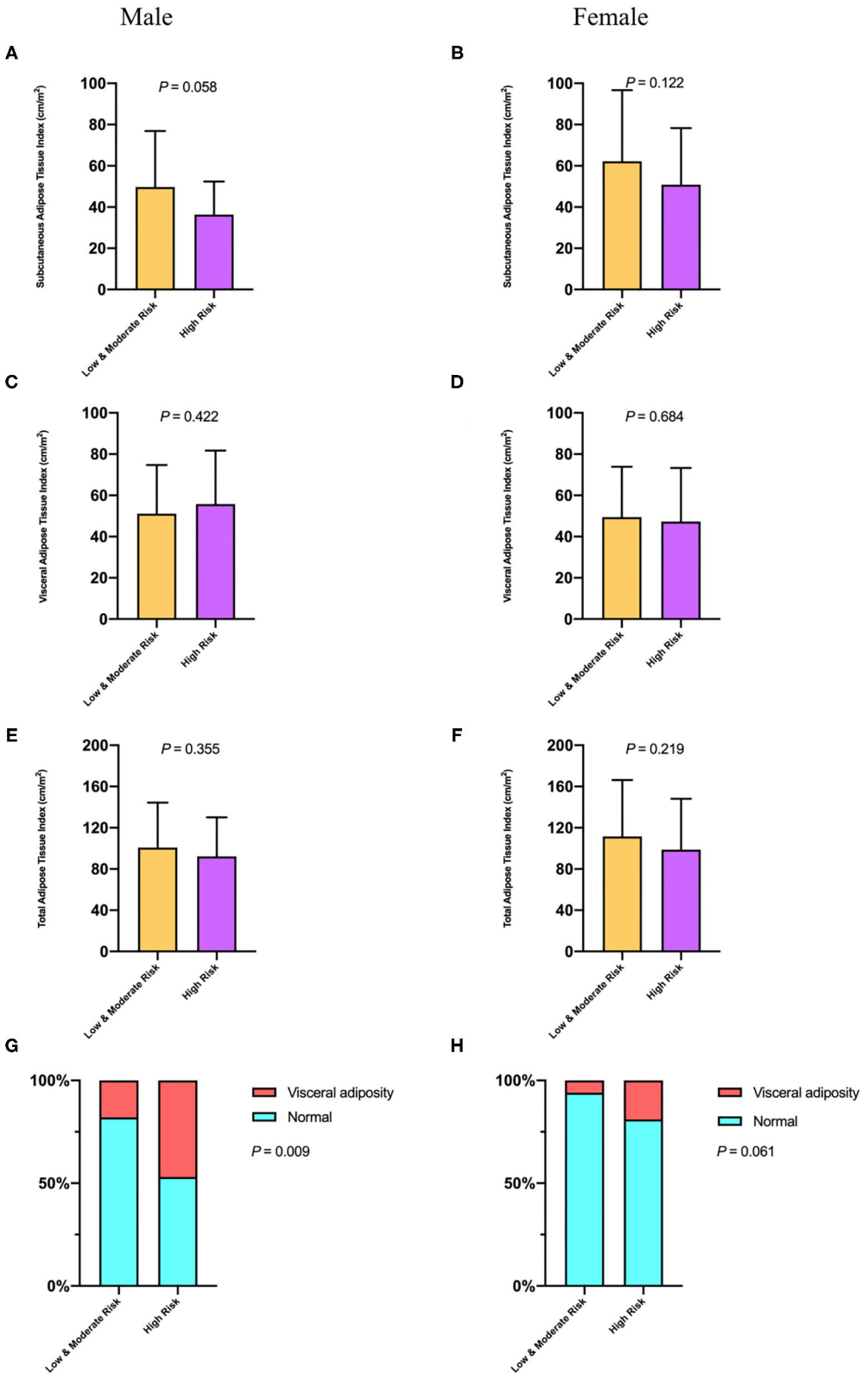

FIGURE 2 | The comparison of subcutaneous adipose tissue index **(A,B)**, visceral adipose tissue index **(C,D)**, total adipose tissue index **(E,F)**, and visceral adiposity **(G,H)** in both genders with distinct malnutrition risk of hospitalized patients with cirrhosis.

the fluid accumulation (e.g., large amount of ascites) might mask weight loss in patients with malnourished condition (6, 28). Consequently, it is recommended to perform a single crosssectional CT image or MRI, rather than BMI, as a non-invasive tool to estimate body composition. Moreover, it is feasible and available to most cirrhotics due to a routine request as screening for hepatocellular carcinoma by using crosssectional imaging.

Intriguingly, several studies have revealed the association between abnormalities in skeletal muscle and malnutrition risk in a wide array of pathological entities. Borges and colleagues showed that the presence of sarcopenia might predict comorbidities in 29%, and nutritional risk in 49% hospitalized patients with cancer (10). Akazawa et al. indicated that higher risk of malnutrition is associated with impaired muscle quality in terms of increased intramuscular adipose tissue of the quadriceps in elder inpatients (29). Notably, another work implicated that patients with cirrhosis and concomitant sarcopenia are predisposed to undernutrition (PG-SGA) and in need for nutritional care (11). Taken together, we and others have demonstrated that assessing body composition components, which includes both muscle and adipose tissue to stratify patients at high malnutrition risk and select more appropriate therapies.

In this work, we further confirmed that a higher VSR associates with high risk of malnutrition in patients with cirrhosis, whereas low subcutaneous adiposity or high visceral adiposity does not. Actually, our previous publication has already suggested that the distribution of adipose tissue, rather than the absolute value, represents a predominant risk factor of prognostication in cirrhosis (7). Marked differences between VAT and SAT have been observed regarding anatomic location, adipocyte size, insulin sensitivity, adipokines profile, and lipolytic capability (13). Adipocytes within VAT are responsible for secreting a variety of cytokines such as IL-1, TNF-α, and toxic-free fatty acids (FFAs) due to active lipolytic effect (30, 31). These FFAs are directly transported to the liver *via* portal vein, consequently resulting in oxidative stress, lipid peroxidation, and hepatocellular inflammation (32, 33). In contrast, SAT has been proved to uptake and deposit triglycerides, plasma FFAs, and responsible for producing leptin in charge of immune response and lipid metabolism (34–36). More recently, our results implicated that immune dysfunction measured by neutrophil-to-lymphocyte ratio (NLR) is associated with malnutrition risk estimated by RFH-NPT in cirrhosis (5). Furthermore, the expression of circulating IL-6 and IL-8 was positively correlated with increased NLR values (37). Taken together, we speculate that VSR might be more closely associated with chronic inflammation in patients with cirrhosis, which promotes the progression of malnutrition (38).

We acknowledge that there are limitations in this work. Firstly, we assured that cutoffs established in this work might not be generalized to other regions and populations. As a matter of fact, a myriad of cutoffs with respect to adipose tissue parameters have already been developed (19, 39). These dramatical disparities might be attributed to different ethnicities, analytic metrics, and also distinct pathologies. Secondly, we were unable to examine metabolic and nutritional profiles such as serum cytokines, nutrients, leptin, and insulin resistance because of the retrospective nature of this work.Thirdly, the sample size was relatively small in our work. Finally, we could not infer the causal relationship between visceral adiposity and the risk of malnutrition. Collectively, there is an urgent need to conduct randomized controlled trial to identify causality between these factors.

CONCLUSIONS

In conclusion, the assessment of adipose tissue distribution by CT might potentiate the estimation of malnutrition risk in cirrhotics. It is pivotal to recognize visceral adiposity and develop targeted therapeutic strategies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tianjin Medical University General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW, YL, MS, and CS equally contributed to the conception and design of the research. GG, WY, and YH contributed to the design of the research. ZY and CL contributed to the acquisition and analysis of the data. XF, BW, JZ, XZ, and KJ contributed to the interpretation of the data. CS drafted the manuscript. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Nutritional Status and Body Composition in Wilson Disease: A Cross-Sectional Study From China

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Background: Abnormal nutritional status is frequently seen in patients with chronic diseases. To date, no study has investigated the detailed characteristics of abnormal nutritional status among Wilson's disease (WD) patients in the Chinese cohort. This study aimed to describe the nutritional status of WD patients, with a particular focus on the differences between patients with different phenotypes.

Methods: The study subjects comprised 119 healthy controls, 129 inpatients (hepatic subtype, $n = 34$; neurological subtype, $n = 95$) who were being treated at the affiliated hospital of the Institute of Neurology, Anhui University of Chinese Medicine. All of the subjects were assessed for body composition by using bioelectrical impedance analysis. All WD patients received anthropometry, nutritional risk screening 2002 (NRS2002), and laboratory test (hemocyte and serum biomarkers) additionally.

Results: Compared with healthy controls, the fat mass and rate of total body and trunk were significantly higher in WD patients ($P < 0.001$), the muscle and skeletal muscle mass of total body and trunk were significantly lower in WD patients ($P < 0.001$). Compared with hepatic subtype patients, the fat mass and rate of total body, trunk, and limbs were significantly lower in neurological subtype patients ($P<0.01$); while there were no significant differences in muscle and skeletal muscle between these two subtypes. The overall prevalence of abnormal nutritional status in WD patients was 43.41% (56/129). The prevalence of high-nutritional risk and overweight in WD patients was 17.83% (23 of 129) and 25.58% (33 of 129), respectively. Compare with patients with high nutritional risk, macro platelet ratio, alkaline phosphatase, the basal metabolic rate ($p < 0.05$), creatinine, trunk fat rate ($p < 0.01$) and appendicular skeletal muscle mass ($p < 0.001$) were significantly higher in patients without nutritional risk ($p < 0.001$). Patients with a high nutritional risk tend to have a lower cholinesterase concentration ($x^2 = 4.227$, $p < 0.05$).

Conclusion: Both patients with H-subtype and N-subtype are prone to have an abnormal nutritional status. Longitudinal studies are required to investigate if

nutritional status and body composition could reflect prognosis in WD patients, and which of these body composition indexes contribute to malnutrition and worse prognosis.

Keywords: Wilson disease, nutritional status, body composition, NRS2002, phenotype

INTRODUCTION

Wilson's disease (WD) is an autosomal recessive copper metabolic disorder characterized by dysfunction of the liver, brain, bone and endocrine system mainly (1). The phenotype can be divided into hepatic subtype (H-subtype) and neurological subtype (N-subtype) according to the primary symptom (2). The prevalence of WD in China was 5.87 in 100, 000, which was higher than western descent (1 in 30,000) (3, 4). WD is potentially treatable, unlike most genetic diseases, and the life expectancy of some patients is about the same as that of normal people if they receive timely and correct treatment. However, there are still quite a few WD patients who have a poor prognosis for the reason of some avoidable factors, including incorrect diet, untimely treatment and irregular medication, etc. (5). Additionally, more unknown factors have not been found and the deterioration of some WD patients cannot be prevented and explained. Therefore, exploring and getting avoid of the controllable factors that may deteriorate the prognosis is being one of the most significant therapeutic targets concerned by neurology physicians. WD is a multisystem disease, which causes injury in not only the brain and liver, but also in renal, cardiac, skin, osteoarticular, or endocrinologic and includes other organ disturbances (6). This may influence the whole body's nutritional status. Additionally, lipid metabolism dysregulation is related to WD (7), which may affect the body composition of patients.

Nutritional status might be a potential factor that influences the prognosis of patients with WD. Abnormal nutritional status can be divided into nutritional deficiency (high nutritional risk) and nutritional overload (overweight) (8). Nutritional status is affected in subjects with Wilson's disease *via* many mechanisms such as the impact of long-lived chronic hepatic damage, renal injury, chelating medications, copper-restricted diet, imbalance of gut flora and dysphagia among patients with severe neurological dysfunction. These factors largely affect the intake, absorption and metabolism of nutrients from daily food. Body composition is another index that can reflect the nutritional status of patients with WD (9). It contains mass and rate of fat, muscle, bone, water and protein. The nutritional status was potentially ameliorated and frequently under-recognized, while it could play a crucial part in WD patients' long-term prognosis.

Nutritional status might be a potential factor that influences the prognosis of patients with WD. It has been proved to play an essential part in Parkinson's disease (PD), Alzheimer's disease (AD), and other chronic diseases. Cova et al. (10) utilized bioelectrical impedance measurements, nutritional scales (nutritional risk screening 2002, NRS2002; mini nutritional assessment, MNA) to explore the differences in nutritional status indexes among healthy individuals, mild cognitive impairment (MCI) subjects and AD patients. They found nutritional status indexes could characterize the process from normal to MCI to AD. Thus, the above nutritional status indicator set could potentially be a non-invasive, convenient, and trackable monitoring tool in the assessment, prevention, and efficacy evaluation of AD. Petroni et al. (11) assessed the nutritional status of patients with advanced-stage Parkinson's disease with body composition analysis, anthropometric measurements and serum biochemical markers. Their results demonstrated that obesity could be common among patients with advanced-stage Parkinson's disease, besides, fat-free mass and muscle mass were continuously consumed during the disease course. Lin et al. (12) reported the correlation between the nutritional status with the severity of PD symptoms. Those patients with poor nutritional status might have a worse prognosis. Weight, body mass index, hemoglobin, and cholesterol can be regarded as regular biomarkers to reflect the nutritional status and disease progression among PD patients.

There appears to be a paucity of medical literature with regard to the prevalence, magnitude, and feature of these nutritional manifestations in Wilson's disease. In this study, we aim to explore the characteristics of nutritional status in patients with WD, with a special focus on the relationship between nutritional status and clinical phenotype.

MATERIALS AND METHODS

Subject and Design

This cross-sectional study was investigated by Sports & Nutrition Information Technology Laboratory, Chinese Academy of Science, and it was carried out in the Center of WD, the affiliated hospital of the Institute of Neurology, Anhui University of Chinese Medicine.

The study protocol was approved by the Ethics Committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences (SWYX-Y-2021-08), and informed written consent from all subjects was obtained by the principal researcher, after self-motivated behavior evaluation of the patients' capacity to provide consent. In those patients under 18-year-old, their assent form was signed by their parents.

We enrolled a total of inpatients admitted from June 2020 to June 2021 with the diagnosis of WD (follow by the standard guidelines developed by the American Association of the Study

Abbreviations: WD, Wilson's disease; HC, healthy controls; H-subtype, hepatic subtype; N-subtype, neurological subtype; PD, Parkinson's disease; AD, Alzheimer's disease; NRS2002, nutritional risk screening 2002; MNA, mini nutritional assessment; MCI, mild cognitive impairment; BMI, body mass index; WHR, waist-to-hip ratio; MUST, Malnutrition Universal Screening Tool; PG-SGA, Patient-generated Subjective Global Assessment; UWDRS, Unified Wilson's Disease Rating Scale.

of Liver Diseases). Healthy controls (HC) were recruited in the same period from communities in Anhui province.

Individuals were excluded if aged >55 years if they had pacemakers, heart defibrillators, bone nails, or other metal/electrical implants and if they were participating in exercise or nutritional intervention programs. To avoid interference from other metabolic factors, patients suffering from ascites, hepatic encephalopathy, diabetes, and hyperthyroidism were excluded.

All study participants underwent an evaluation following a standardized protocol. Collected data included demographic characteristics, medical history, pharmacological history and Unified Wilson's Disease Rating Scale (UWDRS) assessment.

Nutritional Evaluation

The nutritional evaluation was performed using bioelectrical impedance analysis, anthropometry, nutritional risk screening 2002 (NRS2002), and blood test (blood routine examination and serum biochemical test).

Bioelectrical impedance measurements were conducted by a trained investigator and the participants ought to be fasted for at least 3 h. The bioelectrical resistance (R, Ohm) and body composition indexes (mass and rate of fat, muscle, bone, protein, water, etc.) were detected by an eight-electrode impedance analyzer (BX-BCA-100, Broshare Technology, Hefei, China). The accuracy of the body composition model was corrected using the standard of Tanita-980 (Tanita, Tokyo, Japan).

Anthropometric measurements included height, weight, body mass index (BMI), body circumferences (Waist, hip, biceps, mid-arm muscle, calf), skinfold thickness (triceps and subscapular) it was operated by the following standard criteria. All study participants were required to wear light clothing without shoes and socks. The room temperature and humidity were controlled at 24°C and 40%, respectively. Height (cm, to the nearest 1 cm) was measured with an altimeter and weight (kg, to the nearest 0.01 kg) with a standard scale; body mass index (BMI) (kg/m^2) was hence calculated. Body circumferences were obtained with an inelastic plastic-fiber tape measure (to the nearest 0.1 cm); The waist circumferences were measured midpoint between the lowest rib and the upper border of the iliac crest; the hip circumferences were measured at the horizontal section between the pubic symphysis and the most convex part of the back gluteus at the maximum; the calf, biceps and mid-arm muscle were measured at the maximum girth. The skinfold thickness of triceps and subscapular was measured by a skinfold thickness gauge.

NRS2002 is recommended by the ESPEN guidelines and it takes into account the severity of disease and impaired nutritional status (13). In our study, every NRS2002 scale was conducted by an experienced specialist nurse and it should be re-examined by an attending doctor. Impaired nutritional status contains unexplained weight loss, reduced food intake, and BMI. Patients would get a score of 1 if their weight loss >5% in the last 3 months or 0 to 25% reduced food intake of the normal requirement; a score of 2 if weight loss >5% in the last 2 months, BMI was 18.5 to 20.5 kg/m^2 plus impaired general condition, or 25 to 50% reduced food intake of the normal requirement; and a score of 3 if weight loss >5% in the last months, BMI of <18.5 kg/m^2 plus impaired general condition, or 50 to 75% reduced food intake of the normal requirement. The final score of the NRS2002 ranged from 0 to 7, with a score of ≥3 indicating a high nutritional risk.

Blood routine examinations, including red blood cells, leukocytes, hemoglobin, platelets, were measured using a method of fluorescence flow cytometry with an automatic blood analyzer (SYSMEX, XT-1800i). Serum biochemical tests, including aminotransferase, bilirubin, monoamine oxidase, were measured using a colorimetric method with an automatic biochemical analyzer (HITACHI, 7600).

Statistical Analysis

The characteristics of the subjects were described. Continuous variables were presented as mean values and standard deviations, and categorical variables were presented as counts. To compare the malnutrition status, data for NRS2002 were dichotomized into those at moderate or high risk (≥3) and those at a normal level (<3). Subjects with BMI ≥ 25 were defined as overweight. Men with a body fat rate >21.4% and women with a body fat rate >29% were defined as having a high body fat percentage (14). People with high nutritional risk or overweight were defined as having an abnormal nutritional status.

Continuous variables of characteristics among the three groups of participants (different subtypes and nutritional status) were compared using the univariate ANOVA test and student's test. Categorical variables of characteristics between the two groups (H-subtype WD patients and N-subtype WD patients) were compared using the Pearson chi-square test or Fisher's exact probability method. All statistical analyses were conducted using SPSS software (IBM SPSS Statistics Version 17.0). Statistical significance was set at $p < 0.05$.

RESULTS

The 129 WD patients were characterized based on four parameters: bioelectrical impedance analysis (BIA), anthropometry, nutritional status, and serum biochemical biomarkers. HC, patients with H-subtype and patients with N-subtype were 129, 34, and 95 cases each. The mean age of HC, patients with H-subtype, and patients with N-subtype were 28.17 ± 7.56, 28.86 ± 8.47, 25.2 ± 7.36, and 30.15 ± 8.49, respectively. No statistically significant difference in BMI had been found among these three groups. There were no statistically significant differences in gender composition ratio ($x^2 = 2.058$, $p > 0.05$) between H-subtype WD patients (19 males and 15 females) and N-subtype WD patients (66 males and 29 females). Patients with H-subtype were all in the stage of liver fibrosis or compensated stage of cirrhosis, none of them had decompensated cirrhosis with ascites.

The BIA Characteristics of Different Subtypes of WD Patients

Table 1 shows bioelectrical variables in healthy controls (HC), H-subtype Wilson's disease patients and N-subtype patients. No statistically significant differences in BMI between HC and patients with WD. The

TABLE 1 | Bioelectrical variables in healthy controls and patients with WD.

	HC (n = 119)	Overall WD (n = 129)	T-value (HC vs. WD)	H subtype (n = 34)	N subtype (n = 95)	F-value (HC vs. H subtype vs. N subtype)	T-value (HC vs. N subtype)	T-value (H subtype vs. N subtype)
Age (y)	28.17 ± 7.56	28.86 ± 8.47	0.670	25.2 ± 7.36	30.15 ± 8.49	**5.151****	1.813	**−3.017****
Height (m)	1.73 ± 0.07	1.73 ± 0.09	0.162	1.75 ± 0.09	1.71 ± 0.08	**3.167***	−0.82	**2.19***
Weight (kg)	68.38 ± 12.13	68.08 ± 15.2	−0.172	73.18 ± 18.5	66.24 ± 13.46	**3.248***	−1.215	2.005
BMI (kg/m^2)	22.94 ± 3.34	22.78 ± 4.21	−0.346	23.7 ± 5.04	22.43 ± 3.83	1.457	−1.02	1.336
FFM (kg)	55.04 ± 9.29	51.11 ± 10.28	**−3.155****	52.01 ± 12.58	50.77 ± 9.37	**5.165****	**−3.324****	0.526
total body fat mass (kg)	13.41 ± 5.7	16.98 ± 9.4	**3.650*****	21.17 ± 9.54	15.47 ± 8.92	**13.711*****	1.964	**3.138****
Trunk fat mass (kg)	6.73 ± 3.44	9.51 ± 5.71	**4.674*****	12.01 ± 5.63	8.59 ± 5.48	**17.917*****	**2.889****	**3.098****
Left arm fat mass (kg)	0.55 ± 0.22	0.67 ± 0.43	**2.863****	0.82 ± 0.43	0.61 ± 0.41	**9.359*****	1.368	**2.622***
Right arm fat mass (kg)	0.58 ± 0.24	0.71 ± 0.43	**3.145****	0.87 ± 0.44	0.65 ± 0.4	**10.082*****	1.669	**2.637****
Left leg fat mass (kg)	2.75 ± 0.95	3.04 ± 1.47	1.924	3.67 ± 1.57	2.81 ± 1.36	**8.161*****	0.438	**3.031****
Right leg fat mass (kg)	2.82 ± 0.99	3.14 ± 1.51	**1.999***	3.77 ± 1.61	2.91 ± 1.4	**8.01*****	0.54	**2.976****
Total body fat rate (%)	19.28 ± 6.83	24.05 ± 9.82	**4.468*****	28.28 ± 8.82	22.52 ± 9.75	**16.099*****	**2.756****	**3.024****
Trunk fat rate (%)	17.68 ± 7.53	25.57 ± 12.45	**6.090*****	30.73 ± 11.91	23.71 ± 12.16	**24.63*****	**4.233*****	**2.903****
Left arm fat rate (%)	17.56 ± 6.72	20.65 ± 11.85	**2.554***	25.15 ± 11.71	19.03 ± 11.52	**8.394*****	1.109	**2.647****
Right arm fat rate (%)	17.46 ± 7.12	20.08 ± 11.21	**2.215***	24.27 ± 11.25	18.57 ± 10.85	**7.143****	0.865	**2.602***
Left leg fat rate (%)	22.07 ± 6.76	23.57 ± 9.55	1.440	26.96 ± 9.1	22.34 ± 9.44	**4.989****	0.25	**2.466***
Right leg fat rate (%)	22.38 ± 6.84	23.85 ± 9.59	1.406	27.26 ± 9.27	22.62 ± 9.44	**4.934****	0.221	**2.469***
Total muscle mass (kg)	52.17 ± 8.92	48.53 ± 9.85	**−3.041****	49.36 ± 12.05	48.22 ± 8.97	**4.793****	**−3.2****	0.502
Trunk muscle mass (kg)	28.82 ± 4.02	25.38 ± 4.73	**−6.158*****	25.82 ± 5.96	25.2 ± 4.21	**19.166*****	**−6.383*****	0.56
Left arm muscle mass (kg)	2.44 ± 0.66	2.31 ± 0.66	−1.554	2.25 ± 0.66	2.32 ± 0.65	1.369	−1.216	−0.575
Right arm muscle mass (kg)	2.59 ± 0.67	2.4 ± 1.25	−1.457	2.27 ± 0.72	2.43 ± 1.38	1.371	−1.005	−0.64
Left leg muscle mass (kg)	9.09 ± 1.93	9.2 ± 2.02	0.445	9.47 ± 2.41	9.09 ± 1.84	0.575	0.039	0.957
Right leg muscle mass (kg)	9.25 ± 2.06	9.27 ± 2.01	0.045	9.52 ± 2.44	9.16 ± 1.83	0.401	−0.312	0.791
Total skeletal muscle mass (kg)	29.73 ± 5.4	27.53 ± 5.96	**−3.041****	28.03 ± 7.29	27.34 ± 5.42	**4.793****	**−3.199****	0.501
Trunk skeletal muscle mass (kg)	8.63 ± 1.25	6.73 ± 1.5	**−10.857*****	6.89 ± 1.86	6.66 ± 1.34	**58.427*****	**−10.995*****	0.665
Upper limb skeletal muscle mass (kg)	5.53 ± 1.45	5.18 ± 2.03	−1.552	4.98 ± 1.52	5.23 ± 2.17	1.469	−1.131	−0.638
Lower limb skeletal muscle mass (kg)	15.59 ± 3.38	15.69 ± 3.41	0.242	16.15 ± 4.12	15.51 ± 3.11	0.467	−0.14	0.817
ASM (kg/m^2)	7.06 ± 1.26	6.95 ± 1.23	−0.694	6.79 ± 1.29	6.99 ± 1.19	0.565	−0.335	−0.816
Total body water (kg)	38.3 ± 6.65	33.93 ± 7.08	**−5.005*****	33.74 ± 7.82	33.98 ± 6.82	**12.488*****	**−4.654*****	−0.17
Mineral (kg)	2.88 ± 0.39	2.8 ± 0.48	−1.409	2.87 ± 0.56	2.76 ± 0.43	1.791	−1.892	1.16
Protein (kg)	13.57 ± 2.32	14.61 ± 4.42	**2.355*****	15.61 ± 5.29	14.24 ± 4.02	**4.561***	1.465	1.38
Basal metabolic rate (kcal)	1,592.68 ± 253.33	1,483.57 ± 277.17	**−3.228*****	1,540.36 ± 337.94	1,463.23 ± 250.84	**6.289****	**−3.73*****	1.217

*BMI, body mass index; FFM, fat free mass; ASM, appendicular skeletal muscle mass. *P < 0.05, **P < 0.01, ***P < 0.001, statistically significant values are highlighted in bold.*

TABLE 2 | The characteristics of anthropometry parameters of patients with WD.

	H subtype ($n = 35$)	N subtype ($n = 95$)	*T*-value
Height (m)	1.75 ± 0.09	1.71 ± 0.08	**1.998***
Weight (kg)	73.18 ± 18.5	66.24 ± 13.46	1.792
BMI (kg/m^2)	23.7 ± 5.04	22.43 ± 3.83	1.336
Waist circumference (cm)	84.99 ± 14.1	82.99 ± 10.34	0.769
hip circumference (cm)	99.55 ± 9.79	94.38 ± 12.17	**2.258***
WHR	0.86 ± 0.08	0.96 ± 0.83	−0.732
TST (cm)	18.25 ± 7.68	15.06 ± 8.84	1.89
SST (cm)	18.82 ± 7.02	16.87 ± 6.77	1.438
biceps circumference (cm)	26.47 ± 4.58	25.77 ± 3.06	0.839
MAMC (cm)	24.28 ± 3.52	23.78 ± 2.48	0.766
calf circumference (cm)	37.07 ± 4.08	35.99 ± 3.86	1.392

*WHR, waist-to-hip ratio; TST, triceps skinfold thickness; SST, subscapular skinfold thickness; MAMC, mid-arm muscle circumference. *P < 0.05, **P < 0.01, ***P < 0.001, H subtype compared with N subtype. Statistically significant values are highlighted in bold.*

TABLE 3 | The nutritional status of patients with WD.

	H-subtype ($n = 34$)	N-subtype ($n = 95$)	X^2	*P*-value
NRS2002				
Normal nutritional status	26 (76.5%)	80 (84.2%)	1.024	0.312
High nutritional risk	8 (23.5%)	15 (15.8%)		
BMI (overweight)				
Normal weight	22 (64.7%)	74 (77.9%)	2.288	0.13
overweight	12 (35.3%)	21 (22.1%)		
Body fat rate				
Normal body fat rate	12 (35.3)	55 (57.9)	5.123	**0.024**
High body fat rate	22 (64.7%)	40 (42.1%)		

Patients who scored >3 points on NRS2002 scale were defined as high nutritional risk. Patients with BMI ≥25 were defined as overweight. Men with a body fat rate >21.6% and women with a body fat rate >30 were defined as having a high body fat percentage. Statistically significant values are highlighted in bold.

body composition was characterized by fat and muscle mainly.

Most fat parameters (total body fat mass, trunk fat mass, total body fat rate and trunk fate rate, $p < 0.001$; left arm fat mass and right arm fat mass, $p < 0.01$; left arm fat rate and right arm fat rate, $p < 0.05$) of patients with WD was significantly higher than HC. While no statistically significant differences in the fat rate of legs have been found between patients with WD and HC.

On contrary, total muscle mass, total skeletal muscle mass, trunk muscle mass, and trunk skeletal muscle mass of patients with WD were significantly lower than HC (total muscle mass and total skeletal muscle mass, $p < 0.01$; trunk muscle mass, and trunk skeletal muscle mass, $p < 0.001$). However, there was no statistically significant difference has been found in the muscle mass or skeletal muscle mass of limbs between these two groups.

The Characteristics of Anthropometry Parameters of Patients With WD

Table 2 shows the differences in anthropometry parameters between H-subtype WD patients and N-subtype WD patients. No statistically significant difference was found in BMI between these two groups.

Most anthropometry parameters (Waist circumference, triceps skinfold thickness, subscapular skinfold thickness, mid-arm muscle circumference and calf circumference) of H-subtype WD patients were higher than N-subtype WD patients, but these differences had no statistical significance. Only the hip circumferences of H-subtype WD patients were significantly higher than N-subtype WD patients ($t = 2.258$, $p < 0.05$). However, the waist-to-hip ratio of H-subtype WD patients (0.86 ± 0.08) was lower than N-subtype WD patients (0.96 ± 0.83).

Nutritional Status of WD Patients and the Characteristic of Parameters Among Patients of WD With and Without High Nutritional Risk

Table 3 shows the nutritional status of WD patients. Those patients who scored >3 points on the NRS2002 scale were defined as high nutritional risk. Patients with BMI ≥25 were defined as overweight subjects. Men with a body fat rate >21.4% and women with a body fat rate >29% were defined as having a high body fat rate (14).

Additionally, 43.41% (56/129) of the patients with WD were found to have abnormal nutritional status. The prevalence of high-nutritional risk and overweight in WD patients was 17.83% (23 of 129) and 25.58% (33 of 129), respectively. No significant differences in the prevalence of high nutritional risk between H-subtype WD patients and N-subtype WD patients. The incidence of having a high body fat rate in patients with WD was 48.06%, and H-subtype WD patients were more likely to have a high body fat than N-subtype WD patients ($x^2 = 5.123$, $p < 0.05$). A high body fat rate was more common ($x^2 = 10.829$, $p < 0.01$) in female patients with WD (30 of 44) than male patients with WD (32 of 53).

Characteristics of Serum Biochemical Biomarkers and Body Composition of Patients With a High Nutritional Risk

Table 4 shows essential parameters from serum biochemical test, blood routine examination (blood RT) and major body composition parameters among overweight WD patients and WD patients with high nutritional risk and WD patients with normal nutritional status. Compare with patients with high nutritional risk, the ratio of large platelets, alkaline phosphatase, BMR ($p < 0.05$), creatinine, trunk fat rate ($p < 0.01$), and ASM ($p < 0.001$) were significantly higher in patients without nutritional risk ($p < 0.001$).

Among these three groups, significant differences can be found in the ratio of large platelets ($f = 4.734$, $p < 0.05$, overweight < normal nutritional status < high nutritional risk), monoamine oxidase ($f = 5.230$, $p < 0.01$, normal nutritional status < high nutritional risk < overweight), creatinine ($f =$

TABLE 4 | The characteristics of nutritional status among WD patients with and without high nutritional risk.

	High nutritional risk ($n = 23$)	Normal nutritional status ($n = 73$)	Overweight ($n = 33$)	*F*-value	*T*-value (High nutritional risk vs. Normal nutritional status)	*T*-value (High nutritional risk vs. overweight)	*T*-value (Normal nutritional status vs. overweight)
Blood routine examination							
Red blood cells ($\times 10^{12}$/L)	4.37 ± 0.37	6.29 ± 13.97	4.67 ± 0.51	0.378	−0.626	**−2.173***	0.604
Leukocytes ($\times 10^{9}$/L)	5.09 ± 2.00	5.00 ± 1.75	5.17 ± 1.71	0.086	0.189	−0.149	−0.417
Hemoglobin (g/L)	128.00 ± 13.38	134.00 ± 18.13	137.15 ± 15.23	1.809	−1.399	**−2.176***	−0.795
Platelets ($\times 10^{9}$/L)	193.71 ± 114.31	175.61 ± 96.18	196.00 ± 120.43	0.471	0.718	−0.067	−0.861
Macro platelet ratio (%)	36.77 ± 7.07	32.26 ± 7.02	30.65 ± 7.22	**4.734***	**2.547***	**2.943***	0.990
Serum biochemical test							
Total bilirubin (μmol/L)	13.67 ± 8.00	15.24 ± 9.59	12.59 ± 5.54	0.933	−0.599	0.518	1.312
Glutathione aminotransferase (U/L)	28.69 ± 26.48	34.06 ± 27.76	38.35 ± 28.30	0.608	−0.692	−1.101	−0.654
Glutathione transaminase (U/L)	24.63 ± 13.64	26.72 ± 11.12	29.43 ± 18.31	0.661	−0.634	−0.904	−0.700
Monoamine oxidase (U/L)	4.65 ± 1.70	4.51 ± 1.88	5.76 ± 2.01	**5.230****	0.337	**−2.163***	**−3.125****
Creatinine (μmol/L)	61.78 ± 12.22	73.96 ± 22.67	70.00 ± 13.73	**3.562***	**−3.298****	**−2.304***	1.105
Amylase	105.26 ± 80.42	90.79 ± 31.92	74.07 ± 22.13	**3.726***	0.843	1.813	**2.725***
Total protein	64.82 ± 4.22	65.17 ± 4.68	63.26 ± 5.84	1.663	−0.315	1.073	1.766
Albumin	41.45 ± 4.01	41.99 ± 4.44	40.12 ± 3.92	2.123	−0.510	1.213	**2.039***
Globulin	23.28 ± 4.30	23.19 ± 4.53	23.15 ± 4.38	0.007	0.089	0.112	0.041
Albumin/ Globulin ratio	1.85 ± 0.41	1.90 ± 0.50	1.81 ± 0.42	0.5	−0.423	0.422	0.963
Fasting glucose (mmol/L)	5.03 ± 0.77	4.99 ± 0.79	5.15 ± 0.48	0.578	0.245	−0.693	−1.094
Glycosylated serum proteins (mmol/L)	1.89 ± 0.18	1.90 ± 0.26	1.79 ± 0.20	3.064	−0.127	**2.148***	**2.316***
Triglycerides (mmol/L)	0.89 ± 0.35	1.01 ± 0.52	1.13 ± 0.67	1.298	−1.028	−1.686	−0.958
Total cholesterol (mmol/L)	3.96 ± 0.89	4.28 ± 0.96	4.17 ± 1.02	0.982	−1.417	−0.796	0.546
Low density lipoprotein cholesterol (mmol/L)	2.36 ± 0.93	2.71 ± 0.80	2.63 ± 0.83	1.478	−1.717	−1.129	0.439
Alkaline phosphatase (U/L)	104.74 ± 46.94	87.03 ± 28.83	87.22 ± 34.40	2.54	**2.183***	1.614	−0.029
Cholinesterase (U/L)	4,726.78 ± 1,292.50	5,252.74 ± 1,362.34	5,477.67 ± 1,401.34	2.106	−1.634	**−2.036***	−0.780
Ceruloplasmin (mg/L)	58.60 ± 42.81	50.59 ± 22.95	59.25 ± 34.32	1.206	0.859	−0.063	−1.527
Copper oxidase (OD)	0.06 ± 0.05	0.06 ± 0.03	0.07 ± 0.05	2.234	1.342	−0.355	**−2.146***
Serum copper (μmol/L)	3.01 ± 1.70	2.68 ± 1.93	3.28 ± 2.47	1.025	0.757	−0.436	−1.353
25(OH) vitamin D (ng/mL)	16.00 ± 6.87	17.93 ± 8.17	17.23 ± 6.67	0.574	−1.004	−0.658	0.434
Bioelectrical parameters							
Total body fat rate (%)	18.17 ± 0.08	21.68 ± 7.91	33.30 ± 8.54	**30.354*****	−1.835	**−6.588*****	**−6.833*****
Trunk fat rate (%)	7.19 ± 6.44	12.59 ± 9.8	22.69 ± 17.50	**13.218*****	**−3.058****	**−4.655*****	**−3.102****
ASM (kg/m^2)	6.03 ± 0.71	6.8 ± 0.98	7.9 ± 1.39	**22.684*****	**−3.517*****	**−5.921*****	**−4.679*****
Mineral (kg)	2.43 ± 0.42	2.71 ± 0.33	3.24 ± 0.46	**34.061*****	**−2.911****	**−6.718*****	**−6.692*****
Basal metabolic rate (kcal)	1,293.77 ± 218.75	1,424.12 ± 192.23	1,747.38 ± 292.43	**33.048*****	**−2.743***	**−6.305*****	**−5.808*****

*ASM, appendicular skeletal muscle mass. *P < 0.05, **P < 0.01, ***P < 0.001, statistically significant values are highlighted in bold.*

TABLE 5 | The correlation of nutritional risk status and WD treatment.

	Normal nutritional status ($n = 106$)	High nutritional risk ($n = 23$)	*P*
Chelators use			
Before	13 (12.3%)	1 (4.3%)	0.242
After	93 (87.7%)	22(95.8%)	
Zinc agent use			
Before	14 (13.2%)	0	0.062
After	93 (87.7%)	22 (100%)	

3.562, $p < 0.05$, high nutritional risk < overweight < normal nutritional status), amylase ($f = 3.726$, $p < 0.05$, overweight < normal nutritional status < high nutritional risk), total body fat rate ($f = 30.354$, $p < 0.001$, high nutritional risk < NNS< overweight), trunk fat rate ($f = 13.218$, $p < 0.001$, high nutritional risk < normal nutritional status < overweight), ASM ($f = 22.684$, $p < 0.001$, high nutritional risk < normal nutritional status < overweight), mineral ($f = 34.061$, $p < 0.001$, high nutritional risk < normal nutritional status < overweight) and BMR ($f = 33.048$, $p < 0.001$, high nutritional risk < normal nutritional status < overweight).

There are no statistically significant differences that have been found in red blood cells, hemoglobin, glutathione aminotransferase, glutathione transaminase total protein, albumin, globulin, glucose, 25(OH) vitamin D and lipid between patients with high nutritional risk and patients with normal status.

Compared with patients with a high nutritional risk, cholinesterase was higher in patients with a normal nutritional status (high nutritional risk: 4,726.78 ± 1,292.50, normal nutritional status: 5,252.74 ± 1,362.34), however, there was no statistically significant difference between these two groups ($t = -1.634$, $p > 0.05$). Less than 4,400 U/L was defined as having a low concentration of cholinesterase, and patients with a high nutritional risk tend to have a lower cholinesterase concentration ($x^2 = 4.227$, $p < 0.05$).

The Correlation of Nutritional Status and Treatment

Table 5 showed the correlation between nutritional status and treatment. The results showed that there is no significant difference in nutritional risk between before and after medication.

The Correlation of Nutritional Status and Severity

Table 6 showed the correlation of nutritional status and severity, including UWDRS total scores, UWDRS neurological scores, UWDRS hepatic scores and UWDRS mental scores. The results showed that there is no significant difference in UWDRS total scores, UWDRS neurological scores, UWDRS hepatic scores and UWDRS mental scores between patients with and without a nutritional risk.

TABLE 6 | The correlation of nutritional risk status and severity.

	Normal nutritional status	High nutritional risk	*T*	*P*
UWDRS total score	24.91 ± 24.87	31.26 ± 26.26	1.100	0.273
UWDRS neurological score	19.05 ± 21.12	24.26 ± 23.57	1.051	0.295
UWDRS hepatic score	2.42 ± 2.62	3.65 ± 4.05	1.402	0.173
UWDRS mental score	3.44 ± 4.61	3.35 ± 3.88	0.093	0.926

DISCUSSION

Major Findings

This study explored the characteristics of nutritional status of patients with WD, and there were four major findings. First, we detected the body composition of patients with WD by bioelectrical impedance measurements. We find that there are some differences in body composition among healthy individuals, patients with H-subtype and N-subtype, especially in fat rate and muscle mass. Second, we determined the anthropometric data of patients with WD and find that the height, hip circumference, waist-to-hip ratio among patients with H-subtype WD are higher than patients with N-subtype WD. Third, we investigated the abnormal nutritional status among patients with WD, and find that 17.83% of the patients have a high nutritional risk. Finally, the ratio of large platelets, monoamine oxidase, creatinine, amylase and cholinesterase can reflect different nutritional statuses, as well as trunk body fat rate, appendicular skeletal muscle mass, mineral and basal metabolic rate.

Body Composition Factors Associated With Abnormal Body Composition in Patients With WD

While most previous studies have revealed that patients with chronic disease usually have a higher level of body fat and a lower level of muscle (11, 12, 15), we specifically explored the detailed characteristics of patients, to discover how it differs from other chronic diseases. Previous studies reported that patients with WD usually have a high body fat rate and a low muscle mass (16). However, these results cannot characterize the body composition of the different phenotypes of patients with WD comprehensively. In our study, we focus on the differences in body composition among healthy subjects, patients with H-subtype and N-subtype. At the same level of BMI, both trunk and appendicular body fat rates of patients with H-subtype were higher than healthy individuals. While patients with N-subtype only have a higher trunk body fat rate than healthy individuals, whereas there was no difference in appendicular body fat rate compared to healthy individuals. Additionally, the mass of muscle and skeletal muscle in both patients with H-subtype and N-subtype are lower than healthy individuals at the trunk part, while there are no differences at the appendicular section. Different distribution of skeletal muscle and body fat mass may be due to patients with N subtypes having neurological or movement disorders compared to patients with H subtypes.

Anthropometric Factors Related to Abnormal Nutritional Status in Patients With WD

Anthropometric measurements are usually applied in the absence of biochemical conditions and suitable bioelectrical impedance instruments (17–19). Among these parameters, skinfold thickness can reflect the overall level of the fat rate at the section of the trunk and limbs (subscapular skinfold thickness and triceps skinfold thickness, respectively) (20). Additionally, the waist-to-hip ratio reflects the degree of central obesity, which can reveal the mortality risk of subjects (21). Generally, individuals with a high WHR (male> 0.9, female> 0.85) are defined as having an "apple shape," which means tend to have more visceral fat than those who have a pear-shaped, hourglass figure with a lower WHR (22). Previous studies demonstrated that WHR is positively associated with the risk of insulin resistance, hypertension, hyperlipidemia, hypercholesterolemia and other cardiometabolic diseases (23–25). According to our study, we draw the following two inferences. First, no differences had been found in skinfold thickness between patients with H-subtype and N-subtype, which is inconsistent with the results of the previous body composition analysis. This means that anthropometric measurements are less stable and its results are not representative of the actual body composition of patients with WD. Second, most patients have a high WHR and patients with N-subtype are closer to an apple shape than patients with H-subtype. This means that although patients with N-subtype have a lower total fat percentage than B, it has more fat concentrated in the viscera. From nutritional status and comorbidity perspectives, our study suggests that patients with WD need a combined sports nutrition intervention project to control the visceral fat, which can attenuate their cardiovascular-related all-cause mortality and ameliorate their prognosis.

The Relationship Between Nutritional Status and Blood Biomarkers

NRS2002 has a stronger capability in adult patients compared with Malnutrition Universal Screening Tool (MUST) and Patient-generated Subjective Global Assessment (PG-SGA) (26). Thus, it is recommended by the ESPEN guidelines to be a conventional nutritional risk screening tool among patients with chronic diseases (13). In this study, we used NRS2002 to screen the prevalence of malnutrition in patients with WD, and we find that the prevalence of having a high nutritional risk is 17.83%. Besides, 25.58% of the patients are overweight, thus the overall prevalence of abnormal nutritional status among patients with WD is 43.14%. Additionally, patients with H-subtype and N-subtype have the same opportunity to suffer from high nutritional risk. Notably, we find that a high body fat rate is more likely to occur in female patients and patients with H-subtype. Former studies had proved that exercise and nutritional interventions can recover the abnormal nutritional status (27).

We also find that the ratio of large platelets, monoamine oxidase, creatinine, amylase, and cholinesterase have strong correlations with the nutritional status of patients with WD. The ratio of large platelets can enhance the risk of acutely ischemic brain stroke and atherosclerosis, which might be caused by the over-functional-active platelets releasing more active substances (28). According to our study and the above report, patients with WD and a high nutritional risk may have a high risk of atherosclerosis and acute vascular events. Monoamine oxidase is frequently regarded as a parameter to assess the hepatic injury and it may influence the food intake of patients with WD through appetite regulation (29). Hsu et al. (30) reported that higher serum creatinine concentrations were associated with a lower relative risk for malnutrition-related death. In our study, overweight patients and patients with a high nutritional risk have lower serum creatinine concentrations, which match the above study. Su et al. (31) reported that higher serum amylase constantly occurred among patients with atrophic gastritis and it had a correlation with malnutrition. As early as 1989, Ollenschläger et al. (32) recommended cholinesterase as an indicator for nutritional assessment, and they pointed out that the diagnosis of malnutrition can be made when cholinesterase is lower than the normal range or reduced by 10%. According to our research, patients with a high nutritional risk are more likely to have a lower serum cholinesterase concentration.

Limitations and Outlook

There are several limitations to this study. First, although NRS2002 is a well-validated screening tool for abnormal nutritional status, we found it may still not be enough to quantify the abnormal nutritional status for patients with WD. We will pay more attention to selecting or developing better nutrition screening tools specifically for patients with WD in the future. Second, although there are many conveniences testing body composition by BIA, like quick and comprehensive. However, the body composition values calculated by the data model are still less convincing and acceptable compared to Dual- energy X-ray absorptiometry, Computed Tomography and other equipment. Thirdly, this study is a cross-sectional observation focused on the abnormal nutritional status and its detailed characteristics of patients with WD. We have no idea about the correlations between the nutritional status and the prognosis. Longitudinal studies are required to investigate if nutritional status and body composition could reflect prognosis in WD patients, and which of these indexes of body composition and bloody parameters contribute to abnormal nutritional status and worse prognosis. Finally, the guidelines of exercise and nutrition intervention prescription for patients with WD are imperfect. We aim to develop the interventions of exercise and nutrition, and then explore the indications, contraindications, dosage, frequency, adverse reactions and other specific requirements, for earlier clinical application.

CONCLUSION

In conclusion, both patients with H-subtype and N-subtype are prone to have an abnormal nutritional status. However, it is different in the form of abnormal nutritional status between patients with these two subtypes. Patients with N-subtype have a bigger waist-to-hip ratio and a more "apple-shaped" body, suggesting that patients with N-subtype are

at higher risk of suffering from atherosclerosis and acute cardiovascular disease in the future. Among the blood test indicators, we also identified several markers that were associated with nutritional status, including the ratio of large platelets, monoamine oxidase, creatinine, amylase and cholinesterase. Nutritional status is not related to the severity and medication history. Longitudinal studies are required to investigate if nutritional status and body composition could reflect prognosis in WD patients, and which of these body composition indexes contribute to malnutrition and worse prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HG: study concept and design, data validation, statistical analyses, writing—original draft preparation, and revising final manuscript. SW: data validation and proofreading manuscript. YJ, BS, SS, BL, YongsH, YongzH, LG, ZD, and YX: data acquisition. NC: proofreading manuscript. XW: conception and design of the study. ZM: study design and proofreading manuscript. YS: study design, data, acquisition, writing—review and editing and refinement, and supervision. All authors contributed to the article and approved the submitted version.

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CONUT Score Predicts Early Morbidity After Liver Transplantation: A Collaborative Study

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Introduction: Liver transplantation (LT) is burdened by the risk of post-operative morbidity. Identifying patients at higher risk of developing complications can help allocate resources in the perioperative phase. Controlling Nutritional Status (CONUT) score, based on lymphocyte count, serum albumin, and cholesterol levels, has been applied to various surgical specialties, proving reliable in predicting complications and prognosis. Our study aims to investigate the role of the CONUT score in predicting the development of early complications (within 90 days) after LT.

Methods: This is a retrospective analysis of 209 patients with a calculable CONUT score within 2 months before LT. The ability of the CONUT score to predict severe complications, defined as a Comprehensive Complication Index (CCI) ≥42.1, was examined. Inverse Probability Treatment Weighting was used to balance the study population against potential confounders.

Results: Patients with a CCI ≥42.1 had higher CONUT score values (median: 7 vs. 5, *P*-value <0.0001). The CONUT score showed a good diagnostic ability regarding post-LT morbidity, with an AUC = 0.72 (95.0%CI = 0.64–0.79; *P*-value <0.0001). The CONUT score was the only independent risk factor identified for a complicated post-LT course, with an odds ratio = 1.39 (*P*-value <0.0001). The 90-day survival rate was 98.8% and 87.5% for patients with a CONUT score <8 and ≥8, respectively.

Conclusions: Pre-operative CONUT score is a helpful tool to identify patients at increased post-LT morbidity risk. Further refinements in the score composition, specific to the LT population, could be obtained with prospective studies.

Keywords: nutrition, immunology, post-operative morbidity, liver transplant complications, cholesterol, albumin, lymphocyte count

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INTRODUCTION

Liver transplantation (LT) is the cure for a growing number of patients with end-stage liver disease. Many patients who were once deemed too frail are now considered for LT (1). However, due to the necessity to fulfill the gap between offer and demand of liver grafts, increased utilization of extended-criteria donors has led to more risky donor-to-recipient matches (2). These challenging matches contribute to post-operative morbidity and poor long-term outcomes (3).

With the intent to identify frail patients with a greater post-LT risk of complications, sophisticated scores have been introduced focusing on graft function recovery and efficacious retransplantation (4, 5). Malnutrition and immunological status can influence treatment outcomes, with various studies weighing their impact after surgery (6–8). The Controlling Nutritional Status (CONUT) score has been developed to measure both aspects and has been trialed in different settings, including cancer surgery and oncologic treatments (9–12). The CONUT score has been tested with the intent to predict overall survival and hepatocellular cancer (HCC) recurrence after LT and post-operative complications in pancreatic, esophageal, gastrointestinal, and orthopedic surgery (13–16).

However, the ability of the CONUT score to predict post-LT early morbidity and mortality has not been investigated yet. The primary aim of the study was to investigate the role of the CONUT score calculated before LT in predicting the development of severe post-transplant complications as graded by the Comprehensive Complication Index (CCI). The secondary aim was to investigate the role of the pre-LT CONUT score in predicting post-operative mortality within 90 days post-LT.

MATERIALS AND METHODS

Study Design

This is a retrospective bicentric observational study investigating the data of patients undergoing LT.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed to create the study.

Setting

The participant centers were Sapienza University of Rome, Umberto I Polyclinic of Rome, and Catholic Rome University, Gemelli Hospital.

Population

A total of 209 cases transplanted at Sapienza Rome University (period January 2013–December 2020) and Catholic Rome University (period September 2016–December 2020) were considered for the analysis. The only inclusion criterion was the availability of enough data for calculating the CONUT, and the CCI scores were enrolled for the study.

All the study subjects were adult ($\geq$18 years) patients receiving a graft from a deceased-brain donor, including split grafts and retransplants.

Outcomes

The primary outcome of the study was the development of a complex post-operative course defined as a CCI $\geq$42.1. The secondary outcome was the post-LT 90-day mortality. The last follow-up date was May 31st, 2021.

Data Collection

Data were retrospectively obtained from the prospectively collected charts of the patients. The guarantor of the data quality was the Data Manager of the Study Group (QL). Data errors and missingness were identified across the database and solved, when possible, with specific queries.

Definitions

The CONUT score was calculated according to the original descriptions (9–12). The CONUT score is based on serum albumin, cholesterol, and total lymphocyte count. (12) CONUT score ranges from 0 (i.e., normal nutritional status) to 12 (i.e., severe malnutrition) (**Table 1**). The CONUT score was calculated using the last available data from blood tests of patients on the LT waitlist. We arbitrarily decided to select an upper limit of 2 months before LT for calculating the score: all the patients with data older than 2 months before the transplant were excluded from the study.

The CCI is a recently proposed classification for evaluating post-operative complications. This score is more sophisticated respect to the more commonly used Dindo-Clavien classification system (17). The CCI carries the advantage of capturing the burden of the entire morbidity rather than grading only the most severe complication (18). Dindo-Clavien grade I corresponds to 8.7, grade II to 20.9, grade IIIa to 26.2, grade IIIb to 33.7, grade IVa to 42.4, grade IVb to 46.2, and grade V to 100. In the liver transplantation setting, CCI has shown a good prediction ability for 90-day and 1-year graft loss risk (19). The CCI ranges from 0 (i.e., absence of post-operative complications) to 100 (i.e., death) (12, 18). A web-calculator was used for estimating CCI (available at https://www.assessurgery.com). The CCI was calculated using the following original algorithm: $CCI = [\sqrt{(wC1 + wC2 \ldots + wCx)}]/2$.

All the complications collected were summed, even if the same patient received several times multiple administrations of the same medical (i.e., blood transfusion) or interventional (i.e., various radiological or surgical approaches) treatment. In the present study, the entire population was categorized into two groups according to the presence of a low ($<$42.1) or high ($\geq$42.1) CCI value. The CCI threshold value of 42.1 was set according to previously published studies (20). The CCI value was calculated at the time of discharge after LT.

Statistical Analysis

Continuous variables were reported as medians and interquartile ranges (IQR). Categorical variables were reported as numbers and percentages. Mann-Whitney U test and Fisher's exact test were used to compare continuous and categorical variables, respectively.

Missing data relative to study covariates always involved $<$10% of patients. In all the cases, missing data were handled with a single imputation method. In detail, a median of nearby-points imputation was adopted. The median instead of the mean was adopted due to the skewed distribution of the managed variables (21).

With the intent to compensate for the non-randomized design of this retrospective study, the population was "balanced" using Inverse Probability Treatment Weighting (IPTW). With the intent to perform the comparison between low and high CCI groups, twelve potential confounders were included in the model: patient age, patient male sex, HCC, hepatitis C virus (HCV)

TABLE 1 | Controlling nutritional status score calculation.

Variables	Undernutrition status			
	Normal	Light	Moderate	Severe
Albumin (g/dL)	≥3.5	3.0–3.49	2.5–2.9	< 2.5
Points	0	2	4	6
Total lymphocyte count (/mm^3)	>1,600	1,200–1,599	800–1,199	<800
Points	0	1	2	3
Total cholesterol (mg/dL)	>180	140–180	100–139	<100
Points	0	1	2	3
Total CONUT score	0–1	2–4	5–8	9–12

positive status, acute liver failure, waiting list duration, MELDNa, donor age, donor male sex, cold ischemia time (CIT), piggy-back caval reconstruction, cava replacement with veno-venous bypass (VVB).

With the intent to reduce the artificial increase of the sample size, and, therefore, of the type I error rate (namely, the increased number of false positives) caused by the inflated sample size in the pseudo data, we used stabilized weights (SW) according to the formula:

$$\text{SW} = \text{p}/\text{PS} \text{ for the study group;}$$
$$\text{SW} = (1-\text{p})/(1-\text{PS}) \text{ for the control group}$$

where p is the probability of etiology without considering covariates and PS is the propensity score.

Because p-values can be biased from population size, results from the comparisons between covariates subgroups were reported as effect size (D value): values $<|0.1|$ indicated very small differences between means, values between $|0.1|$ and $|0.3|$ indicated small differences, values between $|0.3|$ and $|0.5|$ indicated moderate differences, and values $>|0.5|$ indicated considerable differences (22).

A multivariable logistic regression model was developed in the post-IPTW population for the risk of CCI ≥ 42.1. Odds ratios (ORs) and 95% confidence intervals (95% CI) were reported. A backward conditional method was used for identifying the risk factors for high CCI.

The accuracy of the CONUT score was assessed for the risk of CCI ≥ 42.1 through the Harrel's c statistic. The area under the curve (AUC) and 95% CIs were reported. The model accuracy was compared with five other variables: MELDNa, MELD, D-MELD, waiting time duration, and CIT. Separate AUC of ROC curves were calculated and analyzed for comparing the single components of the CONUT score (albumin, lymphocyte, and cholesterol).

Ninety-day patient death rates were evaluated using the Kaplan-Meier method, and the log-rank test was adopted to compare the obtained survivals.

Variables with a $P < 0.05$ were considered statistically significant. We used the SPSS statistical package version 27.0 (SPSS Inc, Chicago, IL, USA) for the statistical analyses.

TABLE 2 | Comparison between the Low- (<42.1) and the High-CCI (≥42.1) Group.

Variables	CCI <42.1 (n = 151)	CCI ≥42.1 (n = 58)	P
	Median (IQR) or n (%)		
Recipient			
Age, years	58 (51–63)	57 (47–63)	0.26
Male sex	128 (84.8)	54 (93.1)	0.17
Height, cm	170 (165–177)	175 (169–177)	0.13
Weight, kg	76 (65–87)	80 (68–89)	0.52
BMI	26 (23–29)	26 (23–29)	0.75
Waiting time duration, months	4 (1–10)	3 (0–7)	0.10
HCC	85 (56.3)	22 (37.9)	0.02
Underlying liver disease*			
HCV	50 (33.1)	11 (19.0)	0.06
HBV	29 (19.2)	10 (17.2)	0.84
Alcohol	59 (39.1)	21 (36.2)	0.75
NASH	32 (21.2)	15 (25.9)	0.47
Biliary cirrhosis	7 (4.6)	4 (6.9)	0.50
ALF	4 (2.6)	5 (8.6)	0.12
Other	20 (13.2)	7 (12.1)	1.00
T2DM	42 (27.8)	18 (31.0)	0.73
Requiring insulin	26 (17.2)	11 (19.0)	0.84
Arterial hypertension	32 (21.2)	9 (15.5)	0.44
CONUT	5 (3–7)	7 (5–9)	<0.0001
Albumin (g/L)	36 (31–40)	31 (26–35)	<0.0001
Total cholesterol (mg/dL)	129 (91–159)	100 (71–137)	0.001
Lymphocyte count*10^9/L	1.03 (0.71–1.41)	0.86 (0.62–1.29)	0.09
MELD	16 (10–23)	21 (15–30)	0.001
MELDNa	18 (11–25)	23 (17–29)	0.001
D-MELD	853 (517–1,288)	1,128 (723–1,601)	0.002
Donor			
Age, years	58 (45–71)	63 (46–74)	0.42
Male sex	77 (51.0)	23 (39.7)	0.17
Height, cm	167 (160–175)	165 (160–171)	0.30
Weight, kg	72 (65–85)	71 (62–78)	0.37
BMI	26 (23–28)	26 (24–28)	0.95
Cause of death			
CVA	105 (69.5)	39 (67.2)	0.74
Blunt trauma	36 (23.8)	18 (31.0)	0.30
Anoxia	8 (5.3)	1 (1.7)	0.45
Other	2 (1.3)	0 (–)	1.00
T2DM	17 (11.3)	9 (15.5)	0.48
Requiring insulin	5 (3.3)	2 (3.4)	1.00
Arterial hypertension	64 (42.4)	26 (44.8)	0.76
Transplant			
CIT, minutes	420 (370–450)	450 (420–518)	<0.0001
Piggy-back caval reconstruction	116 (76.8)	29 (50.0)	<0.0001
Temporary portocaval shunt	28 (18.5)	13 (22.4)	0.56
Cava replacement with VVB	6 (4.0)	14 (24.1)	<0.0001

**In some cases, more liver diseases were present contemporaneously.*

CCI, comprehensive complication index; IQR, interquartile ranges; n, number; BMI, body mass index; HCC, hepatocellular cancer; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non-alcoholic steato-hepatitis; ALF, acute liver failure; T2DM, type 2 diabetes mellitus; CONUT, Controlling Nutritional Status; MELD, model for end-stage liver disease; Na, sodium; D-MELD, donor-MELD; CVA, cerebrovascular accident; CIT, cold ischemia time; VVB, veno-venous bypass.

RESULTS

Two hundred and nine patients were included in the study population. The median follow-up period after LT was 37 months (IQR = 17–57). During the follow-up, 32/209 (15.3%) patients died, of whom 13 (6.2%) within 90 days from LT. In all the early deaths, the cause was a liver-specific condition (i.e., technical problems in six patients and graft failure in seven). In the late deaths, 13 patients died for liver-specific conditions (biliary complications, vascular complications, liver disease recurrence, acute rejection), while six died due to non-liver-specific conditions.

Patient characteristics are reported in **Table 2**. Several differences were reported between the two groups. Patients with a high-CCI value less commonly had HCC (37.9 vs. 56.3%; $P = 0.02$) and presented a median higher MELD value (21 vs. 16; $P < 0.0001$).

In all the cases, the median values of the CONUT score variables were lower in the high-CCI patients. In detail, median albumin value was 31 vs. 36 g/L ($P < 0.0001$), median total cholesterol was 100 vs. 129 mg/dL ($P = 0.001$), and median lymphocyte count was 0.86 vs. $1.03^{*}10^{9}$/L ($P = 0.09$). Consequently, the CONUT score value was significantly superior in the high-CCI group (median: 7 vs. 5; $P < 0.0001$) (**Figure 1**).

No statistical differences were observed concerning the donor characteristics. As for the transplant surgical procedure, the CIT was longer in the high-CCI group (450 vs. 420 min; $P < 0.0001$). Piggy-back caval anastomosis was observed less commonly in the high-CCI group (50.0 vs. 76.8%; $P < 0.0001$), with higher usage of cava replacement with veno-venous bypass (24.1 vs. 4.0; $P < 0.0001$).

A linear correlation was reported between the CCI and the CONUT values, suggesting a potential connection between these two variables. In detail, a statistical significance was observed ($P < 0.0001$), although the adjusted R squared value showed low values (10.5%) (**Figure 2**).

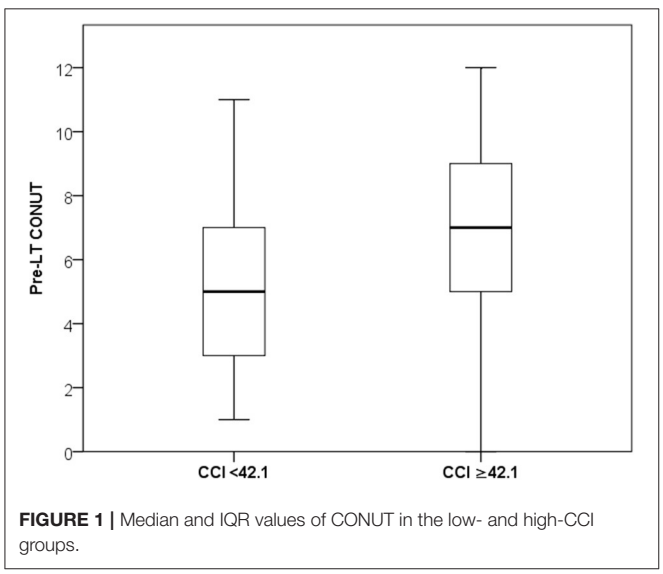

FIGURE 1 | Median and IQR values of CONUT in the low- and high-CCI groups.

To eliminate potential confounders, the two groups were "balanced" for twelve variables emerging as significantly different between the groups. A stabilized IPTW allowed to reduce the initial differences. As reported in **Table 3**, also variables initially showing relevant differences of the means such as MELDNa (*D*-value 0.51), CIT (*D*-value 0.63), and piggy-back caval reconstruction (*D*-value −0.55), all showed small or very small differences after the IPTW. Thanks to the use of a stabilized approach, the sample size of the pseudo population did not significantly differ with respect to the initial unbalanced population (212 vs. 209 cases).

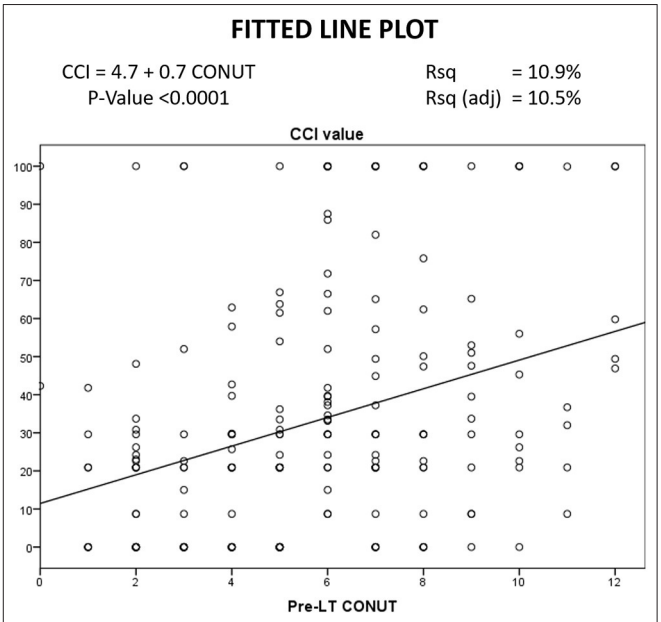

FIGURE 2 | Linear correlation between CONUT and CCI.

TABLE 3 | Effect of IPTW on the variables used for balancing the two groups.

	Cohen's *D*-value	
	Pre-IPTW	**Post-IPTW**
Recipient age	−0.19	−0.07
Recipient male sex	0.29	−0.19
Waiting time duration, months	−0.12	0.06
HCC	−0.37	−0.10
HCV	−0.34	−0.02
ALF	0.23	−0.02
MELDNa	0.51	0.13
Donor age	0.09	−0.08
Donor male sex	−0.23	−0.24
CIT	0.63	−0.15
Piggy-back caval reconstruction	−0.55	−0.05
Cava replacement with VVB	0.09	−0.02

IPTW, inverse probability therapy weighting; HCC, hepatocellular cancer; HCV, hepatitis C virus; ALF, acute liver failure; MELDNa, model for end-stage liver disease sodium; CIT, cold ischemia time; VVB, veno-venous bypass.

The risk factors for a CCI ≥42.1 were investigated in the post-IPTW population using multivariable logistic regression. Twelve different potential risk factors were initially introduced in the mathematical model. Using a backward Wald method, only the CONUT score before LT was an independent risk factor for high CCI. In detail, CONUT showed an OR = 1.39 (95%CI = 1.21–1.58; $P < 0.0001$). In other terms, each increase of one point of CONUT score increased the risk of high CCI by 39% (**Table 4**). All the other patient-, donor-, and transplant-related variables failed to have a relevant role as risk factors for high CCI.

At c-statistics analysis in the pseudo population, the CONUT score was the unique tested variable showing diagnostic ability, with an AUC = 0.72 (95%CI = 0.64-0.79; $P < 0.0001$). All the other potential diagnostic tools measured at the time of LT (i.e., MELD, MELDNa, D-MELD) failed to predict a high CCI, as reported in **Table 5** and **Figure 3**. All of the single variables of the CONUT score showed significant AUC in terms of prognostic ability for the risk of CCI ≥42, in particular albumin AUC was superimposable to CONUT score AUC (data not shown).

When the post-IPTW population was split according to the CONUT value, the 90-day, 1-year, and 5-year patient death rates were 1.2, 4.2, and 9.1%, respectively, when the CONUT value was <8. On the opposite, when a CONUT score ≥8 was observed, the 90-day, 1-year, and 5-year patient death rates increased to 12.5, 14.3, and 27.0%, with a statistically significant difference between the two subgroups (log-rank $P = 0.02$) (**Figure 4A**).

Similar results were observed when only the liver-specific death rates were reported (**Figure 4B**). In detail, the 90-day, 1-year, and 5-year liver-specific death rates were 1.2, 4.2, and 6.6%, respectively, with CONUT scores <8, whilst they increased to 12.5, 14.3, and 20.4% respectively with CONUT scores ≥8. Also in this case, a statistically significant difference between the two subgroups was reported (log-rank $P = 0.09$).

DISCUSSION

Malnutrition and immunologic compromise increase the risk of post-LT complications, particularly after "extended-criteria donor to frail recipient" matches (23, 24). The possibility to pre-operatively predict this potential risk is pivotal for optimizing resource allocation and preserving LT outcomes.

Our study demonstrated the efficacy of the CONUT score in predicting severe post-LT complications, with an OR = 1.39. Moreover, patients with high (≥8) pre-transplant CONUT values showed poor post-operative 90-day as well as long-term patient survival rates. In particular, we encountered a higher rate of liver-specific deaths at all the time points analyzed, highlighting the role of the pre-transplant condition of the recipient.

Our findings align with previous studies exploring the predictive role of the CONUT score in the setting of hepatic (10, 25–27), thoracic, urological, and gastrointestinal oncological surgery (28–32). A study from China ($N = 94$) showed that pre-operative CONUT was the best predictor of overall and recurrence-free survivals in patients resected for hilar cholangiocarcinoma (10).

A multicenter study from Japan ($N = 2461$) similarly showed that the pre-operative CONUT score was predictive of worse overall and recurrence-free survivals in patients resected for HCC, even after propensity score matching analysis (27).

A study from Japan ($N = 204$) suggested that the CONUT score was a strong independent predictor of survival among stage II/III colorectal cancer patients (28).

As for the setting of LT, only a limited number of studies have been published (6, 33).

A study from Italy ($N = 280$) explored the specific impact of CONUT in the LT population with HCC. Of relevance, this study failed to observe any correlation between the CONUT score and post-LT poor survival or tumor recurrence (6). A potential explanation for these results could derive from the super-selection of the explored population. In fact, in LT, HCC patients represent a well-known selected population with a more compensated liver condition and, therefore, a predictable narrower spread of CONUT values.

Another study from the same authors ($N = 324$) investigated the post-LT trend of CONUT in HCC patients, reporting worse values in the early post-LT period than the pre-LT values and a substantial improvement after the post-LT third month (33).

TABLE 4 | Multivariable logistic regression model for the risk of CCI ≥42.1.

Variable	Beta	SE	Wald	OR	95%CI		P
					Lower	Upper	
Pre-LT CONUT	0.33	0.07	23.29	1.39	1.21	1.58	<0.0001
Donor male sex	−0.54	0.34	2.42	0.59	0.30	1.15	0.12
WT duration in months	0.03	0.02	2.31	1.03	0.99	1.07	0.13
Donor age	−0.004	0.01	0.22	0.996	0.98	1.01	0.64
Recipient age	0.003	0.02	0.04	1.003	0.97	1.04	0.84
Recipient male sex	−0.06	0.44	0.02	0.94	0.40	2.21	0.89
Constant	−2.89	1.24	5.43	0.06	–	–	0.02

Variables initially introduced into the model: recipient age, recipient male sex, HCC, HCV, ALF, WT duration, CONUT, donor age, donor male sex, MELDNa, CIT, Piggy-back caval reconstruction.

SE, standard error; OR, odds ratio; CI, confidence intervals; CONUT, Controlling Nutritional Status; WT, waiting time; HCC, hepatocellular cancer; HCV, hepatitis C virus; ALF, acute liver failure; MELDNa, model for end-stage liver disease sodium; CIT, cold ischemia time.

TABLE 5 | C-statistics for the evaluation of CONUT performance for the diagnosis of CCI ≥42.1.

Variables	AUC	SE	95%CI		P
			Lower	Upper	
CONUT	0.72	0.04	0.64	0.79	<0.0001
MELD	0.58	0.04	0.50	0.66	0.06
MELDNa	0.57	0.04	0.49	0.65	0.09
D-MELD	0.53	0.04	0.45	0.62	0.44
WT duration	0.53	0.04	0.44	0.61	0.51
CIT	0.48	0.04	0.39	0.57	0.65

AUC, area under the curve; SE, standard error; CI, confidence intervals; CONUT, Controlling Nutritional Status; MELD, model for end-stage liver disease; Na, sodium; D-MELD, donor-MELD; WT, waiting time; CIT, cold ischemia time.

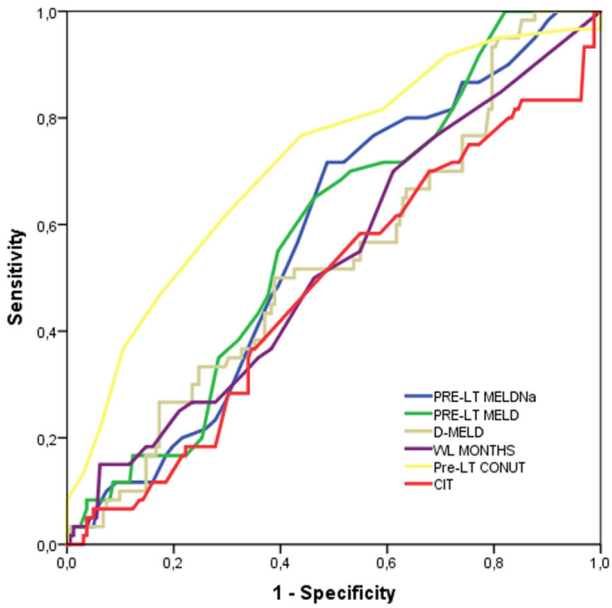

FIGURE 3 | Harrel's c-statistics for the diagnosis of CCI ≥42.1.

Concerning the previously published studies exploring the role of the CONUT score in LT, our study presents some beneficial aspects.

As an example, our analysis was performed on HCC patients and patients with an acute or a severe chronic end-stage liver disease (ESLD). As well known, ESLD causes a reduction in the biosynthetic activity of the liver, translating into lower levels of circulating proteins such as albumin and apolipoproteins. Consequently, the CONUT score reflects the actual liver functional reserve, being particularly useful in the specific setting of patients with more advanced liver disease (34). As a confirmation of this datum, we observed higher CONUT values and lower median levels of cholesterol and albumin in the high CCI group, namely the group comprising more advanced ESLD cases. When comparing the single components of the CONUT score, albumin showed higher predictive ability compared to cholesterol and lymphocyte count, in regards of post-LT morbidity. Similar findings were obtained in the field of thoracic oncologic surgery, with albumin and CONUT having nearly superimposable AUC values, superior to both cholesterol and lymphocyte count AUCs (35).

Another critical aspect to underline is the statistical approach we adopted with the intent to minimize confounding phenomena. Several potential confounders have been identified to bias our results when we compared the two groups with low or high CCI. For example, patients with a lower CCI were more likely to have HCC and a lower median MELD score (i.e., less severe liver disease). Conversely, patients with a higher CCI presented a longer CIT, potentially caused by the increased complexity and longer duration of the hepatectomy (i.e., more complex surgery due to severe liver disease). Thanks to the use of a stabilized IPTW, we were able to "balance" our population for these potential confounders, therefore eliminating the potential bias caused by their effect.

Interestingly, no statistical difference was detected concerning the donor characteristics even before using the IPTW, further emphasizing the prominent role of the initial ESLD severity in determining post-LT complications. However, no firm assumptions can be drawn in these regards due to the abovementioned limitations.

Another relevant aspect of the present study was that the diagnostic performance of the CONUT score in predicting severe post-LT complications was compared for the first time with other commonly used diagnostic tools for organ allocation and donor-recipient match, namely MELD, MELDNa, and D-MELD score. Interestingly, the CONUT score had the best performance as a pre-operative diagnostic tool for predicting a poor post-LT course. The availability of a tool to predict complications is highly desirable and is a topic of central interest in the transplant community, as the complexity of LT procedures continues to increase and more malnourished and immunocompromised patients are evaluated. Similarly to other fields of application, our results confirm the advantages of the CONUT score as a cheap, user-friendly, and pre-operatively available score based on routine blood tests.

Moreover, the pre-transplant CONUT values should consent to target high-risk patients, offering interventions that tackle frailty and sarcopenia before LT (e.g., using nutritional supplementation, immunomodulation, exercise) (36, 37).

As an example, a recent study from Italy reported that an "urgency" model combining MELDNa and sarcopenia should be used to prioritize the sarcopenic patients with an initial MELDNa <20 on the list, further underlying the relevance of the nutritional status in the LT candidates and the scarce ability of the MELD system in capturing the actual complexity of these patients (38).

The use of rehabilitation programs based on multidisciplinary "training" to enhance physical strength and nutritional status has been proven to increase the physiologic reserve before surgery and withstand complications after transplant (39).

The importance of these considerations is even more critical in light of the evolving epidemiology of LT candidates due to the increased prevalence of non-alcoholic steatohepatitis (NASH). A recent study investigating the relationship between frailty and

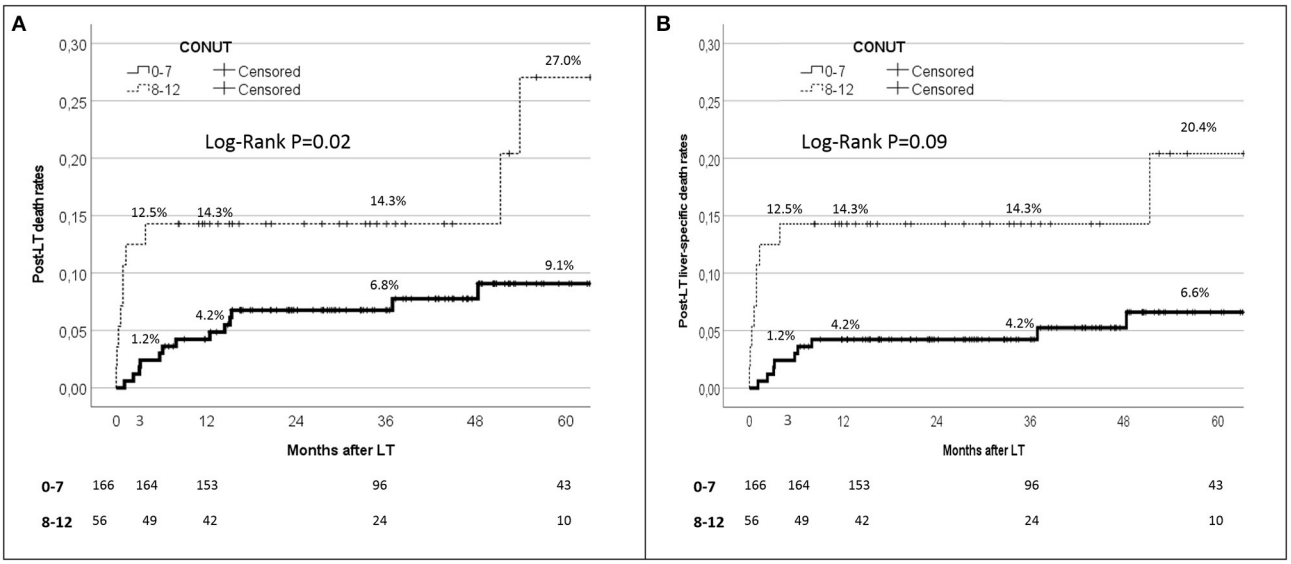

FIGURE 4 | Kaplan-Maier analysis for the 90-day patient survival rates in patient initially with CONUT 0–7 vs 8–12.

cirrhosis etiology revealed that NASH patients were the frailest category of LT candidates, justifying particular attention to the liver functional reserve and malnourishment and immunologic impairment when a patient is transplanted (40).

The CONUT score should play an important role also in the evaluation of the post-LT course, due to the modification of its value in the months after the transplant (33). In this setting, immunosuppression might play a relevant role, mainly impacting on some of the variables composing the CONUT score (e.g., mTOR inhibitors and cholesterol). Further studies are required for the validation of post-transplant CONUT score as a prognosticator of long-term outcomes.

Our study has some limitations. Since this was a retrospective study, the time-point of data collection before LT was heterogeneous. To minimize this heterogeneity, we decided to consider only the blood tests available two months before the transplant. Such a decision impacted the global number of patients we were able to enroll for the study. Many patients transplanted during the study period were not included in the analysis because of outdated tests. The main problem was connected with the cholesterol test, which was not routinely repeated during the LT waitlist. However, despite the consequent sample size reduction, we thought it was a more severe bias to use CONUT calculations based on outdated blood tests (for example, at the time of waiting list inscription), therefore losing the ability of the score to capture the actual nutritional status of the patients at the time of transplantation.

Another already reported limiting factor relates to the number of patients included. Considering our sample size, we were aware that a selection bias could jeopardize the quality of the results of our study. To mitigate such risk, we chose the stabilized IPTW, which allowed us to minimize the effect of potential confounders.

A potential limit to report is that some albumin levels could be partly increased by the intravenous supplementation administered to decompensated ESLD patients. Such practice has been become routine since the supplementation of intravenous human albumin solution was demonstrated to titrate the higher level of prostaglandin PGE2 that is responsible for the macrophage impairment in patients with acutely decompensated cirrhosis (41).

However, only a limited number of patients (<10%) in our series present such a condition. Moreover, other commonly used scores carry similar problems (i.e., MELD and plasma infusion). Thus, we considered this limitation unresolvable in the clinical practice and only marginally impacting on the observed results.

Lastly, due to the lack of sufficient data, we could not investigate the importance of decreasing HDL levels in relation to total cholesterol. It has been observed that HDL levels tend to drop proportionally with the evolution of the severity of the ESLD (42). Further studies are needed to investigate if HDL cholesterol levels might further refine the CONUT score in predicting post-LT outcomes.

In conclusion, our study shows a correlation between the CONUT score and the development of severe complications and 90-day as well as long-term mortality after liver transplantation. The CONUT score proved to be a reliable and easy-to-calculate tool that could be integrated in clinical practice with affordable extra costs. Prospective studies are required to corroborate the present findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GS and QL: conception and design. SA and MR: administrative support. SA, MR, and AWA: provision of study materials or patients. FF, GB, AM, and QL: collection and assembly of data. QL, AWA, and GS: data analysis and interpretation. All authors: manuscript writing and final approval of manuscript.

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Genetic and Life Style Risk Factors for Recurrent Non-alcoholic Fatty Liver Disease Following Liver Transplantation

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Recurrent or *de novo* non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) following liver transplantation (LT) is a frequent event being increasingly recognized over the last decade, but the influence of recurrent NASH on graft and patient outcomes is not yet established. Taking into consideration the long term survival of liver transplanted patients and long term complications with associated morbidity and mortality, it is important to define and minimize risk factors for recurrent NAFLD/NASH. Metabolic syndrome, obesity, dyslipidemia, diabetes mellitus are life style risk factors that can be potentially modified by various interventions and thus, decrease the risk of recurrent NAFLD/NASH. On the other hand, genetic factors like recipient and/or donor PNPLA3, TM6SF2, GCKR, MBOAT7 or ADIPOQ gene polymorphisms proved to be risk factors for recurrent NASH. Personalized interventions to influence the different metabolic disorders occurring after LT in order to minimize the risks, as well as genetic screening of donors and recipients should be performed pre-LT in order to achieve diagnosis and treatment as early as possible.

Keywords: genetic, liver, NAFLD, NASH, liver transplant

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition in Western Europe and USA, but has also an increasing trend in Southern and Eastern European countries and Asia as stated by the HEPAHEALTH Project (1). It is now the most frequent chronic liver disease worldwide (25% of all adults) and represents a major global public health challenge (2) and a cause of significant morbidity and mortality. NAFLD is a liver disease comprising different variants (3) from steatosis (non-alcoholic fatty liver, NAFL), in which plethoric hepatic fat is shown, and non-alcoholic steatohepatitis (NASH), a necroinflammatory form of the disorder manifested by histological inflammation and hepatocyte ballooning that conducts to severe liver fibrosis with end stage liver disease (>20% in NASH patients) and hepatocellular carcinoma requiring liver transplantation (LT). NAFLD/NASH can be present in the patient awaiting LT, but also in the donors because of increased risk of cardiovascular events, the major cause of death in people with NAFLD (4).

Transplant candidates with NASH commonly have certain metabolic comorbidities supplementary to the complexity of managing the complications of chronic liver disease. Obesity escalates the risk of decompensation while on the waiting list and can represent a surgical technical challenge (5). Sarcopenic obesity is multifactorial, affects up to 35% of patients awaiting LT and is associated with increased morbidity and mortality compared to either disease alone, as well as worse survival after LT (6, 7). The overall prevalence of NAFLD and NASH among patients with type 2 diabetes mellitus (T2DM) is 55.5% and, respectively, 37.3% (8), thus T2DM being another factor implicated in prognosis of patients with NASH related cirrhosis awaiting LT and following LT.

Recurrent or *de novo* NAFLD/NASH following LT is a frequent event being increasingly recognized over the past decade (9). The influence of recurrent NASH on graft and patient outcomes is not yet clearly stated. Several data suggest that it does not impact graft and patient survival (10–12), but there is a large variation in the diagnostic modalities, protocol liver biopsies or non-invasive evaluation of fibrosis and follow-up intervals. However, there are publications analyzing the factors that influence the occurrence of NAFLD/NASH after LT and demonstrate the association with adverse post-LT outcomes related to liver and non-liver related events (13–17).

FREQUENCY, PROGRESSION AND SIGNIFICANCE OF POST-TRANSPLANT NAFLD

Recurrent and *de novo* NASH are increasingly being reported in post-LT NASH recipients, and quick diagnosis through non-invasive serological or imaging tests, followed by liver biopsy if needed, will help early intervention to avoid progression of NASH, and its related complications in the post-transplant period.

According to previous studies (14, 17, 18) there are variable prevalence of *de novo* NAFLD or recurrent NAFLD/NASH with different outcomes after LT. Recurrent NAFLD appears to be an earlier, more severe and with negative patient and graft outcomes. The recurrence of NAFLD has been reported to occur in 8.2–62.5% of recipients over variable follow-up periods ranging from <6 months to 10 years, and the rates for steatohepatitis have ranged from 4 to 33% over follow-up periods ranging from 6 weeks to 20 years. The rates of advanced fibrosis have ranged from 0 to 33% (short-term 6–12 months) or even 71.4% at 5 years after LT (14, 19, 20). One study even showed that almost 90% of patients developed recurrent NAFLD, but only 25% of them had advanced fibrosis following LT (21).

Taking into consideration the long term survival of liver transplanted patients and long term complications with associated morbidity and mortality, it is important to define and minimize risk factors for recurrent NAFLD/NASH.

LIFE STYLE RISK FACTORS FOR RECURRENT NAFLD AFTER LT AND POTENTIAL INTERVENTIONS

Metabolic syndrome (MS) has been described in 43–58% of LT recipients. Obesity, dyslipidaemia, diabetes or insulin resistance, as well as certain immunosuppressive agents after LT are frequent predictors of recurrence of NAFLD after transplantation (22).

Obesity

Obesity is encountered in more than one-third of all transplant recipients. Majority of the weight gain occurs during the first 1–3 years (23, 24), but persists to increase over the following years with an enlargement in abdominal girth and body fat content and corresponding low lean body mass. Obesity at 1-year post-transplantation shows a 2-fold increased mortality risk. Interventions to preclude the earliest weight gain might be more promising than later weight-loss endeavors.

Post-LT obesity management should comprise the same algorithm as in other obese persons: diet and exercise, pharmacologic therapy and surgical or endoscopic bariatric procedures. Weight loss is associated with improvement of recurrent NASH and better long term outcome. Weight loss medication can be used in this patient population, but the choice of medication should be individualized.

Orlistat, acting by directly stopping absorption of ∼30% of dietary triglycerides, was evaluated in the post-LT setting and proved to be safe (25), but there are no data regarding its efficacy. Orlistat should be given at least 3 h before or after calcineurin inhibitors and levels should be monitored closely. There are no recorded interactions with antimetabolites or mammalian target of rapamycin (mTOR) inhibitors (26).

Liraglutide, a long-acting glucagon-like peptide-1 (GLP-1), appears to have no interactions with the immunosuppressive therapies and to have also cardio-protective effects in patients with known atherosclerotic disease or heart failure, making it an interesting option in these high risk patients. Following LT, liraglutide can be chosen in patients with diabetes mellitus, end stage renal disease or multiple drugs for different comorbidities, as well as in the early post-LT period to help avoid weight gain and possibly result in modest weight loss (27). Marked weight loss in patients with type 2 diabetes has also been noted in studies of semaglutide, a longer-acting GLP-1 analog, but there are no studies in LT recipients (28, 29).

Phentermine-topiramate suggests having the highest weight loss influence, by directly creating blockade of absorption of ∼30% of dietary triglycerides. However, possible side effects are mentioned such as neuropsychiatric disorders, cardiovascular comorbidities, and drug-drug interactions that could limit their use. There are no known interactions with posttransplant immunosuppressants, but there are no data on the use of phentermine-topiramate following post-solid organ transplant setting (30).

Naltrexon-bupropion was authorized as a weight loss drug in 2014, leading also to improvement of fasting blood glucose and dyslipidemia. There are no data specific to the

benefit of naltrexone-bupropion in the post-transplant setting, but there is no established interaction with post-transplant immunosuppressive medication. However, bupropion is a strong CYP2D6 inhibitor and can elevate the serum concentration of many drugs (26).

There is no specific immunosuppression strategy that has been shown to be useful in preventing weight gain after LT; however, immunosuppression should be tailored to diminish to minimum the risk of metabolic complications (30).

Bariatric surgery is also possible, may be safe and feasible after LT for weight loss, but may be more technically demanding, and is linked with elevated morbidity when compared with non-LT patients (17, 31). However, bariatric surgery should be taken into consideration for treatment of recurrent NAFLD because it ameliorates steatosis and steatohepatitis in most of the patients and improves or resolves liver fibrosis in 30% of patients (32).

Dyslipidemia occurs in 30–60% of LT recipients, being a major risk factor for allograft steatosis and posttransplant cardiovascular-related morbidity and mortality, and often continues despite dietary changes. A fasting lipid profile should be done every year in all LT recipients. mTOR inhibitors produce a stronger dyslipidemic effect compared to calcineurin inhibitors. Sirolimus proved to worsen hyperlipidemia in a dose-dependent manner (33).

Hypertriglyceridemia is the most common dyslipidemic change. Life-style changes should be realized when the low-density lipoprotein cholesterol (LDL-C) level is >100 mg/dL, although dietary modification alone is often inadequate, making pharmacotherapy necessary (24). Different circulating lipid components have varying effects. While circulating triglyceride (TG) levels are associated with the development of hepatic steatosis due to the imbalance between TG synthesis and breakdown process in hepatocytes, LDL-C is closely related to cardiovascular complications. Both lipid components should be addressed to reach different aspects of metabolic syndrome. The therapeutic goal for LDL-C should be below 100 mg/dL (even <70 mg/dL) in order to decrease the high cardiovascular risk in NASH patients after LT. Cholesterol is also a major lipotoxic molecule in NASH development. The gut microbiome represents an environmental factor contributing to the development of NAFLD and there are studies suggesting that dietary cholesterol caused advanced fibrosis by cholesterol-induced gut microbiota changes and metabolomic alterations (34). Thus, cholesterol inhibition and manipulation of the gut microbiota and its related metabolites might represent effective strategies in preventing NAFLD, but no studies are yet in the LT recipients.

Similar to non-transplant patients, statins are the drug of choice being usually well-accepted. Low doses of statins at beginning with slight increase as required and close follow-up should be taken into consideration. Pravastatin and fluvastatin are not metabolized by the CYP3A4 isoenzyme and should be first choice in post-LT recipients. Ezetimibe that acts through inhibition of enterohepatic recirculation of lipids, proved to effectively treat hypercholesterolemia with few side effects and to have no interaction with immunosuppressive agents. However, both pravastatin and fluvastatin are of low potency. Thus, combination therapy using ezetimibe will often be required to reach LDL-C targets. Alternatively, rosuvastatin is a substantially more potent option and is also not metabolized by cytochrome P450 (CYP) 3A4 (35).

Fibrates can be also safely used in patients with high triglyceridemia levels over 600 mg/dL, but caution is required when co-administrated with statins due to high risk of myotoxicity and renal dysfunction. For patients with hypertriglyceridemia, fish oil can be used with minimal side effects except potential increase of low-density lipoprotein level (18, 30).

Diabetes Mellitus

One-third of LT recipients have type 2 diabetes mellitus (post-transplant diabetes mellitus, PTDM), requiring long-term therapy and follow-up. There is lot of evidence that people with T2DM are at high risk of developing NASH, but also that NAFLD may precede and/or develop T2DM, hypertension and atherosclerosis (36). This complex link between NAFLD and T2DM can be extrapolated to post-LT recipients. Treatment of NAFLD/NASH patients could avoid T2DM occurrence and/or progression, but, also the other way around.

Recipients with PTDM are handled just the same as patients with type 2 diabetes mellitus in the general population and the aim is to normalize target values and re-establish metabolic control. Dietary and lifestyle modification are of great importance, but are usually unsatisfactory in this population, with most patients requiring pharmacological therapy with oral agents or insulin.

Metformin and thiazolidinediones, influencing insulin resistance, proved to have benefit on biochemical and metabolic features of NAFLD, but amelioration of patients' histological response or fibrosis was modest and studies were usually short-term, thus liver-related long-term outcomes could not be evaluated (37).

Sodium–glucose cotransporter 2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1RAs) are now accepted as the best therapeutic option for patients with T2DM and cardiovascular disease, heart failure and/or chronic kidney disease. These two types of drugs determine weight loss, making them an attractive option for patients with associated obesity, and offer promising effects in reducing liver fat content (37, 38). However, there are no clinical studies performed in post-LT NAFLD/NASH patients with these two drug classes although this therapeutic approach would be completely justified.

Bariatric surgery has recently proved to be one of the most effective therapeutic options for T2DM through weight-dependent and weight-independent mechanisms (39). Factors associated with diabetes remission consists of duration of diabetes prior to surgery <4 years, higher C-peptide, younger age and use of oral agents or diet to control diabetes (40).

Due to the increased prevalence of NAFLD worldwide, along with a reduced organ pool donation in many countries, usage of donor grafts with steatosis is now rather common. Donor graft steatosis is also a significant risk factor for post-LT recurrence of NASH (41). Defatting strategies, like pharmacological agents (e.g., forskolin, peroxisome proliferator-activated receptor (PPAR) -alpha ligand, hypericin, scoparone,

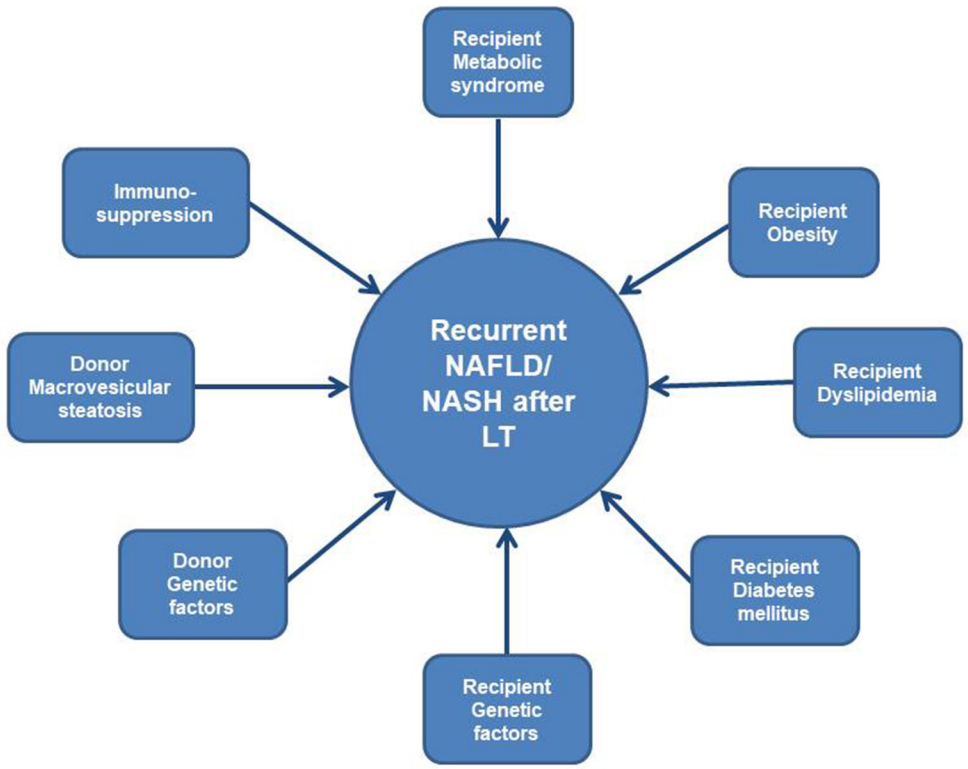

FIGURE 1 | Modifiable and genetic risk factors for recurrent NAFLD/NASH after LT.

PPAR-delta ligand, visfatin, L-carnitine) and hypothermic or normothermic machine perfusion have been shown to decrease hepatocyte steatosis (42). To achieve significant defatting, the protocol of choice should shift the balance toward more efficient TG breakdown (lipolysis) and excretion of related byproducts, as well as minimizing TG synthesis. There is still much research to be done on how best to modulate this lipid metabolism using cocktails of agents or *ex vivo* machine perfusions in order to achieve rapid defatting without adversely affecting viability and other critical liver functions. Short term survival and functionality of steatotic livers for which TG content has been dramatically reduced is already proven (43), however long term prevention of post-LT complications is not yet established.

GENETIC RISK FACTORS FOR RECURRENT NAFLD AFTER LT

There are few studies mentioning genetic influences on NAFLD recurrence post-LT. The range of recurrent NAFLD is wide and causes for this interindividual variability may be at least partially associated to differences in genetic background of both recipient and donor.

Finkenstedt et al. (44) showed that recipient patatin-like phospholipase domain containing 3 (PNPLA3) rs738409 was correlated with graft steatosis according to the 5-year post-LT computed tomography imaging. Kim et al. (45) found that the presence of the rs738409-G risk allele in both donor and recipient was an important risk factor for 1 year post-LT histologically proven NAFLD. Other data by Trunecka et al. (46) proved donor PNPLA3 rs738409 is a powerful risk factor of graft steatosis based on histologic findings on liver biopsy. The actual insight into the role of the p.I148M mutated PNPLA3 protein in liver fat turnover should favor the hypothesis that donor, but not recipient PNPLA3 genotype is critical for fat aggregation in the liver graft (47).

The donor TM6SF2 (transmembrane 6 superfamily member 2) c.499A allele is an independent risk factor of liver graft steatosis following LT in addition to the effects of donor PNPLA3 c.444G allele (48). The TM6SF2 p.E167K (c.499G>A) variant is important in patients with NAFLD, being associated with more severe steatosis, necroinflammation and advanced fibrosis/cirrhosis. Variants in the genes encoding glucokinase regulator (GCKR) and membrane bound O-acyl transferase 7 (MBOAT7) also contribute to the risk of NAFLD, by increasing *de novo* lipogenesis and altering the remodeling of phospholipid.

The study by John et al. (49) newly indicated that recipient adiponectin (ADIPOQ) rs1501299 and rs17300539 polymorphisms are associated with *de novo* NAFLD among patients transplanted for hepatitis C. *De novo* diabetes mellitus, as risk factor for post-LT NAFLD was associated with the following SNPs: recipient angiotensinogen (AGT) rs699; recipient mTOR rs2295080 (only following everolimus use); recipient ADIPOQ rs1501299 and rs822396; donor and recipient small ubiquitin like modifier 4 (SUMO4) rs237025 (50).

Our group recently demonstrated that the allele 1993C of the SNP rs4794067 of gene TBX21 (T-box transcription factor 21), but not CYP3A5*3 genotype may predispose to the development of late significant fibrosis and severe steatosis of the liver graft (51). The functional polymorphism TBX21-1993T/C (rs4794067) increases the transcriptional activity of the TBX21 gene (essential for Th1 polarization) resulting in a preponderance of a Th-2 or Th17 response.

Whenever genetic screening of recipients and donors identifies high risk genotypes for NASH, it is of paramount importance to control the modifiable risk factors and to intensify screening after LT for early detection of NAFLD/NASH.

Screening for genetic risk factors before and after LT is very complex and interrelated (**Figure 1**). Multiple recipient and donor genetic factors are implicated in occurrence of all variants of NAFLD such as: risk factors for insulin resistance, for steatosis, for obesity and dyslipidemia, for metabolisation of immunosuppression, for gut microbiota, thus use of this data in clinical practice is still under investigation and constitutes one of the limitations of this review.

DISCUSSION

NASH remains the fastest growing indication for LT worldwide and recurrent NAFLD is common. There remains a need for long-term studies in this patient population to specifically address approach to diagnosis of recurrent NASH, preventive measures, treatment and implications.

Patients with histologically established posttransplant NASH have elevated risk of poor outcome as one third of them die within 5 years of the diagnosis and 26% develop a cardiovascular event. Almost one third of patients with recurrent NASH may develop bridging fibrosis/cirrhosis at 5 years after LT (17, 18).

Transient elastography (TE) is an ideal, non-invasive and accessible method for diagnosing the stage of hepatic fibrosis post-LT in both viral [hepatitis C virus (HCV) vs. non-HCV] patients (52, 53). Our group proved that LT recipients can very well be evaluated for steatosis and fibrosis by TE with CAP (controlled attenuation parameter) (54). Screening of NASH via TE and CAP should notify the clinicians and patients to this additional comorbidity and the greater possibility for complications related to insulin resistance. Patients who are at high risk of developing MS after LT should receive personalized interventions in order to minimize the risks, and should undergo routine surveillance in order to achieve an earlier diagnosis and treatment. The influence of immunosuppression on the development of MS and NAFLD after LT was extensively discussed in other papers (55, 56) and will not be in the focus of this review. Weight loss through diet, lifestyle modifications, pharmacological agents or bariatric surgery is linked with resolution of NASH and improvement in liver fibrosis, and should be implemented in overweight LT recipients, with an objective of 7–10% decrease in body weight (30). An early diagnosis of MS will restraint associated comorbidities, thereby reducing the risk of cardiovascular events. Strength of our review consists in establishing patients at risk of recurrence of NAFLD through genotypic and phenotypic characterization at transplant that will help to interfere by targeted strategies to prevent recurrence of NAFLD/NASH.

AUTHOR CONTRIBUTIONS

SI, SB, and LG: conceptualization. SI and RI: writing-original draft. CG, VC, SB, and IP: writing-review and editing. IP, SB, and LG: visualization. All authors contributed to the article and approved the submitted version.

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REVIEW
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Impact of Sarcopenia on the Severity of the Liver Damage in Patients With Non-alcoholic Fatty Liver Disease

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An extensive body of the literature shows a strong interrelationship between the pathogenic pathways of non-alcoholic fatty liver disease (NAFLD) and sarcopenia through the muscle-liver-adipose tissue axis. NAFLD is one of the leading causes of chronic liver diseases (CLD) affecting more than one-quarter of the general population worldwide. The disease severity spectrum ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis, and its complications: end-stage chronic liver disease and hepatocellular carcinoma. Sarcopenia, defined as a progressive loss of the skeletal muscle mass, reduces physical performances, is associated with metabolic dysfunction and, possibly, has a causative role in NAFLD pathogenesis. Muscle mass is a key determinant of the whole-body insulin-mediated glucose metabolism and impacts fatty liver oxidation and energy homeostasis. These mechanisms drive the accumulation of ectopic fat both in the liver (steatosis, fatty liver) and in the muscle (myosteatosis). Myosteatosis rather than the muscle mass *per se*, seems to be closely associated with the severity of the liver injury. Sarcopenic obesity is a recently described entity which associates both sarcopenia and obesity and may trigger worse clinical outcomes including hepatic fibrosis progression and musculoskeletal disabilities. Furthermore, the muscle-liver-adipose tissue axis has a pivotal role in changes of the body composition, resulting in a distinct clinical phenotype that enables the identification of the "sarcopenic NAFLD phenotype." This review aims to bring some light into the complex relationship between sarcopenia and NAFLD and critically discuss the key mechanisms linking NAFLD to sarcopenia, as well as some of the clinical consequences associated with the coexistence of these two entities: the impact of body composition phenotypes on muscle morphology, the concept of sarcopenic obesity, the relationship between sarcopenia and the severity of the liver damage and finally, the future directions and the existing gaps in the knowledge.

Keywords: fatty liver, NAFLD, sarcopenia, obesity, muscle-liver axis, myosteatosis, inflammation, sarcopenic obesity

INTRODUCTION

The aging population and the global epidemics of obesity and type 2 diabetes (T2DM) resulted in increased prevalence of several chronic conditions, like non-alcoholic fatty liver disease (NAFLD) and sarcopenia. NAFLD is now recognized as one of the leading cause of chronic liver disease afflicting more than 25.0% of the general population worldwide (1). Sarcopenia, derived from the greek—"*sarcos*" (flesh) and "*penia*" (loss), has been first described in 1989 (2) and is characterized by age-related loss of muscle mass and functional impairment. Since then, numerous studies, definitions and diagnosis criteria have been suggested, leading in 2010 to the development of the first diagnostic consensus of sarcopenia by the European Working Group on Sarcopenia in Older People (EWGSOP) (3). Initially described in older adults (more than 50 millions adults affected in 2010), both sarcopenia and frailty—defined as "decreasing physiologic reserve and increased vulnerability to health stressors" (4)—are now recognized as chronic progressive conditions associated with increased risk of various comorbidities such as chronic liver disease and cardiometabolic disorders, in particular obesity and T2DM, and cancer (5).

Depending on the definitions used, the assessment methods and the study population, sarcopenia is a prevalent condition in patients with cirrhosis (40.0–70.0%) (6, 7) obesity (6.0–43.0%) (8, 9) and NAFLD/non-alcoholic steatohepatitis (NASH) (20.0%) (10). It is likely that NAFLD and sarcopenia share common physiological pathways and are interconnected through the muscle-liver-adipose tissue axis. Skeletal muscle plays a major role in glucose transport and disposal, fatty liver oxidation and energy homeostasis which are all key determinants in the pathophysiology of NAFLD. Ectopic lipid deposition in the skeletal muscle related to increased energy intake causes peripheral insulin resistance (IR) and usually occurs before the onset of NAFLD but is also a common feature of sarcopenia (11). The "metabolic inflexibility" secondary to IR and the crosstalk between the target organs involved (skeletal muscle, liver and adipose tissue) are major determinants in the physiopathology and progression of both conditions (12, 13).

Several studies suggest that sarcopenia is a disease modifier across the spectrum of NAFLD (14). However, the risk of poor clinical outcomes increases when both conditions are associated and therefore is difficult to establish a cause-effect relationship beyond and above the overlap of the common physiopathological pathways. The main topics addressed here are: (i) key mechanisms linking NAFLD and sarcopenia - from IR and low-grade inflammation to myosteatosis and impact of clinical phenotypes on muscle morphology; (ii) clinical evidence linking sarcopenia and NAFLD across the severity spectrum of the liver damage and clinical outcomes; (iii) the concept of sarcopenic obesity (SO); (iv) finally, we will address the future directions and the existing gaps in the knowledge.

COMMON MECHANISMS LINKING SARCOPENIA AND NAFLD

Extensive studies carried out in recent years showed a strong interrelationship between the pathogenic pathways of non-alcoholic fatty liver disease (NAFLD) and sarcopenia through the muscle-liver-adipose tissue axis. The potential interactions between the adipose tissue and the skeletal muscle may play an essential role in the physiopathology and the natural history of NAFLD. The interplay between muscle and liver is influenced by several factors; among them, insulin resistance (IR), obesity, chronic low-grade inflammation, and several hepatokines and myokines have a significant impact on both entities. Other key factors that may significantly impact the muscle-liver crosstalk are vitamin D deficiency, unhealthy/diet composition, oxidative stress, aging, physical inactivity, and several hormonal changes [growth hormone (GH), insulin-like growth factor 1 (IGF-1), testosterone and osteocalcin] (5, 15). Based on the existing data, the association between sarcopenia and NAFLD seems to be independent of IR or obesity (10, 16, 17). **Figure 1** summarizes the key cellular and molecular mechanisms involved in the complex interplay between adipose tissue, sarcopenia, and NAFLD. The dysregulation of the physiological relationship between the skeletal muscle and the liver is bidirectional and potentially plays a role in the progression of NAFLD. However, whether NAFLD directly contributes to sarcopenia or vice versa is still of debate. Further studies are required to specifically focus on the possible mechanisms linking sarcopenia and NAFLD (18).

Insulin Resistance

At the Skeletal Muscle Level

Given that sarcopenia is a multifactorial condition (8, 19), characterized by generalized and progressive loss of skeletal muscle mass (SMM), reduced physical performance and strength (3), it may be exacerbated by the association with some comorbidities that have common pathophysiological backgrounds. Moreover, a condition known as sarcopenic obesity (SO) proves that the detrimental effects of sarcopenia are magnified when associated with obesity. As such, IR is considered not only the core common pathological mechanism responsible for developing sarcopenia, but also a pivotal mechanism in the development and progression of NAFLD/NASH (20, 21).

In addition, the skeletal muscle is the major participant in the whole-body insulin-mediated glucose homeostasis and has the highest requirement of postprandial glucose in an insulin-dependent manner. In the settings of normal skeletal muscle physiology, insulin stimulates glycogen synthesis in the liver and muscle. It also binds to the transmembrane insulin receptor and activates the Protein Kinase C (PKC) pathway, supporting the translocation of the glucose membrane transporter-4 (GLUT4) and thus facilitating glycogen synthesis. Moreover, insulin plays a major role in protein metabolism, promotes the transport of amino acids (AA) in tissues, improves protein synthesis and the inhibition of proteolysis (22, 23), mainly via the p38 mitogen-activated protein kinase (MAPK) and

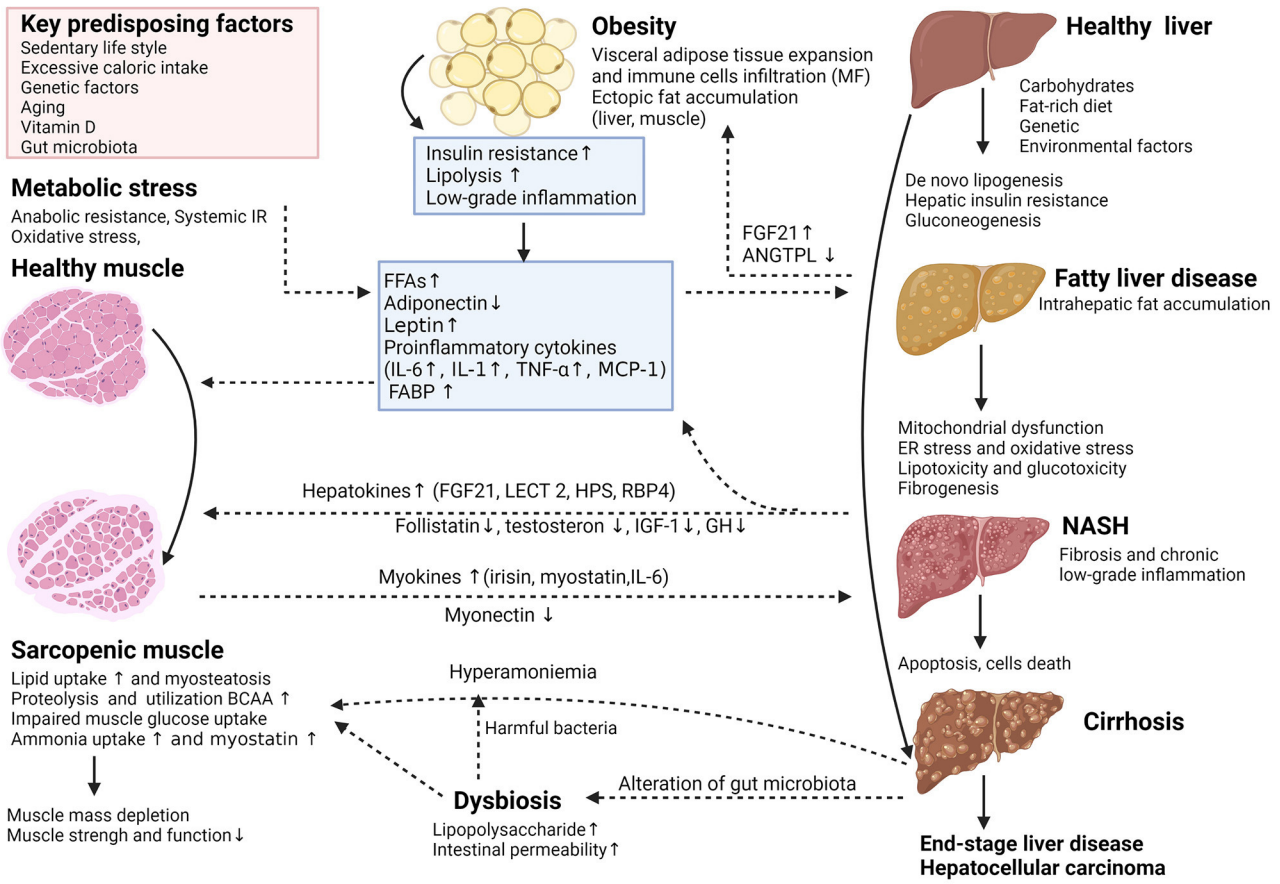

FIGURE 1 | (Created with BioRender.com). Key cellular and molecular mechanisms involved in the complex interplay between adipose tissue, sarcopenia, and the nonalcoholic fatty liver disease (NAFLD). The potential interaction between adipose tissue and skeletal muscle plays an essential role in the pathophysiological and natural course of NAFLD. Adipose tissue dysfunction is characterized by inflammation and adipokine disturbances, subsequent ectopic fat deposition and insulin resistance (IR). In insulin-resistant subjects, insulin fails to promote glycogen synthesis and favors adipose tissue lipolysis, redirecting substrate to "*de novo*" lipogenesis and accelerates proteolysis. These deregulations trigger further insults in hepatocytes through increased inflammation, lipotoxicity, mitochondrial dysfunction, oxidative and endoplasmic reticulum stress and anabolic resistance, which can all contribute to the progression of NAFLD. The release of multidirectional molecular signals comprising myokines and hepatokines regulates a range of systemic metabolic processes including skeletal muscle and hepatic IR, escalating dysfunction of the adipose-muscle-liver axis. Other mechanisms like dysbiosis related to changes in gut microbiota may have additional detrimental effects. Thus, dysregulation of the complex physiological relationship between skeletal muscle and the liver is reciprocally unfavorable and supports each other in a vicious circle, potentially playing a causative role in NAFLD incidence or progression. ANGPTL4, angiopoietin-like 4; BCAA, branched-chain amino acids; ER, endoplasmic reticulum; FABP, fatty acid-binding protein; FFA, free fatty acid; FGF21, fibroblast growth factor 21; GH, growth hormone; HPS, hepassocin; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; LECT2, leukocyte cell-derived chemotaxin-2; MCP-1, monocyte chemoattractant protein 1; MF, macrophages; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RBP4, retinolbinding protein 4; TNF-α, tumor necrosis factor-alpha.

the mammalian target of rapamycin p70S6 kinase (mTOR/p70S6) pathways (24–26).

Skeletal muscle is significantly involved in the energy metabolism. In a healthy subject, fasting periods are characterized by a decrease in insulin levels and by the use of fatty acids (FA) as the preferred fuel substrate (27). After a meal, insulin levels rise, favoring cellular glucose uptake and shifting the fuel source from FA to glucose. *Metabolic flexibility* represents this capacity of recognizing the appropriate fuel substrate and periodically shifts between FA (during fasting) and glucose (postprandial) in order to produce energy (28, 29). The absence of appropriate periodic shifts in glucose and fat oxidation in accordance with the energy state results in *metabolic inflexibility* (30). In these settings, insulin pulsatile secretion is impaired and leads to hyperinsulinemia and insulin resistance (30, 31). IR can develop before NAFLD, as a result of excess adiposity, or may occur subsequent to NAFLD, due to hepatic lipid infiltration leading to impaired glucose and triglycerides metabolism (32). Myosteatosis is a consequence of excessive circulating FA and IR. Muscle cell lipid infiltration is associated with reduced protein synthesis (33, 34).

The skeletal muscle protein synthesis depends on the proper stimulation by anabolic hormones (insulin, GH, IGF-1, sex hormones), AA availability and muscle contractibility

(mechanical stimulation) (35, 36). Protein catabolism develops in the context of energy, AA or hormonal deficiencies, physical inactivity, and inflammatory processes (35, 37). The main causes of anabolic resistance include older age, obesity, IR reduced AA availability and systemic inflammation. Very important, in hepatic disorders (NAFLD, NASH, cirrhosis), the decline in the muscle mass is often caused by IR. This leads to anabolic resistance, the loss of protein synthesis in the skeletal muscle (particularly structural muscle proteins, including myosin) and reduced insulin-mediated suppression of protein catabolism which exacerbate proteolysis and lipolysis (38–40). As IR promotes a decrease in SMM, it also worsens sarcopenia and further favors the increased fat accumulation (myosteatosis) (41, 42). As such, the diminished additional SMM reduces insulin-mediated glucose disposal and contributes to IR, promoting gluconeogenesis which, in turn, may exacerbate proteolysis and muscle depletion, resulting in a vicious cycle (43–45). Several studies evaluated the regulatory action in protein synthesis of leucine and arginine. Leucine is involved in protein turnover through protein synthesis stimulation, proteolysis inhibition, and activation of the mammalian target of rapamycin complex 1 (mTORC1) (46, 47). Arginine is an AA used as a substrate for nitric oxide synthesis, which increases the muscle blood flow and further promotes glucose uptake, fatty acid oxidation and lipolysis (48). Several interventional studies showed that AA supplementation may ameliorate sarcopenia by improving the lean body mass (LBM) and the Liver Frailty Index (5, 49). Moreover, anabolic resistance may also be alleviated with exercise (50).

Skeletal muscle lipid levels (or intramyocellular lipids/IMCL) develop particularly when the inflow of free fatty acids (FFAs) exceeds the oxidative capacity of the skeletal muscle. It is now accepted that IMCL accumulation is negatively correlated with insulin sensitivity, suggesting the involvement of IMCL in the development of lipid-induced IR, probably due to a disbalance in the skeletal myocellular lipid influx, lipid metabolism, and oxidative capacity (51). Based on available evidence, the accumulation of bioactive DAG species, at specific intracellular sites, rather than the total levels of IMCL, could have had a role in lipid-induced IR by activating the serine phosphorylation of insulin receptor substrates-1 (IRS-1), and interfering with phosphoinositide 3-kinase (PI3K) activation (24, 52). The accumulation of lipid intermediates causes lipotoxicity by promoting reactive oxygen species (ROS) production and endoplasmic reticulum (ER) stress, which, in turn, lead to impaired mitochondrial dysfunction (53) and create a vicious cycle of lipotoxicity (54). More recent data suggest that intramyocellular lipid dynamics [decreased triglyceride (TAG) synthesis or increased TAG lipolysis, and reductions in oxidative capacity] may represent essential causes in regulating lipid-induced IR (51). Moreover, intermyocellular adipose tissue (IMAT) and IMCL secrete myostatin, C-C motif chemokine ligand 2 (CCL2), tumor necrosis factor-alpha (TNF-α), interleukin-1 alpha (IL-1α), and interleukin-6 (IL-6), thus inducing IR and lipotoxicity (55).

At the Hepatic Level

IR plays an important role in NAFLD pathogenesis. IR triggers adipokine-induced liver damage through increased inflammation, oxidative and ER stress, mitochondrial dysfunction, anabolic resistance, and increased deposition of ectopic fat. All of the above mentioned mechanisms are responsible for the progression of NAFLD (56, 57) and account for the overlap between the muscle and the liver damage.

The hepatic fat content is the strongest predictor of IR both in the skeletal muscle and in adipose tissue. In addition, hepatic steatosis and IR are correlated independently of adiposity, which indicates that liver fat, rather than body fat in general, is responsible for this association (58–61). Consequently, DAGs levels are strong predictors of hepatic IR (62, 63) and trigger IR through the activation of the PKC pathway.

The presence of hepatic steatosis is an independent predictor for incident T2DM (64–66). As already shown (67), adipose tissue IR was correlated with the severity of muscle and liver insulin sensitivity, as well as with hepatic steatosis. Moreover, their presence worsens with the progression of glucose intolerance, which may aggravate lipotoxicity, explaining the higher difference between adipose tissue IR, presented by individuals with T2DM and with or without NAFLD. As higher muscle mass is related to greater insulin sensitivity and lower risk of prediabetes, sarcopenia may be an early predictor of diabetes susceptibility, independently of obesity (68). Thus, sarcopenic patients had higher amounts of total body fat mass (FM), more components of metabolic syndrome (MetS), HOMA-IR index, and higher C-reactive protein (CPR) levels, compared to those without sarcopenia (10). Furthermore, a negative relationship was found between HOMA-IR, liver attenuation index and skeletal muscle mass index (SMI), in opposition to a positive correlation between HOMA-IR and CPR, suggesting a potential inflammatory role of the muscle-liver axis.

Consequently, the current therapeutic focus is to reduce the low-grade inflammation through lifestyle changes and physical activity, by promoting visceral fat and fatty liver reduction in association with greater energy expenditure and increased SMM (69). Given the limited expandability of the adipose tissue, excessive lipids accumulation occurs in other compartments such as the liver and skeletal muscle. Thus, during aerobic exercise, the stored lipids are used as a fuel source (70), which can lead to decreased levels of myostatin (15). Myostatin has been suggested as a diagnostic biomarker to predict obesity-associated comorbidities, due to its increased concentration in skeletal muscle and its significant correlation with IR severity (71).

The Role of Adipose Tissue in Sarcopenia and NAFLD

Adipose tissue may interfere with the liver and muscle through the secretion of various adipokines, such as leptin and adiponectin. Hyperleptinemia is positively correlated with fat mass (FM) (72) and may facilitate IR, liver inflammation and fibrosis (73). It has been shown that circulating leptin levels were higher in patients with NAFLD than in normal subjects, and were positively correlated with the severity of the liver damage

(74). Increased leptin levels are found in sarcopenic patients despite their low body FM. The appendicular skeletal muscle mass (ASM) was independently and negatively correlated with leptin even after adjusting for body FM (75).

Adiponectin, another major adipokine, is a protein exclusively secreted from the adipose tissue and negatively correlates with fat accumulation. Adiponectin promotes insulin sensitivity by facilitating glucose uptake in the skeletal muscle and adipose tissue and increases fatty acid oxidation (76, 77). Accordingly, it is likely that adiponectin triggers the preferential use of FFA as a fuel in the skeletal muscle (76–78). Additionally, adiponectin exerts an anti-inflammatory effect (79) and has a hepatoprotective role in liver inflammation and cell injury (80–82).

To sum up, the complex interplay between the adipose tissue, the skeletal muscle and the liver is leading to the development of NAFLD and its progression to NASH, but also to the loss of the skeletal muscle mass and worsening sarcopenia.

Chronic Low-Grade Inflammation

Both NAFLD and obesity are now recognized as subclinical inflammatory conditions (83, 84). Obesity enlarged adipose tissue secretes adipokines and proinflammatory cytokines which facilitate infiltration of macrophages (MF) and other inflammatory cells. MF are the major source of inflammatory mediators and the importance of macrophage-mediated inflammation is recognized as a cause of IR (84, 85). Thus, MF change the phenotype to M1 and liberate pro-inflammatory factors such as TNF-α, interleukin-1 beta (IL-1β), IL-6, and CCL2 (5, 24, 86, 87) resulting in toxic effects on myocytes and ultimately sarcopenia. It seems that these cytokines induce muscle atrophy by favoring apoptosis and upregulating proteasomal decay of filament proteins (5, 24, 76). IL-6 and TNF-α are able to inhibit the anabolic hormone IGF-1 activity and to induce IR, leading to a catabolic state and reduced myogenesis (76, 88). TNF-α might also induce apoptosis in muscle cells (89). In addition, IL-6 may be secreted by the skeletal muscle during the physical exercise and after its outflow in the blood stream increases liver gluconeogenesis and adipose tissue lipolysis (76, 90). These data were supported by a study showing that CRP and IL-6 were positively correlated with body mass index (BMI) and FM and were inversely correlated with fat-adjusted ASM (91). However, no significant associations were found between CRP and IL-6 levels and obesity or sarcopenia showing that the role of inflammatory cytokines in the development of SO is poorly understood. Similarly, NAFLD is accompanied by hepatic inflammation and IR (92). MF and Kupffer cells contribute to the overall inflammatory environment of the liver and are thought to contribute to decreased hepatic insulin sensitivity by secreting pro-inflammatory molecules that activate pathways involved in insulin signaling. Inflammatory cytokines favor "*de novo*" lipogenesis (92, 93) and increased intra hepatocyte levels of ceramide which may decrease insulin signaling (92, 94) by inhibiting the activation of AKT/PKB (protein kinase B).

Thus, the development of chronic inflammation and oxidative stress induced by the multidirectional molecular signals of cytokines secreted in an excessive manner (80, 81) could aggravate the dysregulation of the muscle-liver axis, resulting in loss of the muscle mass (14) but also playing a causative role in NAFLD progression (5).

Other Key Factors

Nonetheless, muscle-liver crosstalk is influenced by numerous other factors, such as vitamin D deficiency, hormonal changes (GH; IGF-1; testosterone levels), low physical activity, aging, and diet composition (5, 15).

Hepatokines

Hepatokines are liver-secreted proteins that signal through autocrine, paracrine and endocrine signaling to impact hepatic and non-hepatic metabolic processes. The hepatocyte protein secretome undergoes marked changes in response to liver steatosis (95). The most studied hepatokines are: hepassocin (HPS), adropin, angiopoietin-like protein 4 (ANGPTL4), sex hormone-binding globulin (SHBG), fetuin-A and -B, retinolbinding protein 4 (RBP4), selenoprotein P, fibroblast growth factor 21 (FGF21), leukocyte cell-derived chemotaxin 2 (LECT2). HPS or hepatocyte-derived fibrinogen-related protein can mediate IR in both liver and skeletal muscle and increases hepatic lipid accumulation and NAFLD activity scores (96). Furthermore, increased HPS levels in hepatocytes induce IR in the skeletal muscle through the epidermal growth factor receptor/ c-Jun N-terminal kinases (JNK) pathway (97). Increased Fetuin-A in steatosis stimulates pro-inflammatory cytokine production from adipocytes and MF but also may cause IR (98, 99). Administration of FGF21 in mice improves hepatic and peripheral insulin sensitivity (100), suppresses lipolysis in adipose tissue (101), and reduces triglyceride and DAG levels in liver and skeletal muscle (102, 103). However, these beneficial effects on obesity, T2DM, and fatty liver disease seem to contrast with the high levels of FGF21 identified in these disorders, being currently difficult to specify whether this reflects a resistance to FGF21 or it is a compensatory reaction to basal metabolic stress (95).

LECT2 positively correlates with obesity and severity of liver steatosis and mediates skeletal muscle IR and hepatic IR (103–105). RBP4 is secreted by hepatocytes and adipose tissue and is increased in steatosis. RBP overexpression is related to inflammation and IR in mice and humans and has been validated as a biomarker for a series of metabolic diseases, including T2DM and obesity (106, 107). Selenoproteins P induces peripheral and hepatic IR and is considered a biomarker for a range of disorders including NAFLD, obesity, T2DM, and cardiovascular disease (CVD) (108). Withal, the liver-specific deletion of the gene encoding selenoprotein P enhances insulin signaling in muscle and liver and improves whole-body glucose tolerance (109). Positive metabolic actions are registered for adropin, a hepatokine that improves insulin sensitivity, hepatic steatosis and reduces adiposity. Low levels of adropin are linked to whole-body adiposity, hepatic steatosis, IR, and CVD (110–112). ANGPTL4 is generally produced in hepatic and adipose tissue and is decreased in NAFLD. ANGPTL stimulates adipose tissue lipolysis, increases plasma levels of lipids and may cause liver steatosis (113, 114).

Altogether, hepatokines impact hepatic and non-hepatic metabolic disorders and are important drivers of metabolic processes, especially of the IR, directly influencing the pathophysiology of the different components of the muscle-liver axis.

Myokines

Myostatin is a myokine known to play a crucial role in the negative regulation of muscle mass. Increased levels of myostatin promote protein catabolism, inhibit growth of skeletal muscle and associate with obesity and IR (15). It has been shown that deletion of myostatin in mice increases muscle mass and reduces adiposity, increases insulin sensitivity and glucose uptake and protects from hepatic steatosis (115–117). Myostatin has both local and endocrine effects that can link sarcopenia and NAFLD via a complex process involving several cellular signaling pathways, resulting in the downregulation of the expression of myogenic factors, the decrease in protein synthesis, and the activation of proteasome–ubiquitin ligases (5, 115). Follistatin is a specific inhibitor that binds myostatin and inhibits its activity by preventing its attachment to the receptor. It has been recently shown that myostatin may be a key molecular mediator of muscle-liver crosstalk. Myostatin modulates the biologic properties of human stellate cell (HSC) in a profibrogenic fashion via activation of JNK and might be a novel muscle-to liver pathway implicated in the pathogenesis of hepatic fibrosis in NAFLD (118).

Other myokines that might link sarcopenia and NAFLD are irisin and myonectin. Irisin improves glucose metabolism, increases adipocyte energy expenditure, modulates the expression of enzymes that inhibit lipid accumulation and reduces weight and has a positive effect on hepatic steatosis (24, 119, 120).

Myonectin promotes fatty acid uptake and links skeletal muscle to lipid homeostasis in the liver and the adipose tissue in response to alterations in the energy state, revealing a novel myonectin-mediated metabolic circuit (121).

Vitamin D

Recent studies suggest that vitamin D deficiency or its impaired signaling are involved in metabolic disorders, related to both muscle and the liver (5, 122). It has been shown that vitamin D nuclear receptors (VDRs) are present in human skeletal muscle. Also, the vitamin D signaling by VDRs is involved in myogenesis, myoblast proliferation, and differentiation and in the skeletal muscle growth. Vitamin D levels are considerably lower in individuals with sarcopenia, independently of the presence of obesity (17, 123). Supplementation in vitamin D increases the expression of VDRs in skeletal muscle and improves sarcopenia (124), reinforcing the link between the two entities. Large meta-analyses suggested that daily vitamin D supplementation was beneficial for muscle strength, gait and balance (125) decreasing the risk of falling, especially in those with a baseline vitamin D level of <25 nmol/L (126). On the other hand, recent studies (127) suggest that vitamin D deficiency is independently associated with the severity of the injury in NAFLD. Altogether, these data strengthen the hypothesis that vitamin D is a key mediator in the nexus of NAFLD and sarcopenia and may be a potential promising therapeutic target.

Physical Activity and Unhealthy Diet (Lifestyle Changes)

Reduced physical activity correlates independently with sarcopenia in patients with NAFLD (128). The physical exercises required to improve sarcopenia in NAFLD patients should aim to enhance muscle strength, muscle mass, and physical performance. A meta-analysis evaluating the effects of the physical exercise on sarcopenia in patients with NAFLD revealed that endurance and combined (endurance and resistance) exercises improved physical function but have no effect on the muscle mass (129). Endurance exercises increase oxygen consumption, mitochondrial synthesis and skeletal muscle capillaries, improving the cardiovascular system and energy levels (130, 131). Still, the best type of training that improves all three parameters of sarcopenia needs to be defined.

An unbalanced diet, rich in lipids, fructose or sucrose, plays an important role in the occurrence of NAFLD, by favoring the development of subcutaneous and visceral obesity, hypertension, IR, dyslipidemia and hyperuricemia (132, 133). Interestingly, a week of high-fructose diet (>1.5 g/kg/day) can double the intrahepatocellular lipids. During the same time lapse, a fat-rich diet has a similar effect, leading to a 90.0% increase of intrahepatic fat, while a high glucose intake (3.0 g/kg/day) causes a 60.0% increase (134). Nevertheless, a reduction of fructose intake by 50.0% improves the hepatic fat content, plasma transaminases, BMI, and the glucose metabolism (135). The nutritional management in the presence of SO involves FM reduction combined with an increase in muscle mass and strength. These cases should benefit from protein supplementation in order to prevent the catabolism and the muscle loss (136). Patients with sarcopenia and NAFLD cirrhosis require a protein intake of 1.5–2.0 g/kg/day (137). Moreover, in cases of SO, a fairly hypocaloric diet and a late evening snack (50.0 g carbohydrate ± 20.0 g protein) to minimize overnight fasting and prevent muscle destruction, are advised (138, 139).

Hormonal Imbalance

It is now widely accepted that older age is correlated to the development of NAFLD in the general population (43). Aging is also associated with a decrease in anabolic hormones (GH, IGF-1, testosterone), which further impacts muscle loss (140). Aging and ectopic fat deposition cause a decline in GH levels, which consequently downregulates the PI3K-AKT/PKB-mTOR pathway, leading to lower levels of IGF-1 and impaired protein synthesis in the muscles (141–143). Plasma IGF-1 levels are positively correlated with LBM and muscle activity and negatively associated with FM. IGF-1 binding proteins (IGFBP), which are produced by the liver, also affect IGF-1 biological activity (144). Moreover, reduced GH and IGF-1 levels favor ectopic fat storage in the liver, contributing to NAFLD development (142, 145).

Under normal circumstances, testosterone increases muscle mass by promoting protein synthesis, skeletal muscle cell expression of androgen receptors, and IGF-1 secretion (36). However, through an increased aromatase activity, obesity favors

testosterone aromatization to estradiol, causing hypogonadism (146). Hypogonadism favors central obesity by increasing TNF-α and IL-6, thus contributing to SO (140, 147). Similarly, in menopausal women, the low levels of estrogen and high levels of follicle-stimulating hormone (FSH) and androgens promote SO (148, 149).

CLINICAL EVIDENCE LINKING NAFLD AND SARCOPENIA

The largest amount of data linking sarcopenia and NAFLD comes from cross-sectional cohort studies, most of them performed in Asian populations. Several large meta-analyses showed that the risk of NAFLD, NASH and significant fibrosis was increased 1.5–2.5 fold among individuals with sarcopenia (150–153). Conversely, subjects with NAFLD have significantly lower SMI when compared to controls (153). Although these studies outline the bidirectional relationship between sarcopenia and NAFLD, the causal relationship is difficult to establish. The main limitations of these studies come from the significant heterogeneity in the study population and in the definitions and methods used to diagnose both NAFLD and sarcopenia. These shortcomings explain at least in part the differences in the prevalence of sarcopenia across studies (**Supplementary Table 1**). Most of the studies defined sarcopenia by low-muscle mass, determined by various methods—dual energy-ray absorptiometry (DXA), bioelectric impedance analysis (BIA), CT, and MRI. Despite an international consensus to include muscle strength when available (3), only a minority of studies assessed muscle function and composition.

Muscle composition, in particular muscle fat infiltration, also called myosteatosis, is a major determinant not only for muscle strength and function, but also for metabolic and liver-related clinical outcomes (144, 154, 155). Thus, adverse muscle composition (AMC) has been widely studied, in an attempt to elucidate the sarcopenia paradigm in NAFLD (144, 156, 157), as shown in **Figure 2**. Patients with NAFLD have more frequent AMC when compared with patients without NAFLD. A recent study has shown that the skeletal muscle fat index (SMFI) as a reflection of absolute high muscle fat content, rather than a low muscle mass, is strongly and independently associated with the severity spectrum of NAFLD and progressively increases from patients without fatty liver to patients with isolated steatosis, NASH without fibrosis and NASH with significant fibrosis respectively, $p < 0.0015$ (155). Another study has shown that NAFLD patients with low muscle mass and high intramuscular fat represent a distinct clinical phenotype with significantly worse metabolic outcomes, particularly with respect to T2DM and coronary heart disease (156). Conversely, patients with NAFLD and normal muscle composition had similar metabolic outcomes as patients with normal liver and normal muscle composition. Very important, this study showed that only a proportion of patients with NAFLD and AMC had sarcopenia according to the classical definition which strengthens the evidence that this particular "unhealthy" phenotype is clearly underestimated by the current methods used to diagnose and assess sarcopenia (156). Altogether, these data suggest that muscle fatty infiltration might be a potential marker associated with the severity spectrum of NAFLD. Remarkably, a recent experimental study showed that myosteatosis is strongly correlated with the severity of the liver damage and discriminates between simple steatosis, NASH and normal liver (144). Thus, myosteatosis has been suggested as a new non-invasive biomarker of NAFLD/NASH, able not only to predict the severity of the liver damage but also to identify early changes muscle composition (144, 157, 158).

The differences in the study population with respect to ethnicity and gender directly impact the body composition and thus limits the generalizability of the results. Most of the studies have been performed on Asian populations (10, 14, 17) and only a minority have been performed in Europe (159) or in the US (160–162). One study performed in the US has shown that the prevalence of sarcopenia was higher in females, in Hispanics and non-Hispanics whites (>40.0%) compared to non-Hispanic Black and non-Hispanic Asian (<10.0%) (161). Several Asian studies derived from the Korean National Health and Nutrition Examination Survey (KHANES) 2008–2011 showed that patients with sarcopenia have a two- to five-fold increased risk of NAFLD (10, 45) and two-fold increase risk of significant fibrosis (45) independently of obesity, IR, MetS and liver enzymes. However, most of these studies used non-invasive methods to diagnose NAFLD and to assess its severity (either biological biomarkers or imaging methods) (14, 155, 159, 160, 163) (**Supplementary Table 1**) which may lead to the misclassification of NASH and advanced fibrosis.

More recently, the association between sarcopenia and the severity spectrum of NAFLD has been confirmed in a cohort of 309 patients with available liver histology. The prevalence of sarcopenia almost doubled along the severity spectrum of NAFLD and ranged from 9.0% in controls to 18.0% in patients with isolated fatty liver, and 35.0% in patients with NASH. Among individual histological features of NASH, sarcopenia was associated with the amount of steatosis and ballooning but not with lobular inflammation. Patients with sarcopenia also had higher prevalence of significant fibrosis (≥F2, 46.0 vs. 25.0%, $p < 0.001$). Sarcopenic patients with NAFLD have a two-fold increased risk for NASH (OR 2.46; 95% CI, 1.35–4.48) and significant fibrosis (OR 2.01; 95% CI, 1.12–3.61). This association persisted after adjustment for classical confounders (age, sex, BMI, and T2DM) but was slightly attenuated after adjusting for HOMA-IR and high-sensitivity C-reactive protein (hs-CRP) which further highlights the role of IR and inflammation as a potential link between the two entities (14).

The association between sarcopenia and the amount of steatosis and fibrosis has been confirmed in a Caucasian population by a recent study by Petta et al. (159) and persisted after adjustment for confounders. The prevalence of sarcopenia gradually increased with the fibrosis stages (fibrosis F0, 22.2%), to F1 (34.9%), to F2 (43.7%), F3 (66.6%) and finally F4 (60.0%) ($p = 0.002$). Moreover, in the presence of sarcopenia, the prevalence of severe fibrosis was higher both in patients with visceral obesity (46.0 vs. 30.9% in non-sarcopenic, $p = 0.05$) and in non-obese patients (44.4 vs. 7.1%, $p = 0.002$). Contrary to the Korean study, in this study, the significant

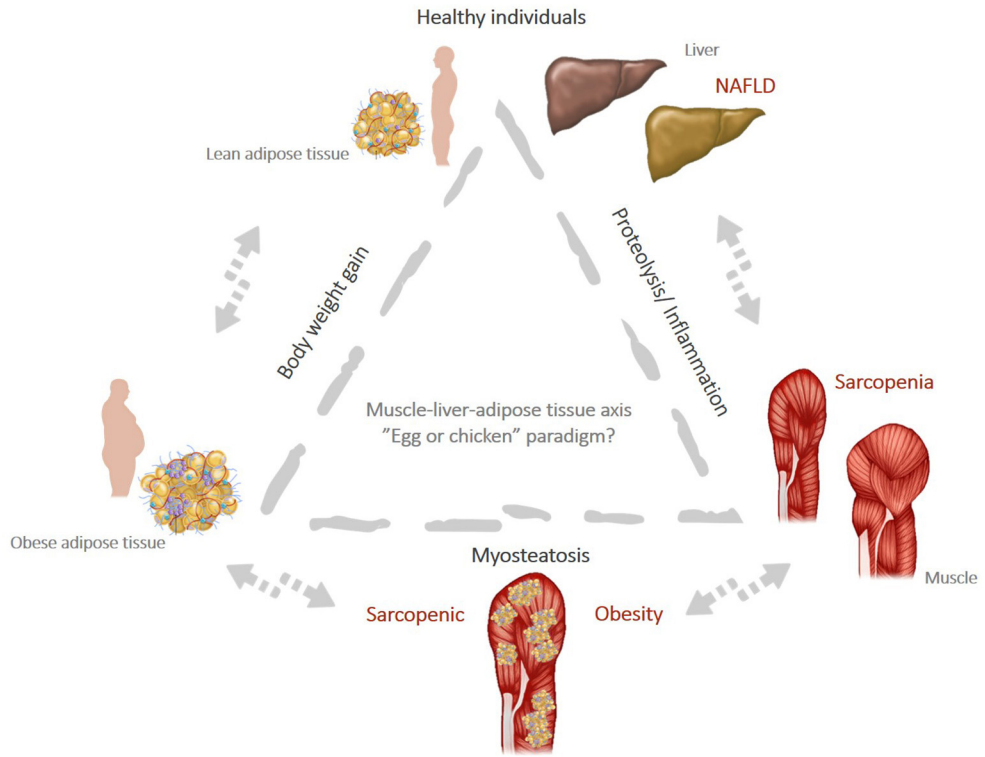

FIGURE 2 | (Created with PowerPoint Microsoft). Muscle-liver-adipose tissue axis: the "egg or chicken" paradigm: weight gain leads to obesity and favors the onset of chronic inflammation that can trigger increased muscle wasting (proteolysis) and promote "*de novo*" lipogenesis pathways. This increase the risk of NAFLD and sarcopenia and favor the milieu for myosteatosis, a condition strictly associated with SO.

association between sarcopenia and NASH was not maintained after adjusting for demographic and metabolic risk factors (159). These differences highlight not only the heterogeneity in the populations studied (Asian vs. Caucasian) and the definition used, but also suggest that sarcopenia and NAFLD are linked through obesity and IR and probably potentate each other. Indeed, sarcopenia reduces glucose uptake and increases insulin resistance which in turn accelerates fibrosis progression. It is under debate whether these findings should prompt us to include the assessment of sarcopenia in the initial evaluation of patients with NAFLD or vice versa and prospective follow-up studies are mandatory. In line with that, several longitudinal studies support the assumption that sarcopenia is an independent risk factor for the progression of the liver damage and is able to predict clinical outcomes and liver-related mortality.

A large longitudinal cohort study with a 10-year follow-up showed that age-related decrease in ASM and body composition were associated with an increased risk of incident NAFLD, particularly in non-obese subjects. At baseline, individuals with incident NAFLD had higher BMI, FM, and ASM despite a lower ASM adjusted by body weight (ASM-to-weight); during follow-up, they gained more weight, had a lower ASM-to-weight, and had a more important decrease in ASM (43). Another Korean study showed that baseline ASM-to-weight was inversely associated with incident NAFLD and positively associated with the resolution of pre-existing NAFLD. Furthermore, increasing ASM-to-weight had a positive impact both on the risk of incident NAFLD and the resolution of existing NAFLD even after adjusting for metabolic confounders (164). Several longitudinal follow-up studies showed that sarcopenic patients with NAFLD had poor clinical outcomes and increased risk in overall (OR 1.28; 95% CI 1.06–1.5) and specific mortality, particularly cardiovascular, cancer, and diabetes related mortality (162). Another recent study analyzing the NHANES 1999–2004 dataset reported a 78.0% increased risk in all-cause mortality and a 320.0% increase in cardiac-specific mortality in patients with sarcopenia-related NAFLD (128).

Finally, in patients with compensated-advanced chronic liver disease (c-ACLD), sarcopenia is highly prevalent [40.0% in cirrhotic patients (165, 166), and rises up to 70.0% in candidates to liver transplantation—LT] (**Supplementary Table 2**). Using the Fried Frailty Index, 25.0% of candidates to LT were classified as frail. In patients with cirrhosis, the annual rate of skeletal muscle loss increases with the severity of liver disease from 1.3% in Child A cirrhosis to 6.0% in Child C patients (167). In patients with c-ACLD, both sarcopenia and frailty, significantly increase the overall mortality (more than two-to-five-fold increase) the risk of cirrhosis decompensation (hepatic encephalopathy—HE, ascites), the length of hospital stay (168) and were associated with higher medical costs (169).

Sarcopenia is an independent predictor of mortality in cirrhotic patients even after adjusting for MELD score or the presence of portal hypertension (170). The addition of sarcopenia to the MELD score improved the prediction of short-term survival; The MELD-psoas model outperformed the MELD score and performed similarly to MELD-Na in predicting survival (171). Several studies described a dose-dependent and bidirectional relationship between sarcopenia or frailty and the clinical outcomes in cirrhosis. Thus, a single unit increase in the Fried Frailty Index was associated with a 45.0% increase in mortality on the waiting list for LT (172). Conversely, an increase in HGS by 1.0 kg or the improvement in the gait speed by 0.1 m/s decreased the wait-list mortality by 11.0 and 28.0%, respectively (173). Although sarcopenia and frailty are probably interrelated through a bidirectional relationship, each entity captures different risks and thus explains the different impact on the clinical outcomes in the same patient. Although both sarcopenia and frailty have been largely explored in patients with advanced liver disease, only a minority of studies specifically focused on NAFLD-related cirrhosis (**Supplementary Table 2**).

The limited number of patients with NAFLD-related cirrhosis, most of whom are coming from LT centers, is mainly due to the fact that NAFLD was for a long time an underrecognized condition and patients were listed both in *U*nited *N*etwork for *O*rgan *S*haring (UNOS) and European Liver Transplant Registry (ELTR) registries as either "cryptogenic cirrhosis" or "other metabolic etiologies" (174). One study reported that patients with NAFLD-related cirrhosis have a 6-fold increased risk of having sarcopenic obesity (44) which in turn is associated with more severe liver disease and worse outcomes (175). These results are not consistent across all studies. For example, one study from Mayo Clinics, found that patients with NAFLD-related cirrhosis listed for LT had a higher prevalence of frailty (49.0%) and myosteatosis (78.0%) and a lower prevalence of sarcopenia (22.0%). The higher prevalence of frailty in patients with NAFLD is probably related to the phenotype of NAFLD candidates to LT (older age, clustering of cardiometabolic comorbidities) which leads to disability, dependency and impaired cognitive function. Frailty is associated with longer hospital stays and increased risk of removal from the waiting list but had no impact on the overall survival after LT (175). The course of sarcopenia following LT is controversial and most of the studies have found little or no improvement probably in a relationship with post-LT complications or immunosuppressive therapy (176, 177).

SARCOPENIC OBESITY: A SEPARATE AND DIFFERENT ENTITY?

Aging is characterized by specific alterations in body composition, particularly by an increase in FM and a decrease in SMM, without evident changes in BMI. The concept of "sarcopenic obesity—SO" was first mentioned by Heber et al. (178) in 1996 and defined as reduced LBM associated with increased FM as determined by BIA. In 2000, Baumgartner (179) described SO in relationship with the decline in physical activity and energy expenditure as the interplay between obesity and sarcopenia defined by DXA. The global prevalence of SO is 11.0% in the general population and rises up to 23.0% in subjects ≥75 years old as reported by a recent meta-analysis (180) but can vary widely depending on the population studied (181, 182).

Situated at the confluence between the actual trends in aging population and the increasing prevalence of obesity, SO is now an emerging health problem responsible for an increased disability in daily activities and reduced quality of life. The morbidity and mortality risk related to SO is greater than risk related to either obesity or sarcopenia alone (183–185). For example, in the British Regional Heart Study, a 6-year prospective study of ≥4,000 men aged 60–70 years, the mortality risk was 55.0% and increased in subjects with both sarcopenia and obesity compared with those with sarcopenia or obesity alone (186). Similar results were found among Japanese Americans elderly men from the Kuakini Honolulu Heart Program (187). It has been shown that compared to patients with sarcopenia or obesity alone, patients with SO are at increased risk of MetS irrespective of ethnicity (Asian, OR 8.28; 95% CI 4.45–15.4, and mixed population Asian and Caucasian, OR 11.59; 95% CI 6.72–19.98, respectively) (188, 189). Several cross-sectional studies also reported an increased risk of hypertension (OR 6.42; 95% CI 4.85–8.48), dyslipidemia (OR 2.82; 95% CI 1.76–4.51) (190), diabetes (OR 2.16; 95% CI 1.08–3.27) (191), or diabetes related complications (OR 6.52; 95% CI 1.47–28.8) (192).

Concerning the cardiovascular risk associated with SO, the results are controversial. Some studies reported an overall increased risk for early atherosclerosis as defined by coronary artery calcifications (OR 1.92; 95% CI 1.16–3.18) (193) and 10-year cardiovascular risk determined by Framingham score (OR 2.49; 95% CI 1.53–4.06) (194) in subjects with SO vs. sarcopenia or obesity alone. Data from the US NHANES database including 11,317 participants also demonstrated that SO was associated with an eight-fold increased risk of cardiovascular disease in both metabolically healthy and unhealthy individuals (195). The British Regional Heart Study, conducted in older men of 60–79 years of age, although found a significant association between SO and cardiovascular mortality, failed to demonstrate an association between SO and coronary heart disease events. Ultimately, the association between SO and individual cardiovascular risk factors—atherogenic lipids profile with low high-density lipoprotein cholesterol (HDL-C) and high triglyceride levels, increased hs-CRP and IR acts together and increases the overall cardiovascular risk and mortality (196).

The risk factors for SO identified by most of the studies are older age (180), sex-related hormonal changes (i.e., postmenopausal increase in visceral fat as a result of low estrogen levels, testosterone deficiency) (197), ethnicity (higher prevalence in Hispanics and non-Hispanic whites compared with non-Hispanics blacks) (181), physical inactivity and clustering of the comorbidities (198). These risk factors are all common among patients with NAFLD and favors SO. The clinical phenotype of NAFLD is typically characterized by older age and clustering of cardiometabolic comorbidities—T2DM, obesity and its complications, and cardiovascular disease—which result in the impairment of the effort capacity and decreased physical activity.

On the other hand, a sizable proportion of patients with NAFLD experienced repetitive restrictive dietary interventions to lose weight which often result in loss of the LBM but gain in FM because of concomitant physical inactivity. Therefore, it is not surprising that the prevalence of SO is higher in patients with NAFLD (ranges from 18.0 to 77.0%) than reported in the general population (144, 158) (**Supplementary Table 3**). By altering lipid muscle metabolism, IR and the inflammatory pathways, obesity and sarcopenia promote lipotoxicity and not only potentate each other in a kind of vicious circle as discussed above, but also have a negative impact on the natural course of NAFLD both in terms of the evolution of the cardiometabolic conditions and progression of the liver damage. Conversely, the presence of NAFLD significantly impacts the changes in body composition. This has been shown in a small North American weight loss interventional trial which evaluated the relationship between NAFLD and NAFLD resolution and body composition in obese individuals. Despite similar changes in BMI during follow-up, patients with NAFLD had a greater reduction in visceral adipose tissue area (VATA). Furthermore, participants with NAFLD resolution had an even more significant reduction in the VATA while no significant changes occurred in the SMM (199). These findings suggest a possible protective role of FM in preserving muscle mass in weight loss settings but further studies are warranted.

Because of the overlapping in the physiopathological pathways, it is under debate whether the impact of SO on the liver-related outcomes is more than additive. Both sarcopenia and obesity are risk factors for fibrosis progression. As shown in a recent study from the NHANES database from 2017 to 2018 (161), patients with SO had a higher prevalence of significant fibrosis (20.9 vs. 9.4%), and cirrhosis (7.5 vs. 2.6%) than those without these conditions. Even after adjustment for confounders, patients with SO have a two-fold higher risk of having NAFLD-associated significant fibrosis. SO is found in 20.0 to 40.0% of LT candidates. One study has shown that patients with end-stage NAFLD listed for LT have a six-fold increased risk of SO (44).

Both sarcopenia and obesity are established risk factors for the development of hepatocellular carcinoma (HCC) and are predictors for tumor recurrence and overall survival (200, 201). A retrospective study of 465 Japanese patients who underwent liver resection for HCC found that preoperative SO more than doubles the risk of death and HCC recurrence after hepatectomy (202). These results have been confirmed by another Japanese study that showed that patients with SO have a significant decrease in overall and recurrence-free survival at 3- and 5 years despite similar 1-year survival. This study also showed that patients with SO also have more advanced liver lesions with higher rates of multiple tumors, microvascular invasion, moderate differentiation, and satellite nodules (57). A more in-depth analysis of body composition has shown that sarcopenia, intramuscular fat content and visceral adiposity were independent predictors of mortality in patients with HCC. These data emphasize that body composition rather than BMI has significant prognostic value in HCC patients (203).

As most of these data are coming from Asian population with HBV-related cirrhosis, their generalizability to a Caucasian population with NAFLD is limited. Whether the impact of SO on liver and HCC-related outcomes is magnified in the context of the pro-inflammatory and IR milieu which characterizes patients with NAFLD should be determined by future prospective studies.

GAPS IN KNOWLEDGE AND FUTURE DIRECTIONS

Sarcopenia and SO are perfect models to illustrate the changes in body composition that occur with aging and result in distinct clinical phenotypes which specifically impact clinical outcomes. Simple clinical tools like BMI or waist circumference (WC) measures are widely used to evaluate nutritional status (204) but are unable to capture the differences in body composition, such as the amount and the distribution of muscle and fat mass. As an example, it is now widely accepted that low BMI is linked to higher mortality rates in relationship with a low SMM and not with a low FM. These data underline the clinical relevance of the body composition and bring into attention a challenging new paradigm also called the "obesity paradox." This new concept suggests the possible protective role of the FM and reveals the preservation of the lean body mass as an important therapeutic goal (205). This concept is particularly important in patients with NAFLD because weight loss and lifestyle changes have a central role in the management of the disease (206). Preventing lean mass wasting should be a therapeutic goal in patients with NAFLD who are particularly exposed to reduced physical activity and sedentary behavior (207) due to the associated comorbidities. Because a lot of the molecules now tested in randomized clinical trials in NAFLD target multiple metabolic pathways to improve liver damage and some of them are associated with changes in body weight, the assessment of the body composition is now one of the secondary outcomes to assess drug efficacy in the ongoing clinical trials in NAFLD (208). While the assessment of body composition gains more and more recognition in NAFLD clinical trials, its use in routine clinical practice is still limited and warrants increased awareness of the clinical practitioners.

The assessment of body composition has been extensively investigated in sarcopenia and SO aiming to improve the diagnosis of muscle disease in many settings. However, this triggered a plethora of various diagnostic tools and different cut-offs which resulted either in underestimating or overestimating the prevalence of these conditions according to the populations studied (209). The cut-off that is better correlated with clinical outcomes has to be determined by future longitudinal prospective follow-up studies for each diagnostic tool. Among the diagnostic methods employed to assess muscle mass and quality (210), some of them (CT, MRI) can also be performed to evaluate the presence of hepatic steatosis which makes possible a single examination for both conditions.

Another gap in the knowledge with important clinical implications is to distinguish between each of (1) the quantitative assessment of the SMM and body composition resulting into sarcopenia or SO (2) assessment of the muscle strength resulting

in functional impairment and frailty and (3) assessment of the muscle composition resulting in myosteatosis. A better understanding of the clinical and prognostic information brought by each of these measures will allow for more individualized recommendations to use one test or another depending upon the clinical phenotype and management strategies used for each patient.

Although the association between sarcopenia and NAFLD is largely supported by the existing literature, there is insufficient evidence to argue for a direct causal relationship between these two entities beyond the common pathophysiological pathways. In a kind of an "egg and chicken story" it is unclear whether sarcopenia is coming first and represents a risk factor for NASH progression, or it is rather a complication of NAFLD which occurs later along with the worsening of the liver damage (**Figure 2**). The studies published to date suggest that the two entities are interconnected through a bidirectional relationship and each one increases the risk for the other, in some kind of a vicious circle. Thus, the crosstalk between muscle, liver and adipose tissue plays a central role in shaping the body composition and defines a new clinical phenotype concept called *sarcopenic NAFLD*, which might account for the heterogeneity of the NAFLD phenotypes. This concept is partially supported by clinical and experimental data suggesting that sarcopenia and NAFLD are both consequences of lipotoxicity and ectopic lipid storage in skeletal muscle (myosteatosis) and hepatocytes. Yet, it has to be determined whether screening for sarcopenia should be implemented in routine clinical practice in patients with NAFLD. Hypothetically, screening for sarcopenia and assessing muscle composition in NAFLD would allow to better stratify patients according to the clinical phenotype and identify those at higher risk of disease progression and severe clinical outcomes. A first line screening for sarcopenia could be easily done in clinical practice using available questionnaires such as GLIM criteria (*Global Leadership Initiative on Malnutrition*) (211), a worldwide consensus for categorizing the various forms of malnutrition based on phenotypic (body morphologies) and etiologic (food intake and disease burden) criteria. European Association for the Study of the Liver (EASL) guidelines clearly state that obesity does not rule out malnutrition and recommend to perform a rapid nutritional screen for sarcopenia in all patients with cirrhosis and to complete a detailed assessment in those at high risk of malnutrition (139).

Finally, in patients with NAFLD, a more subtle analysis of the clinical phenotypes would potentially lower the heterogeneity of the population included in NAFLD clinical trials, thus increasing the chances to prove drugs efficacy. This would be particularly helpful in the actual landscape of drug development in NAFLD with a lot of molecules that failed to prove their efficacy despite promising results in experimental and preliminary phase I and II clinical trials. On the other hand, sarcopenia is a modifiable risk factor through lifestyle interventions. Thus, the identification of *sarcopenic NAFLD* phenotype will allow a better counseling of the nutritional interventions with a focus on diet composition and physical exercise to avoid skeletal muscle loss and weight regain which is also known as the "accordion effect" (137). Subtle changes in body composition might help to restore homeostasis in the muscle-liver-adipose tissue axis and promote a long-term sustained weight loss which is a key to modify the natural course of NAFLD.

AUTHOR CONTRIBUTIONS

RP designed, wrote, and revised the manuscript. CS and VZ equally contributed to writing the manuscript. HS performed literature research and entered data. TM and VR critically revised the manuscript. All authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.774030/full#supplementary-material

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Effects of Branched-Chain Amino Acids on Parameters Evaluating Sarcopenia in Liver Cirrhosis: Systematic Review and Meta-Analysis

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Introduction: Sarcopenia is a major element of malnutrition in liver cirrhosis (LC) and is present in 30–70% of this population, being associated with a poor overall prognosis due to related complications such as hepatic encephalopathy, ascites, and portal hypertension. This systematic review and meta-analysis aimed to evaluate the effects of branched-chain amino acids (BCAA) supplementation on several parameters used to assess sarcopenia in LC.

Materials and Methods: A comprehensive systematic electronic search was performed in PubMed, EMBASE, Scopus, Cochrane Library, and ClinicalTrials.gov databases using predefined keywords. We included full articles that satisfied the inclusion and exclusion criteria. Quality assessment of included studies was conducted using Cochrane Collaboration's tool and NHLBI quality assessment tools for interventional and observational studies, respectively. The principal summary outcome was the mean difference (MD) in the evaluated parameters. We performed a pre- and post-intervention analysis and comparison between two intervention groups (BCAA vs. controls) of the evaluated parameters when applicable.

Results: A total of 12 studies involving 1,225 subjects were included in our qualitative synthesis and five in our quantitative synthesis. At baseline vs. post-intervention assessment, subjects receiving BCAA supplementation were found to have a significant improvement in skeletal muscle index (SMI) (−0.347 [95% CI −0.628–0.067; *p*-value 0.015]) and mid-arm muscle circumference (MAMC) (−1.273 [95% CI (−2.251–0.294; *p*-value 0.011]). However, no improvements were reported in handgrip (-0.616 [95% CI −2.818–1.586; *p*-value 0.584]) and triceps subcutaneous fat (1.10 [95% CI −0.814–3.014; *p*-value 0.263]).

Conclusion: Following BCAA supplementation, several parameters used to evaluate sarcopenia in LC patients were found to be improved, including SMI and MAMC. Nevertheless, no improvements were seen in handgrip and triceps subcutaneous fat.

Results should be interpreted with caution due to the limited methodological quality of the included studies.

Keywords: branched-chain amino acids (BCAA), sarcopenia, liver cirrhosis, anthropometric parameters, skeletal muscle index (SMI), mid-arm muscle circumference (MAMC), systematic review, meta-analysis

INTRODUCTION

Sarcopenia is a syndrome proposed by Rosenberg in 1989, defined as an age-related muscle mass reduction and abnormalities in muscle function, including muscle strength and physical performance (1, 2). Sarcopenia can be categorized as primary when associated with aging or secondary when related to an underlying condition such as systemic diseases, including chronic liver disease (CLD), being one of the main causes of secondary sarcopenia (3, 4).

Sarcopenia is a major element of malnutrition in liver cirrhosis and has been reported to be prevalent in 30–70% of this population (5). Several causes lead to the development of sarcopenia in patients with cirrhosis. These include malabsorption, dysregulated metabolism, reduced nutritional intake, hormonal alterations, increased loss of muscle, and hyperammonemia (6, 7). The overall prognosis in patients with cirrhosis is affected by sarcopenia due to other related complications such as hepatic encephalopathy, ascites, and portal hypertension (8, 9). The importance of sarcopenia is that it is associated with reduced quality of life, survival, mobility, and cardiopulmonary performance, as well as unfavorable metabolic outcomes and increased infection rates when compared to non-sarcopenic patients (3, 4).

Most studies evaluating sarcopenia were conducted in community-dwelling elderly patients for whom several consensus definitions have been published (3, 4, 10). Nevertheless, applying the existing consensus definition in liver cirrhosis patients is challenging due to muscle mass changes that develop in this population influencing the measurements, possibly due to altered hepatic function and water retention in peripheral edema and ascites. Furthermore, a clear consensus defining sarcopenia in liver cirrhosis patients remains required. Several tests have been used to assess sarcopenia in cirrhosis (11). **Figure 1** summarizes the frequently used tests to evaluate sarcopenia, including muscle mass, function, and strength evaluation (11).

Although the clinical significance of sarcopenia in cirrhosis has been widely recognized, effective therapies are still to be discovered, mainly due to the limited available data describing the mechanisms relating sarcopenia to cirrhosis, a condition believed to be associated with a state of anabolic resistance (12, 13). Studied therapeutic approaches include diets rich in protein and fiber, nutrients supplementation with branched-chain amino acids (BCAAs), minerals, and vitamins, as well as exercise (14–16).

The concentration of BCAAs in plasma and skeletal muscle are reduced in cirrhosis (17, 18). BCAAs have been shown to be helpful as a nutritional supplement in liver cirrhosis (19–21). Several clinical trials reported the efficacy of BCAAs for nutritional status, general status, hepatic encephalopathy, and quality of life (22–25). Therefore, it is expected that BCAA supplementation may be considered a useful therapeutic modality in treating decreased muscle mass and strength that accompany secondary sarcopenia.

This comprehensive systematic review and meta-analysis aimed to investigate the effects of BCAA supplementation in observational and interventional studies on several parameters used to assess sarcopenia in patients with liver cirrhosis, including muscle mass, function, and strength evaluation such as mid-arm muscle circumference (MAMC) and skeletal muscle index (SMI), as well as handgrip strength and triceps subcutaneous fat assessment.

METHODS

This systematic review and meta-analysis was written according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist 2020 (26).

Data Sources and Search Strategy

We aimed to review the currently available evidence published on PubMed, EMBASE, Scopus, Cochrane Library, and ClinicalTrials.gov, trying to answer the PICOS research question: identifying the population of patients with liver cirrhosis, with a BCAA supplementation intervention, vs. a comparator (maltodextrin), or a before after design, to observe several parameters assessing sarcopenia, as outcomes, including SMI and MAMC, as well as handgrip strength and triceps subcutaneous fat, in interventional or observational studies. A description of the conducted search strategy is provided in **Supplementary Material 1**. Moreover, we performed a manual search for missed publications by screening the references of included articles to minimize the risk of missing relevant studies. The conducted search included published articles from inception up to 14 July 2021. No search filters or restrictions were applied in regards to duration, country, or language. Afterward, a screening assessment was conducted by evaluating titles and abstracts for appropriateness. Articles that were selected based on the inclusion and exclusion criteria were evaluated through a full-text review. Eligibility of the evaluated studies was performed independently by two authors (A.I. and D.C.L.), and data extraction from eligible studies was performed by two authors (C.B. and A.F.), while resolving any discrepancies by mutual consensus.

Eligibility Criteria

Inclusion criteria of original articles were as follows: (1) Full article interventional (clinical trials, RCTs) or observational studies (observational cohort population-based/ hospital-based, cross-sectional or case-control designs), evaluating

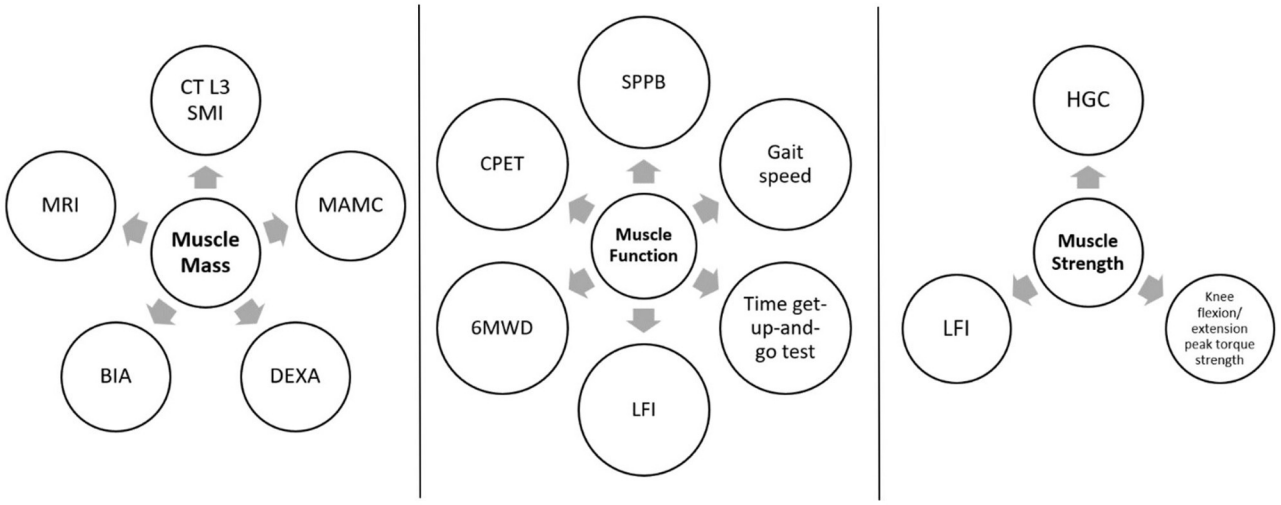

FIGURE 1 | Tests used to evaluate sarcopenia, assessing muscle mass, function, and strength. CT, computed tomography; SMI, skeletal muscle index; MRI, magnetic resonance imaging; MAMC, mid-arm muscle circumference; DEXA, dual-energy X-ray absorptiometry; BIA, bioimpedance analysis; SPPB, short physical performance battery test; LFI, Liver Frailty Index; 6MWD, 6-minute walk distance; CPET, cardiopulmonary exercise testing; HGC, Handgrip strength.

the effects of BCAA supplementation on anthropometric and functional parameters assessing sarcopenia in patients with cirrhosis; (2) Liver cirrhosis evaluated using liver biopsy or imaging techniques such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), codes such as International Classification of Diseases (ICD), or as per study definition; (3) Parameters assessing sarcopenia according to each studies definition; (4) Human studies only; and (5) Studies published in English, French, German or Romanian languages.

Exclusion criteria were as follows: (1) Subjects with end-stage liver disease who received a liver transplant; (2) Presence of hepatocellular carcinoma; (3) Editorials, letters to the editor, case reports, conference abstracts, literature, and systematic reviews, practice guidelines, commentaries, clinical trial registrations, abstracts published without a full-text article; and (4) Experimental studies.

Risk of Bias Assessment in Individual Studies

The risk of bias for randomized controlled trials was assessed using the Cochrane Collaboration's tool (27). The quality assessment was based on randomized sequence generation, treatment allocation concealment, blinding, and completeness of the outcome data, in addition to selective outcome reporting and other sources of bias.

For non-RCT studies, we used the National Heart, Lung, and Blood Institute (NHLBI) quality assessment tools (28). Two NHLBI tools were used, the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies, as well as the Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group.

We used these evaluation tools to evaluate bias risk and internal validity in individual studies in a similar manner. The risk of bias in individual studies was evaluated by two authors independently (A.I. and D.C.L.). In case of disagreement, a consensus was reached through a discussion.

Summary Measures and Synthesis of Results

The principal summary outcome was the mean difference (MD) of several parameters, including SMI, MAMC, handgrip, and triceps subcutaneous fat. For the summary outcomes, we computed the estimates of the random effects using restricted maximum likelihood to estimate the heterogeneity variance, since we assumed clinical variability between the studies. We conducted the data analyses of the meta-analysis using R with Metafor package (OpenMeta [Analyst]) (29, 30). Between-study heterogeneity was evaluated using the χ^2 based Q-test and I^2. According to the recommendations of the Cochrane Handbook (31) for identifying and measuring heterogeneity, we estimated I^2 values of 0% to 40% as not important; 30% to 60% as moderate heterogeneity; 50–90% as substantial heterogeneity; and 75% to 100% as considerable heterogeneity.

The estimated total effect size analysis was calculated using the random-effects model and MD. In studies that reported medians and interquartile ranges, we calculated the mean and standard deviation (SD) to perform statistical analyses of the obtained data (32). In studies reporting results at baseline and post-intervention data, the mean change and SD change were used if they were reported. Still, in case they were not reported, they were calculated based on the before and after values according to the Cochrane Handbook recommendation using the correlation coefficient from the same study or imputed from a similar study (27). Data was reported from each study as the estimated MD

with a 95% confidence interval (CI). A statistically significant *p*-value was considered when <0.05. The analyses were conducted if two or more studies evaluated similar groups and reported the same outcome using mean +/– SD or median (IQR). We also performed baseline and post-intervention analysis when available in single studies. For baseline and post-intervention analysis, we only included groups that received solely BCAA supplementation. We were not able to perform publication bias assessment due to the limited number of included studies.

RESULTS

General Results

Figure 2 outlines the PRISMA flow diagram describing the performed search strategy. The initial search yielded 191 articles (PubMed $n = 29$, EMBASE $n = 70$, Scopus $n = 56$ articles, ClinicalTrials.gov $n = 23$ articles, Cochrane Library $n = 13$ articles). A total of 63 studies were removed after being detected as duplicates. After excluding the duplicates, 128 articles underwent a preliminary screening by assessing the title and abstract for inclusion and exclusion criteria fulfillment. During the screening phase we excluded 110 articles. Eighteen articles were sought for retrieval, out of which the full text of one article was not found (we contacted the authors by email, but we didn't receive any feedback). We performed a thorough reading and evaluation of the full texts for further eligibility assessment of the remaining 17 articles. We excluded six out of these articles with reasons as follows: (1) no clear BCAA group (33, 34), (2) abstract without full text (35), (3) involving hepatocellular carcinoma (HCC) patients (36), (4) not involving cirrhosis patients (37), and (5) outcome influenced selection (38). Accordingly, the qualitative synthesis included 11 articles, out of which five were included in the quantitative synthesis (38–49).

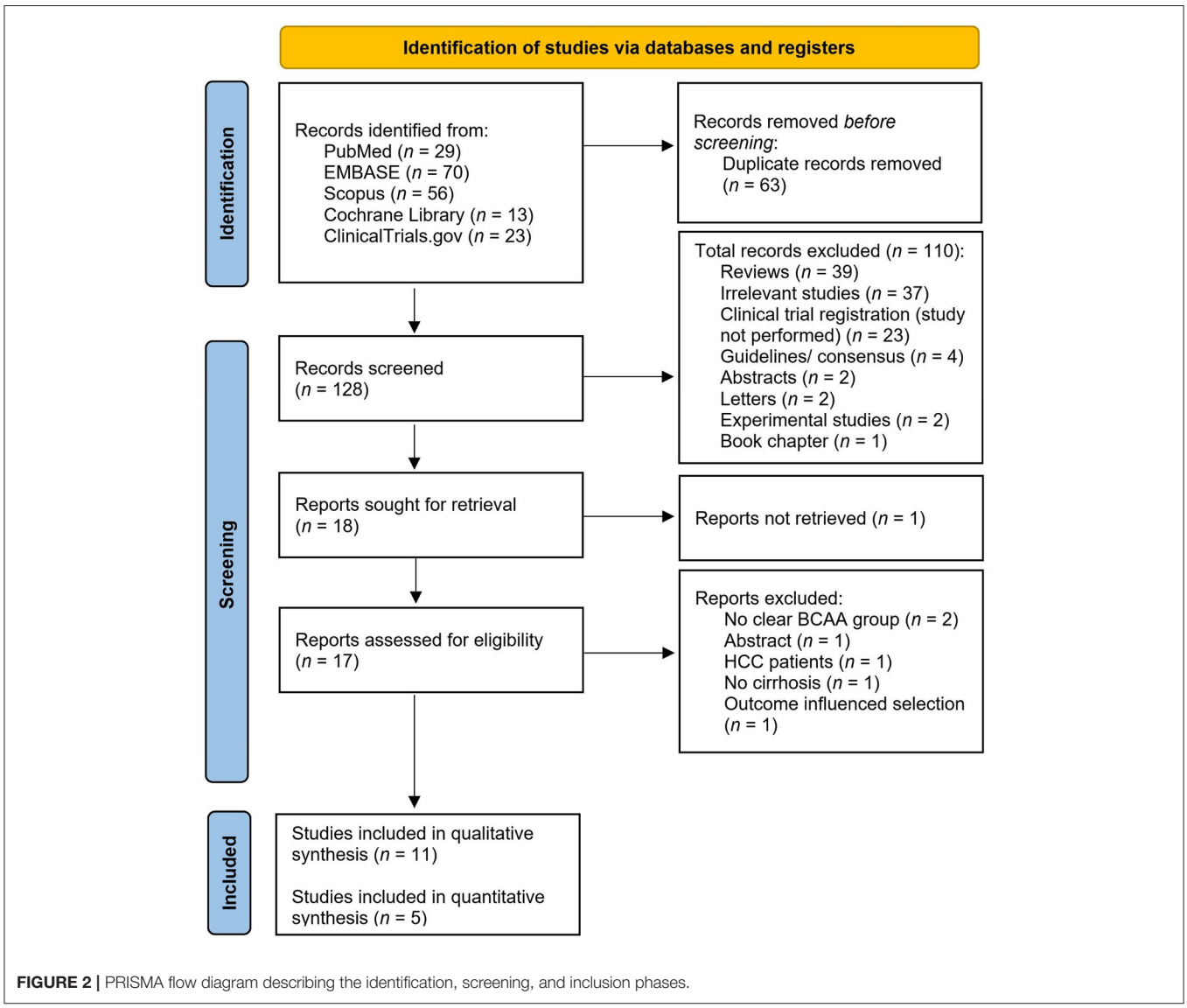

FIGURE 2 | PRISMA flow diagram describing the identification, screening, and inclusion phases.

Study Characteristics

The main characteristics of included studies are summarized in **Table 1**. This systematic review and meta-analysis included a total number of 1,215 individuals (394 individuals in RCTs, 821 individuals in observational studies).

Four studies had an interventional study design (39, 40, 46, 49), five studies had a prospective study design (42–45, 47), and two had a retrospective design (41, 48). Seven studies were undertaken in Asia (Japan $n = 7$), two in Europe (multicenter $n = 1$, Spain $n = 1$), and two in the Americas (USA $n = 1$, Mexico $n = 1$).

Effects of BCAA Supplementation on Sarcopenia in Patients With Cirrhosis

Several parameters were evaluated in the included studies, assessing the effects of BCAA supplementation in sarcopenic patients with liver cirrhosis, including muscle mass, function, and strength, as demonstrated in **Supplementary Table 1**. These parameters included MAMC, SMI, skeletal muscle area, handgrip strength, tricipital skinfold thickness, bicipital skinfold thickness, suprailiac skinfold thickness, subscapular skinfold thickness, midarm muscle area, midarm fat area, fat mass, fat-free mass.

Skeletal Muscle Index Improvement (Baseline and Post-intervention)

A total of two studies reported mean +/– SD or median (IQR) for the SMI (cm^2/m^2) involving baseline values and post-BCAA supplementation (45, 49). **Figure 3** summarizes the obtained results regarding SMI, which was evaluated using CT scan and bioelectrical impedance analysis.

The pooled analysis assessing the SMI in baseline minus post-BCAA supplementation groups demonstrated that SMI significantly increased after the BCAA supplementation, the difference being of −0.347 (95% CI −0.628−0.067), *p*-value of 0.015. Very low heterogeneity was reported with an $I^2 = 0\%$ and *p*-value 0.589.

Mid-Arm Muscle Circumference Post-intervention (BCAA vs. M-DXT)

A total of two studies reported mean +/– SD for the MAMC (cm) comparing BCAA group vs. maltodextrins (M-DXT) group (39, 40). **Figure 4** summarizes the obtained results regarding MAMC.

In the pooled analysis assessing the MAMC in BCAA group vs. M-DXT group, we observed overall larger MAMC post-intervention values in the M-DXT group compared to BCAA group, but they did not reach the statistically significant threshold, the mean difference between the groups being−0.443 (95% CI −0.994–0.240), *p*-value of 0.116. Substantial heterogeneity was reported with an $I^2 = 75.63\%$ and *p*-value 0.043.

Mid-Arm Muscle Circumference Improvement (Baseline and Post-intervention)

A total of two studies reported mean +/– SD for the MAMC (cm) comparing baseline values and post-BCAA supplementation (40, 46). **Figure 5** summarizes the obtained results regarding MAMC.

The pooled analysis assessing the difference in MAMC values between baseline and post-BCAA supplementation groups demonstrated that MAMC values significantly increased after the BCAA supplementation, the MD being of −1.273 (95% CI −2.251−0.294), *p*-value of 0.011. Substantial heterogeneity was reported with an $I^2 = 90.98\%$ and *p*-value <0.001.

Handgrip Change (Baseline and Post-intervention)

A total of two studies reported mean +/– SD or median (IQR) for handgrip (kg) comparing baseline values and post-BCAA supplementation (40, 49). **Figure 6** summarizes the obtained results regarding handgrip.

In the pooled analysis assessing the difference in handgrip between baseline and post-BCAA supplementation we observed an increase in handgrip, albeit non-statistically significant, with an overall MD of −0.616 (95% CI −2.818–1.586), *p*-value of 0.584. Substantial heterogeneity was reported with an $I^2 = 59.29\%$ and *p*-value 0.117.

Handgrip was evaluated in one individual study separately, comparing BCAA group vs. DXT group, assessing baseline vs. post-intervention difference. We observed a larger increase in handgrip (baseline vs. post-intervention) in BCAA group compared to DXT group, albeit non-statistically significant, with an MD of −1.0 (95% CI −2.674–0.674), *p*-value of 0.244.

Triceps Subcutaneous Fat Change (Baseline and Post-intervention)

Triceps subcutaneous fat was evaluated in one individual study separately, comparing BCAA group vs. controls, assessing baseline vs. post-intervention difference. We observed a non-statistically significant decrease in triceps subcutaneous fat (baseline vs. post-intervention) in BCAA group compared to controls, with an MD of 1.10 (95% CI −0.814–3.014), *p*-value of 0.263.

Quality Assessment

Four articles were evaluated using the Cochrane Collaboration's tool (39, 40, 46, 49), five articles using the NHLBI Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (38, 41–43, 48), and three articles using the NHLBI Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group (44, 45, 47), as demonstrated in **Supplementary Tables 2–4**.

Several issues were reported regarding bias in the assessed articles. Regarding RCTs evaluated in our review, although all four included RCTs had a low risk for selection bias related to random sequence generation, two of them presented an unclear risk of allocation concealment bias (46, 49). Moreover, one article presented a high risk of performance bias related to blinding of the participants and personnel as well as detection bias evaluated as outcome assessment blinding (46), while another was evaluated as unclear for both parameters being assessed (49). Incomplete outcome data, considered as attrition bias, was high in two included RCTs (40, 46). All RCTs included in this review had an unclear risk of bias regarding reporting bias and other possible sources of bias (39, 40, 46, 49).

TABLE 1 | Characteristics of studies included in the systematic review and meta-analysis.

No.	Study, setting	Study type	Population included	BCAA/BCAA + Intervention	Comparison	Number of patients included	Study participant's characteristics	Measurements	Main findings
1	Marchesini et al., 2003; Setting: Europe	RCT, double-blind, multicenter	Patients with liver cirrhosis, portal hypertension, with Child–Pugh score ≥7	BCAA (14.4 g/day composed of 1.2 g L-leucine, 0.6 g L-isoleucine, and 0.6 g L-valine, 225 Kcal/day) for 12 months	Lactoalbumin OR Maltodextrins for 12 months	*n* = 59 BCAA group/ 56 Lactoalbumin group/*n* = 59 Maltodextrins group	59 Y in BCAA group/60 years in Lactoalbumin group/59 Y in Maltodextrins group; 36.8% F	Midarm circumference (to the nearest millimeter) using a tape meter; skinfold thickness using a caliper	Significant ↑in triceps skinfold thickness and midarm fat area in BCAA group
2	Les et al., 2011; Setting: Spain	RCT, double-blind, multicenter	18–85 years, with liver cirrhosis and hospitalized for hepatic encephalopathy, compliant with a standard diet 2 weeks before inclusion	BCAA (30 g/day, 120 Kcal/day) composed of leucine: 13.5 g, isoleucine: 9 g, valine: 7.5 g or MDX, for 56 weeks	Maltodextrin	*n* = 58 BCAA group/*n* = 58 Maltodextrin group	64.1 Y in BCAA group/62.5 Y in Maltodextrin group; 24% F	Midarm muscle circumference; muscular strength by handgrip (methods of measurements not provided)	Midarm muscle circumference and handgrip ↑in patients in the BCAA group
3	Hanai et al., 2015; Setting: Japan	Retrospective cohort study	>18 years with liver cirrhosis	[a]BCAA 12 g/day, 4 g of BCAA per sachet composed of 952 mg L-isoleucine, 1904 mg L-leucine, and 1144 mg L-valine, for >12 months	NA	*n* = 94 BCAA group/*n* = 36 non-BCAA group	66 Y in BCAA group/64 Y in non-BCAA group; 42% F	SMI using CT scan	A possible association between BCAA administration and ↑outcome in four sarcopenic patients with liver cirrhosis was observed
4	Tsien et al., 2015; Setting: Cleveland, USA	Prospective study	Patients with alcoholic cirrhosis, with Child–Pugh score ≤7 and healthy controls	Leucine enriched BCAA, 15 g composed of BCAA/LEU (7.5 g L-leucine, 3.75 g L-isoleucine, 3.75 g L-valine), single administration in patients with cirrhosis	Leucine enriched BCAA, 15 g single administration in healthy controls	*n* = 6 patients with cirrhosis/*n* = 8 healthy controls	54 Y in patients with cirrhosis group/45 Y in healthy controls; 35.7% F	Body composition characteristics assessed using dual-energy Xray absorptiometry. Muscle expression of myostatin, mTOR targets, autophagy markers, protein ubiquitination and intracellular amino acid deficiency sensor by muscle biopsy	Impaired mTOR1 signaling and ↑autophagy in skeletal muscle of alcoholic cirrhosis patients is acutely reversed by leucine enriched BCAA

(Continued)

TABLE 1 | Continued

No.	Study, setting	Study type	Population included	BCAA/BCAA + Intervention	Comparison	Number of patients included	Study participant's characteristics	Measurements	Main findings
5	Hiraoka et al., 2017; Setting: Japan	Observational, prospective (pre-post intervention)	Patients with liver cirrhosis	BCAA (protein 13.5 g, 210 kcal/day including L-leucine 1922.5 mg) + walking exercise (additional 2,000 steps daily)	NA	*n* = 33 (one group)	67 Y; 60.6% F	Muscle volume using bioelectrical impedance; handgrip strength using a hand dynamometer; leg strength using a position controllable cycle ergometer	BCAA supplementation and walking exercise were found to be effective and easily implemented for ↑muscle volume and strength in liver cirrhosis patients
6	Uojima et al., 2017; Setting: Japan	Single-center, prospective study (pre-post intervention)	Patients >20 years, with liver cirrhosis, with albumin level <3.5 g/dl after standard nutrition therapy for at least 28 days	BCAA (2*50 g/day, 420 Kcal/day), one package of BCAA (50 g) was composed of 13.5 g of protein, including L-leucine, L-isoleucine, and L-valine, which provided 210 kcal of energy; for 24 weeks	NA	*n* = 82 (one group)	69 Y; 44% F	SMI using bioelectrical impedance analysis; hand grip using a grip dynamometer	BCAA supplementation ↑low muscle strength in patients with chronic liver disease, but did not increase muscle mass during the treatment period
7	Kitajima et al., 2017; Setting: Japan	Observational, prospective; (pre-post intervention)	Patients with liver cirrhosis	BCAA granules (25–35 Kcal/kg/day and protein intake to 1.0–1.4 kg/day), each packet of BCAA contained 952 mg L-isoleucine, 1904 mg L-leucine, and 1144 mg L-valine; for 48 weeks	NA	*n* = 21 (one group)	71.3 Y; 57.1% F	Skeletal muscle volume using CT scan and bioelectrical impedance analysis; intramuscular adipose tissue content using CT scan	BCAA were associated with ↑albumin levels in patients with hypoalbuminemia and were related to maintained skeletal muscle mass
8	Ruiz-Margáin et al., 2018; Setting: Mexico	RCT, open-label	Patients 18–65 years, with liver cirrhosis	BCAA (110 g/day) + High-protein, High-fiber diet, composed of 3.38 g of L-leucine, 2.75 g of L-isoleucine, and 2.5 g of L-valine, totaling 500 Kcal for 6 months	High-protein, High-fiber diet	*n* = 37 BCAA group/*n* = 35 control group	54.9 Y in BCAA group/47.8 years in Control group; 80.6% F	Triceps skinfold thickness and mid-arm muscle circumference	↑in muscle mass and a decrease in fat mass in the BCAA group, but not in the control group

(Continued)

TABLE 1 | Continued

No.	Study, setting	Study type	Population included	BCAA/BCAA + Intervention	Comparison	Number of patients included	Study participant's characteristics	Measurements	Main findings
9	Hiraoka et al., 2019; Setting: Japan	Observational, prospective (pre-post intervention)	Patients with liver cirrhosis and BCAA supplementation (12.45 g/day)	Levocarnitine (1000 mg/day) + exercise (plus 2000 steps/day), for 6 months	NA	$n = 18$ (one group)	68.4 Y, 44.4% F	Muscle volume using bioelectrical impedance analysis; hand grip using a grip dynamometer; Leg muscle strength using position controllable cycle ergometer	No significant changes in the ratios of handgrip strength, leg strength, and muscle volume after 6 months
10	Hanai et al., 2020; Setting: Japan	Observational, retrospective cohort study	Patients >20 years, with liver cirrhosis	[a]BCAA (6.1 g)-enriched powder mix daily use composed of 1.602 g of L-valine, 2.037 g of L-leucine, and 1.923 g of L-isoleucine (213 kcal)	No administration	$n = 87$ BCAA group/$n = 436$ No-BCAA group	69 Y BCAA group/66 years No-BCAA group; 54.7% F	SMI	Nocturnal BCAA supplementation was associated with a significant ↓in the risk of death
11	Okubo et al., 2021; Setting: Japan	RCT, open-label	≥20 years, with decompensated cirrhosis and treated with BCAA for at least 6 months	Vitamin D, 2000 IU for 12 months	No administration	$n = 15$ Vitamin D group/$n = 17$ Control group	73 Y Vitamin D group/70 years Control group; 59.4% F	Grip strength using a grip force meter; skeletal muscle volume using bioelectrical impedance analysis	In Vitamin D group: SMI values significantly ↑; median change rates in the SMI were +5.8%; prevalence of sarcopenia significantly ↓from 80% to 33%

BCAA, branched-chain amino acid; SMI, skeletal muscle index; SMA, skeletal muscle area; CT, computed tomography; F, females; NA, not applicable; RCT, randomized-controlled trial; Y, years; ↑, increased; ↓, decreased; [a]*non-interventional retrospective studies where BCAA were previously administered according to current guideline in Japan.*

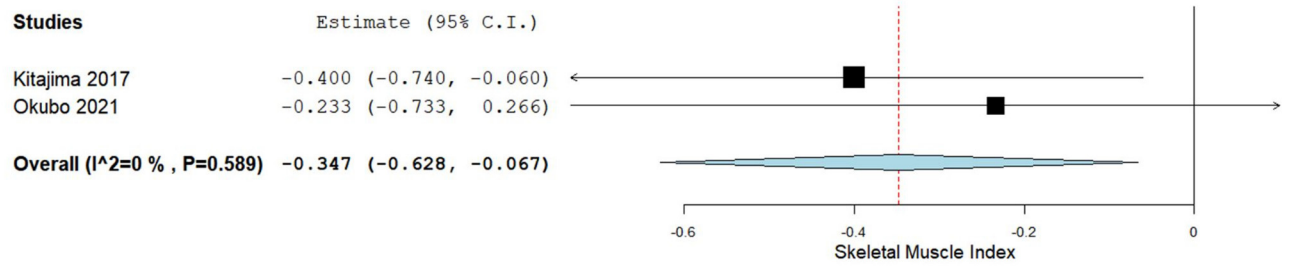

FIGURE 3 | Skeletal muscle index difference between baseline and post-BCAA supplementation groups.

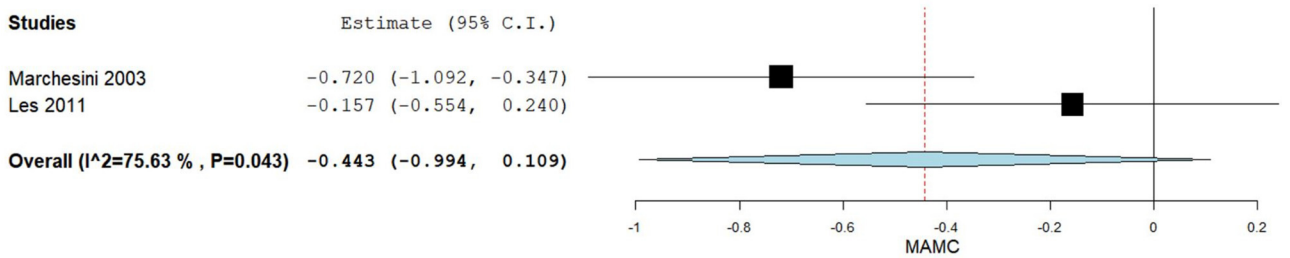

FIGURE 4 | Mid-arm muscle circumference post-intervention in BCAA group vs. M-DXT group.

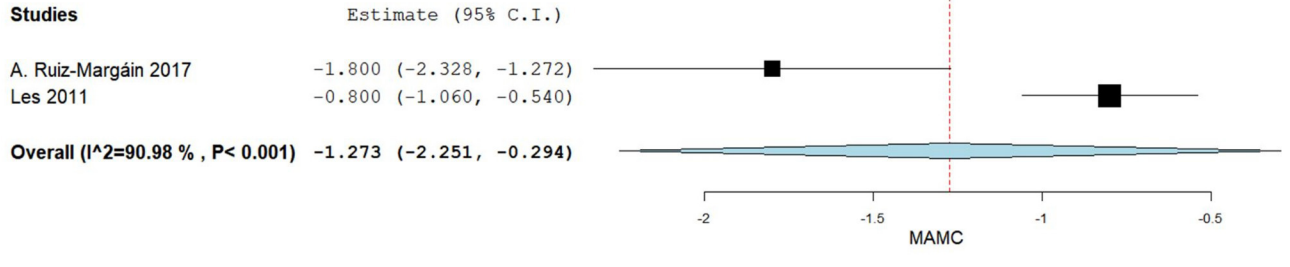

FIGURE 5 | Mid-arm muscle circumference difference between baseline and post-BCAA supplementation.

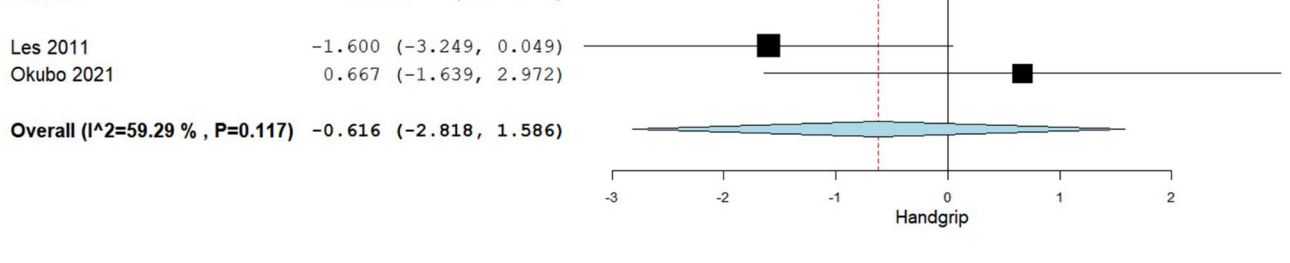

FIGURE 6 | Handgrip difference between baseline and post-BCAA supplementation.

Studies evaluated using the NHLBI quality assessment tool for observational cohort and cross-sectional studies were mainly rated as "fair" in four studies (38, 41, 43, 48) and one rated as "poor" (42). Generally, all included articles presented a clearly stated objective or research question. The study population was clearly specified and defined in three studies (38, 41, 48). The time frame was considered sufficient to reasonably expect seeing an association between BCAA supplementation and changes in

parameters in only one study (41). None of the five articles evaluated using this tool clearly stated that assessors were blinded to the exposure status of the participants. Only two studies assessed potential confounding variables and performed statistical adjustments for their impact (38, 48).

A total of three studies comparing baseline with post-intervention values were evaluated using the NHLBI Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group, with one article rated as "good" (44), one as "poor" (45), and one as "fair" (47). All three studies had a clear objective or research question, as well as clearly described eligibility criteria for the study population. All three articles used statistical methods to examine the outcome measure from before and after the intervention (44, 45, 47). Only one study did not assess the outcome measure multiple times after the underwent intervention (45).

DISCUSSION

Recently, the interest in sarcopenia in liver cirrhosis has increased significantly. Several published reviews evaluated the effects of interventions, including BCAA supplementation, on sarcopenia in patients with cirrhosis (11, 50, 51). However, none evaluated the effects of BCAA on anthropometric parameters assessing sarcopenia in this group population through a meta-analysis. To the best of our knowledge, this is the first systematic review and meta-analysis to evaluate the effects of supplementation with BCAAs on parameters assessing sarcopenia in liver cirrhosis. We included a total of eleven studies in our qualitative synthesis with a total population of 1,215, mainly Asian individuals, and to a lesser extent, Caucasians and Hispanics. They participated in four RCTs and seven observational studies. Furthermore, we included five studies in our quantitative synthesis, in which we demonstrated a significant improvement in SMI and MAMC parameters following BCAA supplementation, compared to non-BCAA group or baseline values, without significant improvement in handgrip and triceps subcutaneous fat. Although M-DXT was shown to slightly increase MAMC more than BCAA supplementation, no statistically significant difference was found between both groups. Moreover, handgrip was slightly better in subjects receiving BCAA supplementation compared to DXT, but without a statistically significant difference between both groups.

The new guidelines emphasize the importance of performing a functional evaluation and muscle mass quantification for evaluating sarcopenia (52). Anthropometric measures such as MAMC and triceps subcutaneous fat have been used and remain of significant importance in daily practice, reported to correlate with lean muscle mass and body fat, with an acceptable predictive value (53, 54). Nevertheless, measurement errors can occur due to lack of inter-observer agreement, as well as reduced accuracy in case of fluid overload (55). Several tools have been proposed to quantify muscle mass in clinical practice, including hand grip strength, assessed by recording the mean value of the dominant arm gripping a dynamometer in three consecutive measurements, and the chair stand test (CST), evaluated by counting the number of times the patient is able to rise fully to a standing position and subsequently sitting down in 30 seconds, without using their hands (56). Although hand grip strength was reported as an independent factor of mortality (57), on cross-sectional imaging, it was found to weakly correlated with muscle mass and quality (58). Currently, the skeletal muscle area (SMA) can be obtained using CT scan with a specific software (56). Afterwards, skeletal muscle index (SMI) in cm^2/m^2 can be obtained, being easily performed as the abdomen is evaluated to diagnose liver cirrhosis, while being able to discriminate between ascites and soft tissues.

Recently, researchers have attempted to find strategies that can help decrease the increased anabolic turnover rate of muscle that is age-related (59). Protein is made up of amino acids, possibly inducing a muscle protein anabolic response that is according to the availability of BCAAs, including leucine, isoleucine, and valine (37, 60). In elderly subjects, anabolic resistance and delayed absorption of amino acid absorption can be seen (61). Decreased muscle mass and function, as well as weaker muscle strength in the elderly have been associated with decreased BCAA levels (62). Moreover, aerobic exercise was found to contribute to the inductions of mitochondrial biogenesis and dynamics, mitochondrial metabolism restoration, as well as decreases the catabolic genes expression and increases muscle protein synthesis (63). Furthermore, an important strategy for preventing muscle wasting includes resistance exercise that was reported to strengthen muscle mass and function through stimulating muscle hypertrophy and improving muscle strength (64). Therefore, combining aerobic and resistance exercises can provide a greater benefit, providing a partial solution to sarcopenia. Accordingly, several studies reported that BCAA supplementation, including leucine, isoleucine, valine, or essential amino acids, in addition to aerobic and low-intensity resistance training can attenuate sarcopenia and stimulate muscle protein synthesis, even in the bedrest confined elderly subjects (37).

We believe that our results need to be further discussed. Firstly, most involved participants were from Asia, with a limited number of Caucasians and Hispanics. Moreover, ethnicities such as African Americans were not included in any of the studies evaluating parameters of sarcopenia in patients with cirrhosis. Therefore, the obtained results cannot be generalized to other ethnicities that have not been evaluated yet in the currently published evidence.

In our meta-analysis, we demonstrated that several parameters, including SMI and MAMC were improved following BCAA supplementation. Nevertheless, handgrip and triceps subcutaneous fat did not improve significantly. Although current evidence demonstrated that supplementation with BCAAs such as leucine, valine, and isoleucine could ameliorate protein synthesis, lipid, and glucose metabolism, as well as insulin resistance and hepatocyte proliferation, in addition, reduce oxidative stress in hepatocytes in liver cirrhosis, several published studies reported no significant improvement in muscle strength or mass post-intervention with BCAA supplementation (51, 65). This can be partially explained by the short intervention duration and small sample size (40, 66–68), or a specific subgroup of LC with albumin $\leq$3.5 g/dL (44, 45). Furthermore, administration timing, dose, and nutritional

education regarding BCAA supplementation are also considered essential factors that might lead to suboptimal results if not properly performed. The present systematic review and meta-analysis evaluates parameters used to assess sarcopenia and did not assess sarcopenia as its presence or absence. However, it is important to assess the effects of BCAAs on these parameters, used to evaluate sarcopenia in patients with liver cirrhosis. Any findings in this respect can help gather evidence on how to treat sarcopenia as well.

According to the quality assessment of included studies in our systematic review and meta-analysis, most evaluated studies using both NHLBI quality assessment tools were rated as "poor" and "fair," while only one study was evaluated as "good." Moreover, half of the included RCTs presented an unclear risk of allocation concealment bias, in addition to unclear risk of reporting bias and other possible sources of bias in all included RCTs. Another point to consider is that several studies did not perform statistical adjustments for potential confounding variables. Therefore, the obtained results from studies with poor and fair methodological quality should be cautiously interpreted.

Most included studies were of observational design and not interventional. Therefore, more interventional studies are required in order to confirm the possible causality between BCAA supplementation and improvements in parameters assessing sarcopenia in patients with cirrhosis. Furthermore, we were not able to perform subgroup analysis evaluating variables according to the etiology of liver cirrhosis or administrated BCAA supplementation due to limited available data.

Our systematic review and meta-analysis has several limitations that need to be addressed. Multiple included studies in this review are of observational design. Therefore, causality between BCAA supplementation and improvement or worsening of sarcopenia assessed parameters in cirrhosis cannot be confirmed or negated according to these studies. Although several parameters assessing sarcopenia, including muscle mass, function, and strength evaluation, were conducted in the included studies in our review, most studies had a different grouping of the involved participants or interventions performed, leading to a very limited number of studies that were possibly included in our quantitative synthesis. Furthermore, due to a limited number of published articles assessing several parameters used to assess sarcopenia in liver cirrhosis patients receiving BCAA supplements, we could only assess two studies for each association. The pre-post comparisons encountered in some meta-analyses are subject to possible biases, being less desirable to classic between-arms comparisons. Short intervention intervals and small sample sizes can also lead to suboptimal results. Accordingly, future research involving larger populations with longer interventional intervals involving BCAA supplementation is deemed necessary. Due to possible methodological flaws in included studies, results should be interpreted with caution.

Nevertheless, our systematic review and meta-analysis also has several important strengths. We believe that this topic is of important clinical significance due to the increased prevalence of liver cirrhosis and secondary sarcopenia, leading to more complications and higher morbidity and mortality rates. In this review, we point out several problems in current studies that should be remediated in future studies. We also performed a comprehensive search in several electronic databases while meta-analytically summarizing the current literature regarding this topic in a non-biased manner. To the best of our knowledge, this is the first systematic review and meta-analysis to assess the effects of BCAA supplementation on sarcopenia evaluated parameters in liver cirrhosis.

CONCLUSIONS AND FUTURE DIRECTIONS

Improvements in several parameters used to assess sarcopenia in liver cirrhosis patients, including skeletal muscle index, mid-arm muscle circumference, were seen following BCAA supplementation. However, no improvements were seen in handgrip and triceps subcutaneous fat. Nonetheless, due to the imperfect methodological quality of the evaluated articles, interpretation of the obtained results should be performed with caution.

Future interventional studies, mainly better methodologically conducted RCTs, with larger sample sizes and longer interventional intervals evaluating the effects of BCAA supplementation on parameters used to assess sarcopenia in liver cirrhosis patients from different ethnicities, remains necessary. Possible improvements in quality of life, survival, mobility, and cardiopulmonary performance, in addition to reduced infection rates and favorable metabolic outcomes in liver cirrhosis patients with sarcopenia, can be obtained if future RCTs confirm our reported findings. Moreover, a clear consensus defining sarcopenia in liver cirrhosis patients is required.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

D-CL and DD had the idea of the manuscript and made substantial contributions to the conception and critically revised the manuscript for important intellectual content. AI and D-CL independently applied the search strategy, performed the study selection, and risk of bias assessment. CB and AF performed the data extraction. AI drafted the manuscript. D-CL, CB, AF, S-LP, and DD contributed to the writing of the manuscript. All authors revised the final manuscript and approved the final version.

SUPPLEMENTARY MATERIAL

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Association of Serum Vitamin C With NAFLD and MAFLD Among Adults in the United States

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Background and Aims: Despite the remarkable progress of metabolic dysfunction-associated fatty liver disease (MAFLD), formerly named non-alcoholic fatty liver disease (NAFLD), the disease remains poorly improved. Since increased oxidative stress and inflammation contribute to the initiation and progression of fatty liver disorders, vitamin C (VC), an antioxidant agent, might be a suitable treatment option for MAFLD. However, the lack of clinically confirmed benefits makes clinicians challenging to recommend antioxidant supplements for MAFLD individuals.

Methods: Herein, the nationally representative National Health and Nutrition Examination Survey 2017–2018 data were collected to evaluate the potential association between the serum VC levels with the risk of different categories of NALFD and the newly proposed MAFLD terminology. Hepatic steatosis was defined as controlled attenuated parameter scores $\geq$ 263 dB/m, whereas liver fibrosis (LF) status was defined as F0–F4, with the cutoff values of median liver stiffness being 6.3, 8.3, 10.5, and 12.5 (KPa), respectively. A cross-sectional analysis was performed to calculate the odds rate and determine the potential beneficial effects of VC.

Results: A total of 4,494 participants aged more than 18 years and conducted transient elastography examinations were included. Our findings demonstrated that participants with increased serum VC status were more likely to be female predominant, more educated, and moderate drinkers. Interestingly, female participants tended to have a lower prevalence of NAFLD, MAFLD, LF, and liver cirrhosis (LC) after stratification by gender. Moreover, our results revealed that participants from the quartile three group (quartile 3: 50.5–67.0 μmol/L) experienced a slightly lower risk of MAFLD than the risk of NAFLD. Of note, the serum concentration of VC (quartile 2: 30.9–50.5 μmol/L) inversely associated with LF and LC was lower than the serum VC level (quartile 3) associated with NAFLD and MAFLD. Notably, individuals from the quartile 3 group experienced a statistically significant 32.5, 42.0, 45.7, and 71% decrease in risk of NAFLD, MAFLD, LF, and LC, respectively.

Conclusion: In summary, our findings suggested an inverse association between serum VC levels and NAFLD, MAFLD, LF, or LC. Additionally, adjustment of VC supplementation according to age, gender, and ethnicity may be a promising candidate for these diseases.

Keywords: non-alcoholic fatty liver disease, liver fibrosis, liver cirrhosis, vitamin C, metabolic dysfunction-associated fatty liver disease

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a public health problem affecting approximately a quarter of the global population and has been the fastest-growing cause of liver cancer in the United States (1, 2). Despite remarkable progress, this condition remains poorly improved, and effective therapeutic strategies remain elusive. According to the recent consensus, international experts redefined NAFLD as metabolic dysfunction-associated fatty liver disease (MAFLD) *to* establish more clear diagnostic criteria (3). Compared with NAFLD, MAFLD is a broader disease entity that requires the presence of metabolic abnormalities, including obesity and diabetes. The proposed new term from NAFLD to MAFLD is not simply a change to a more appropriate name but also a shift in the populations who meet the criteria for one but not the other. This change highlights the unmet clinical need to investigate the association between promising treatments with those only meeting criteria for MAFLD but not the traditional NAFLD. Accordingly, determining the association of potential treatment strategies with both NAFLD and MAFLD may help to deepen our understanding and application of this new concept (4).

Non-alcoholic fatty liver disease encompasses a continuum of liver disorders, ranging from hepatic steatosis to steatohepatitis (NASH), liver fibrosis (LF), and liver cirrhosis (LC) (5, 6). It is estimated that ∼37% of NASH will develop fibrosis, and subsequently, 10–20% of them will develop cirrhosis. Within 5–7 years, 40–60% of cirrhosis can develop into liver failure, and 2.4–12% of cirrhosis eventually progress into hepatocellular carcinoma (HCC) within 3–7 years (7). Although the prognosis is poor, recent studies have shown that mild to moderate LF is reversible, developing after years of NASH with hepatic inflammation. Furthermore, it is generally assumed that the transition from steatosis to NASH is crucial for disease progression, leading to cirrhosis and HCC. For this reason, researchers have focused on steatohepatitis to develop new preventing and reversing strategies. Mechanically, progression from steatosis to NASH and hepatic fibrosis is driven by a series of liver damage resulting from lipid deposition, reactive oxidative species (ROS), nitrogen oxides overload, endoplasmic reticulum stress, and inflammation, which ultimately lead to the activation of hepatic stellate cells, fibrogenesis, and extracellular matrix deposition (8).

In view of the antioxidant function of vitamin C (VC), it could be beneficial in NASH. Previous studies have demonstrated a vicious cycle of deficient balance between oxidant generation and antioxidant defense, leading to liver dysfunctions. Recent studies reported that free fatty acids typically overload in steatosis, resulting in continuous adaptation and further remodeling of structure, mitochondrial bioenergetics, and energy metabolism. Furthermore, the fatty liver tends to be vulnerable to injury, especially when challenged by oxidative stress and lipid peroxidation. The dysfunctional mitochondria in NAFLD are concurrent to incomplete lipid oxidation, leading to the accumulation of lipotoxic lipids, which further activates inflammation, promoting the transition from steatosis to NASH (9–11). Therefore, ROS and inflammation are critical factors in the stepwise progression from simple steatosis to LF and LC. Thus, VC potentially contributes to the alleviation of ROS imbalance and its concomitant pro-inflammatory actions postulated to initiate NASH or cirrhosis.

However, it is difficult for clinicians to recommend the use of antioxidative substances due to the paucity of data on clinically confirmed or definitive physical benefits of VC supplements among patients with MAFLD. Moreover, based on the National Health and Nutrition Examination Survey (NHANES) III data, a recent study found that MAFLD had a greater risk for all-cause mortality, while NAFLD showed no association (3). Hence, assessing the serum VC levels associated with different categories of NAFLD and the proposed term MAFLD may illuminate the potential utility of antioxidative substances between the two entities.

To our knowledge, this is the first study determining the association between serum VC levels with different categories of NALFD and MAFLD using a representative national cohort.

METHODS

Study Population

Data for the current study were collected from NHANES 2017–2018, in which liver ultrasound Transient Elastography (TE) examination was undertaken. NHANES is a nationally representative cross-sectional study designed to examine demographic, socioeconomic, health, and nutrition information. Detailed characterization of NHANES has been reported in previous studies (12). A total of 9,254 participants completed the survey during 2017–2018. However, in the current study, individuals aged <18 years and without complete TE were excluded ($N = 4{,}508$). In addition, subjects with unavailable data for the controlled attenuated parameter (CAP) or median liver stiffness (LSM) were excluded from the current study ($N = 1$). In addition, participants with missing data on VC were excluded from analysis ($N = 251$). As a result, 4,494 participants were included in the final analysis (**Figure 1**). Written informed consents were acquired from all study participants and the study protocols were approved by the Research Ethics Review Board of the National Center for Health Statistics. In addition, specific informed consent was not required because of the secondary analysis of public data. The current report was also written based on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (13).

Primary Exposure

During NHANES 2017–2018, participants aged 6 years and older were eligible for serum VC examination. A detailed description of laboratory methodology for serum VC detection has been

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline Phosphatase; ALB, albumin; BMI, body mass index; CAP, controlled attenuation parameter; *CI*, confidence interval; CRP, C-reactive protein; GGT, gamma glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; LF, liver fibrosis; LC, liver cirrhosis; LSM, median liver stiffness; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; TC, total cholesterol; TB, total bilirubin; VC, vitamin C.

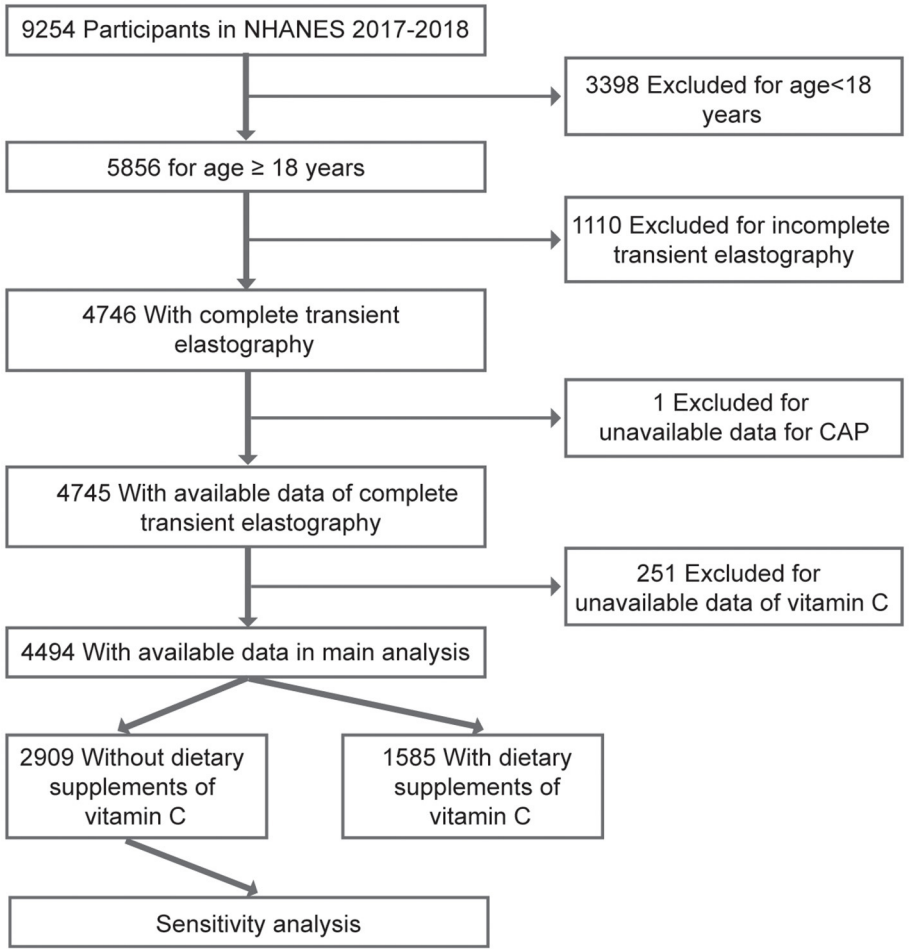

FIGURE 1 | A flowchart showing the selection of study participants. CAP, controlled attenuation parameter.

reported in previous studies (14, 15). A total of 6,740 participants, aged older than 6 years, completed this examination, whereas 695 participants failed to complete the examination. Serum VC levels were categorized into evenly distributed quartiles (<30.9 μmol/L, 30.9–50.5 μmol/L, 50.5–67.0 μmol/L, and ≥67.0 μmol/L).

Outcomes

Liver ultrasound TE using FibroScan model 502 V2 Touch was first undertaken on NHANES 2017–2018 participants to examine hepatic steatosis and stiffness. TE is a widely used and reliable method to evaluate liver steatosis and fibrosis (16, 17). Participants aged over 12 years old were eligible except for persons (pregnant, could not lie, or had an implanted electronic device/lesion at the examination site). Only subjects with complete tests [fasting time ≥3 h, complete stiffness tests ≥10 measures, and interquartile range (IQR) of liver stiffness/LSM <30%] were included in the current study. Of 4,494 included participants, 3,311 (73.68%) used a medium (M) probe while 1,183 (26.32%) used a large (XL) probe. Herein, hepatic steatosis was defined as CAP scores ≥ 263 dB/m (18), whereas LF status was defined as F0–F4, with the cutoff values of LSM being 6.3, 8.3, 10.5, and 12.5 (KPa), respectively (19).

Non-alcoholic fatty liver disease was diagnosed as the presence of hepatic steatosis without significant alcohol consumption (>3 drinks/day in men and >2 drinks/day in women) and/or viral hepatitis (hepatitis B virus [HBV] or hepatitis C virus [HCV] infections). Individuals with HCV or HBV infections were identified based on positive diagnostic tests (20, 21) or self-reported infection. MAFLD was defined on the basis of steatosis with at least one of the following conditions: (i) body mass index (BMI) ≥ 25 kg/m^2; (ii) type 2 diabetes which was defined as having a self-reported history of diagnosis with type 2 diabetes or glycohemoglobin ≥ 6.5% (22); (iii) at least 2 of metabolic risk abnormalities below, which included: (i) waist circumference ≥88 cm for women and ≥102 cm for men, (ii) high blood pressure (≥130/85 mmHg) or drug treatment for hypertension, (iii) plasma triglycerides ≥1.70 mmol/L or drug treatment for hyperglyceridemia, (iv) plasma HDL-cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women or drug treatment for

hypercholesterolemia, (v) prediabetes (fasting glucose 5.6–6.9 mmol/L or hemoglobin A1c 5.7–6.4%, (vi) homeostasis model assessment of insulin resistance score ≥2.5, and (vii) plasma high sensitivity C-reactive protein (CRP) level >2 mg/L (3, 23). Significant LF and LC were defined as LSM ≥ 6.3 KPa (fibrosis grade ≥ F1) and LSM ≥ 12.5 KPa (fibrosis grade ≥ F4), respectively (19, 24).

Covariates

In the current study, covariates were ascertained based on known confounders from previously described methods and clinical practice. First, dietary supplements of VC taken from multivitamins or other medications during the past 30 days were considered as "yes/no" variable or in daily (0, 1–60, 61–120, 121–500, or ≥500 mg), or monthly (0, 1–1,800, 1,801–3,600, or ≥3,600 mg) doses (15). The level of dietary VC intake by food was categorized into evenly distributed quartiles (<18.5 g/d, 18.5–47.1 mg/d, 47.1–106.5 mg/d, and ≥106.5 mg/d) and adjusted in the final model. Then, demographic factors including age, gender, and race were selected. The current study classified age into three categories, namely 18–39, 40–59, and 60–80 years. In NHANES 2017–2018, race/ethnicity was classified into Hispanic (such as all Hispanics), non-Hispanic White (such as whites with no Hispanic origin), non-Hispanic Black (such as blacks with no Hispanic origin), non-Hispanic Asian (such as Asians with no Hispanic origin), or other races, including Alaska Natives or American Indians, Native Hawaiians or other Pacific Islanders, and multiracial individuals. Furthermore, BMI (weight/height^2) was categorized as under/normal weight (<25.0 kg/m^2), overweight (25.0–30.0 kg/m^2), and obesity (≥30.0 kg/m^2).

In addition, alcohol consumption was classified as none, moderate (1 drink/day for women or 1–2 drinks/day for men), heavy (2–3 drinks/day for women or 3–4 drinks/day for men), or binge (≥4 drinks/day for women or ≥5 drinks/day for men) according to definitions from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) in the National Institute of Health. Smoking was classified based on serum cotinine levels into low (<0.015 ng/ml), moderate (0.015–3 ng/ml), and high levels (>3 ng/ml) (25). Moreover, based on Physical Activity Guidelines recommendation of ≥75 min/week of vigorous or ≥150 min/week of moderate physical activity, participants were classified into three groups, namely active (≥the level of recommended activity), less active (<the level of recommended activity), and inactive (no physical activity) (26). The poverty income ratio (ratio of family income to poverty threshold) and was categorized as <1.3, 1.3–1.8, and >1.8. Furthermore, the level of education for participants (more than high school, high school, and less than high school) was also established through interviews.

Statistical Analysis

Continuous variables were described as weighted mean ± SD and compared using weighted linear regression. Categorical variables were expressed as weighted percentages (95% *CI*) and compared using the chi-square test. Multivariate logistic regression models were constructed to assess the association between VC and NAFLD, MAFLD, LF, or LC. The final model was adjusted for most or all these variables, including gender, age, race, the poverty income ratio, level of education, BMI, serum cotinine levels, daily alcohol consumption, history of diabetes, HBV infection, HCV infection, physical activity status, dietary supplements of VC taken, and level of dietary VC intake by food for different diseases. In addition, subgroup analysis was performed to evaluate the influence of age, gender, race, or BMI on the outcome.

Given that some participants may have high serum VC concentrations because they took high doses of supplements of VC by other multivitamins or medications, we thus adjusted for VC supplementation in the main analysis using the yes/no variable, and daily or monthly doses in a sensitivity analysis. Still for sensitivity analysis, logistic regression was performed again in which we excluded the participants from included subjects who took any additional supplements of VC.

All statistical analyses were undertaken by R software (http://www.R-project.org, The R Foundation, Austria), Empowerstats (http://www.empowerstats.com, X&Y Solutions, Inc, CA, USA), and STATA 16.0 (StataCorp, College Station, TX, USA). Appropriate examination weights were applied to represent the complex survey design. Moreover, two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Participants

A total of 4,494 participants were included, of whom 49.15% were male and 50.85% were female, with an average age of 47.13 years old. Overall characteristics of the study subjects by quartiles of serum VC are summarized in **Table 1**. There were 37.23% NAFLD, 47.98% MAFLD, 21.23% significant LF, and 3.08% LC, among all participants. Statistically significant differences were observed in most outcomes across quartiles of serum VC concentrations, except for HBV infection, aspartate aminotransferase (AST), and total bilirubin (TB) ($p > 0.05$). Besides, those with higher levels of serum VC tended to be female predominant, non-Hispanic White, moderate drinkers, and had the lowest BMI. Subjects with increased serum VC levels were female predominant, more educated, moderate-drinkers, and had the lowest serum cotinine level (<0.015 ng/ml). Conversely, participants with decreased serum VC levels were 40∼59 years, male predominant, less educated, and had a higher prevalence of diabetes and obesity. Moreover, the current study observed inverse associations of serum VC levels with the CRP level and prevalence of MAFLD and LF. In contrast, no significant trends were observed for physical activity levels.

Given that our findings on gender predominant are of particular interest, data were further stratified by gender, suggesting a lower prevalence of NAFLD, MAFLD, LF, and LC among women (**Supplementary Table 1**).

Associations Between VC and NAFLD or MAFLD

Participants with higher blood VC levels had a decreased risk of NAFLD or MAFLD. Associations of serum VC levels with the risk

TABLE 1 | General characteristics of participants ($n = 4494$) stratified by vitamin C (quartiles1-4, umol/L) in the NHANES 2017–2018.

Characters	Total ($n = 4494$)	Quartiles 1 (<30.9) ($n = 1121$)	Quartiles 2 (30.9–50.5) ($n = 1124$)	Quartiles 3 (50.5–67.0) ($n = 1096$)	Quartiles 4 (≥67.0) ($n = 1153$)	*p*-Value
Age (years)	47.13 ± 17.49	45.94 ± 16.74	45.26 ± 16.81	46.20 ± 17.18	50.73 ± 18.49	<0.001
18∼39	38.37 (36.27–40.52)	37.77 (33.67–42.04)	43.13 (38.86–47.50)	40.96 (36.54–45.52)	32.43 (28.63–36.47)	
40∼59	34.09 (31.92–36.34)	37.20 (32.88–41.74)	34.44 (30.14–39.00)	33.28 (28.92–37.95)	31.60 (27.48–36.03)	
60∼80	27.53 (25.65–29.50)	25.04 (21.62–28.80)	22.43 (19.08–26.18)	25.76 (22.02–29.90)	35.97 (32.01–40.12)	
Gender						<0.001
Men	49.15 (46.93–51.38)	56.73 (52.36–61.00)	54.39 (49.96–58.75)	54.16 (49.57–58.68)	32.74 (28.74–37.00)	
Women	50.85 (48.62–53.07)	43.27 (39.00–47.64)	45.61 (41.25–50.04)	45.84 (41.32–50.43)	67.26 (63.00–71.26)	
Race/ethnicity						<0.001
Hispanic	16.44 (15.27–17.68)	11.94 (10.05–14.12)	21.67 (18.95–24.66)	18.16 (15.70–20.90)	14.61 (12.56–16.93)	
Non-Hispanic White	62.61 (60.71–64.47)	68.11 (64.57–71.44)	52.34 (48.01–56.64)	59.94 (55.84–63.91)	68.74 (65.41–71.88)	
Non-Hispanic Black	10.54 (9.75–11.39)	10.52 (9.07–12.16)	13.43 (11.60–15.49)	10.64 (9.05–12.48)	7.97 (6.70–9.46)	
Non-Hispanic Asian	5.67 (5.18–6.21)	3.48 (2.76–4.38)	7.08 (5.93–8.43)	6.27 (5.26–7.46)	5.99 (5.06–7.08)	
Other races[a]	4.74 (3.91–5.73)	5.96 (4.23–8.35)	5.49 (3.70–8.06)	4.99 (3.45–7.15)	2.69 (1.72–4.19)	
Education						<0.001
More than high school	60.93 (58.80–63.02)	50.49 (46.10–54.88)	59.56 (55.24–63.74)	64.28 (59.93–68.41)	68.93 (65.04–72.57)	
High school or equivalent	27.61 (25.66–29.64)	35.19 (31.08–39.54)	26.48 (22.68–30.66)	26.54 (22.68–30.78)	22.35 (19.02–26.09)	
Less than high school	11.39 (10.40–12.46)	14.31 (12.04–16.94)	13.74 (11.58–16.24)	9.13 (7.55–11.00)	8.68 (7.18–10.46)	
Not recorded	0.07 (0.03–0.16)	-	0.21 (0.07–0.62)	0.05 (0.01–0.21)	0.03 (0.00–0.25)	
Poverty-income ratio						<0.001
<1.3	18.19 (16.85–19.61)	22.48 (19.73–25.49)	19.44 (16.56–22.68)	15.73 (13.25–18.58)	15.32 (13.02–17.94)	
1.3–1.8	8.20 (7.35–9.15)	9.52 (7.70–11.72)	8.24 (6.53–10.33)	8.24 (6.61–10.22)	6.89 (5.51–8.58)	
>1.8	63.57 (61.60–65.51)	57.76 (53.63–61.79)	60.92 (56.72–64.97)	67.96 (64.08–71.61)	67.29 (63.57–70.81)	
Not recorded	10.04 (8.88–11.32)	10.23 (8.07–12.89)	11.40 (8.87–14.55)	8.07 (6.20–10.44)	10.50 (8.32–13.17)	
BMI group						<0.001
<25	27.60 (25.63–29.65)	23.73 (20.23–27.63)	23.31 (19.68–27.37)	25.69 (21.80–30.00)	36.76 (32.65–41.08)	
25–30	31.50 (29.47–33.60)	21.96 (18.65–25.67)	30.99 (27.07–35.19)	39.11 (34.66–43.76)	33.91 (29.93–38.13)	
≥30	40.40 (38.24–42.60)	53.36 (48.97–57.70)	45.34 (40.98–49.78)	34.89 (30.69–39.33)	28.95 (25.25–32.95)	
Not recorded	0.50 (0.33–0.77)	0.96 (0.48–1.88)	0.36 (0.14–0.92)	0.31 (0.14–0.66)	0.38 (0.15–1.01)	
Physical activity level						<0.001
Inactive	50.77 (48.54–53.00)	49.74 (45.34–54.14)	46.94 (42.56–51.36)	49.34 (44.73–53.96)	56.39 (52.03–60.65)	
Less active	7.43 (6.31–8.73)	5.93 (4.27–8.18)	7.94 (5.71–10.94)	8.61 (6.25–11.75)	7.33 (5.30–10.05)	
Active	41.80 (39.61–44.02)	44.33 (40.00–48.75)	45.12 (40.74–49.58)	42.05 (37.56–46.68)	36.28 (32.19–40.58)	
Daily alcohol drinking status						<0.001
Non-drinkers	7.53 (6.50–8.71)	7.49 (5.52–10.08)	6.82 (5.25–8.82)	7.22 (5.18–9.98)	8.48 (6.48–11.03)	
Moderate-drinkers	29.66 (27.58–31.82)	26.48 (22.50–30.89)	27.55 (23.53–31.97)	31.72 (27.43–36.34)	32.58 (28.64–36.79)	
Heavy-drinkers	14.48 (13.00–16.09)	14.77 (11.84–18.27)	15.70 (12.65–19.32)	12.95 (10.37–16.07)	14.57 (11.85–17.79)	
Binge-drinkers	32.79 (30.72–34.94)	34.86 (30.89–39.05)	36.68 (32.46–41.12)	33.61 (29.29–38.22)	26.7 (22.92–30.86)	
Not recorded	15.54 (14.15–17.03)	16.40 (13.62–19.61)	13.25 (11.17–15.64)	14.50 (11.94–17.50)	17.66 (14.69–21.09)	
History of diabetes						<0.001
Yes	12.82 (11.54–14.22)	16.57 (13.68–19.93)	12.87 (10.62–15.51)	11.31 (8.96–14.19)	10.62 (8.43–13.29)	
Having HBV infection						0.105
Yes	0.94 (0.62–1.41)	0.70 (0.39–1.28)	1.31 (0.52–3.27)	1.33 (0.66–2.66)	0.47 (0.25–0.87)	
Having HCV infection						0.011
Yes	2.49 (1.83–3.38)	2.36 (1.57–3.53)	3.09 (1.67–5.64)	1.58 (0.79–3.13)	2.95 (1.52–5.64)	
Dietary VC supplement						<0.001
Yes	38.03 (35.85–40.27)	13.24 (10.29–16.87)	33.25 (29.05–37.74)	40.6 (36.08–45.29)	63.37 (59.19–67.36)	
Daily dose of supplement of VC, mg						<0.001
None	74.68 (72.60–76.65)	94.96 (92.92–96.44)	82.60 (78.57–86.01)	74.21 (69.83–78.16)	48.96 (44.65–53.28)	

(Continued)

TABLE 1 | Continued

Characters	Total (n = 4494)	Quartiles 1 (<30.9) (n = 1121)	Quartiles 2 (30.9–50.5) (n = 1124)	Quartiles 3 (50.5–67.0) (n = 1096)	Quartiles 4 (≥67.0) (n = 1153)	p-Value
1–60	10.08 (8.81–11.52)	2.73 (1.70–4.36)	9.67 (7.21–12.86)	11.48 (8.88–14.72)	16.13 (13.17–19.61)	
60–120	5.03 (4.04–6.26)	0.86 (0.37–2.00)	3.85 (2.28–6.44)	6.71 (4.46–9.96)	8.45 (6.17–11.48)	
121–500	5.62 (4.60–6.84)	0.58 (0.25–1.32)	2.21 (1.01–4.77)	5.29 (3.45–8.05)	13.65 (10.81–17.09)	
>500	4.59 (3.70–5.68)	0.87 (0.34–2.20)	1.66 (0.82–3.34)	2.30 (1.42–3.71)	12.81 (9.97–16.30)	
Monthly dose of supplement of VC, mg						<0.001
None	67.90 (65.74–69.98)	89.95 (86.74–92.45)	71.67 (67.40–75.58)	65.05 (60.46–69.37)	46.31 (42.04–50.63)	
1–1800	15.28 (13.75–16.94)	7.32 (5.18–10.26)	16.58 (13.59–20.09)	17.08 (13.93–20.76)	20.05 (16.85–23.69)	
1800–3600	5.79 (4.75–7.06)	1.55 (0.71–3.36)	6.19 (4.13–9.18)	8.07 (5.59–11.50)	7.38 (5.42–9.96)	
>3600	11.03 (9.66–12.57)	1.18 (0.57–2.42)	5.56 (3.76–8.16)	9.81 (7.42–12.86)	26.27 (22.49–30.43)	
Dietary VC intake by food, mg/d						<0.001
0–18.5	22.73 (20.96–24.61)	36.05 (31.97–40.33)	23.24 (19.63–27.27)	18.21 (15.08–21.82)	13.86 (11.23–16.97)	
18.5–47.1	25.62 (23.66–27.68)	33.68 (29.63–37.99)	29.16 (25.25–33.40)	23.15 (19.16–27.67)	17.19 (14.00–20.94)	
47.1–106.5	24.44 (22.53–26.45)	17.65 (14.52–21.29)	26.48 (22.51–30.86)	24.37 (20.68–28.49)	29.20 (25.31–33.41)	
>106.5	21.68 (19.94–23.52)	8.27 (6.13–11.08)	15.62 (13.08–18.55)	28.66 (24.65–33.03)	33.16 (29.29–37.26)	
Not recorded	5.53 (4.72–6.47)	4.35 (2.81–6.67)	5.51 (4.26–7.11)	5.62 (4.22–7.43)	6.60 (4.88–8.86)	
Laboratory parameters						
Smoking (serum cotinine levels, ng/ml)						<0.001
<0.015	38.17 (35.97–40.42)	27.60 (23.52–32.09)	34.35 (30.23–38.71)	42.38 (37.80–47.09)	47.61 (43.29–51.97)	
0.015–3	37.41 (35.31–39.56)	35.32 (31.24–39.62)	40.29 (36.04–44.69)	36.37 (32.13–40.84)	37.90 (33.88–42.09)	
≥3	24.04 (22.27–25.90)	36.86 (32.89–41.01)	24.47 (20.95–28.37)	20.83 (17.43–24.70)	14.47 (11.77–17.68)	
Not recorded	0.37 (0.15–0.90)	0.23 (0.09–0.58)	0.89 (0.20–4.00)	0.42 (0.18–0.95)	0.01 (0.00–0.10)	
ALT (U/L)	23.26 ± 17.77	25.13 ± 22.03	24.21 ± 17.75	22.14 ± 12.84	21.71 ± 16.99	<0.001
AST (U/L)	22.31 ± 13.41	22.96 ± 18.55	22.15 ± 11.94	22.06 ± 10.16	22.05 ± 11.24	0.304
ALB (g/L)	41.02 ± 3.21	40.36 ± 3.36	40.99 ± 3.26	41.30 ± 3.00	41.40 ± 3.12	<0.001
ALP (U/L)	76.34 ± 25.33	82.37 ± 30.58	77.45 ± 24.94	73.62 ± 21.67	72.19 ± 21.92	<0.001
GGT (U/L)	29.71 ± 40.06	36.16 ± 55.87	34.02 ± 43.07	24.79 ± 25.32	24.46 ± 27.20	<0.001
TC (mmol/L)	4.88 ± 1.04	4.89 ± 1.07	4.92 ± 1.11	4.80 ± 0.97	4.91 ± 1.02	0.017
TB (umol/L)	8.14 ± 4.83	7.89 ± 4.81	8.11 ± 4.62	8.40 ± 4.98	8.17 ± 4.88	0.095
CRP (mg/L)	3.72 ± 7.16	5.14 ± 9.05	3.99 ± 8.43	3.10 ± 5.64	2.72 ± 4.46	<0.001
Platelet (×10^9/L)	244.79 ± 61.42	247.07 ± 62.84	248.08 ± 62.13	244.39 ± 63.13	240.15 ± 57.40	0.010
Transient Elastography						
Median stiffness (kPa)	5.66 ± 4.73	6.64 ± 6.79	5.48 ± 3.97	5.34 ± 4.36	5.18 ± 2.71	<0.001
Controlled attenuation parameter (dB/m)	262.47 ± 62.70	277.89 ± 66.53	266.90 ± 60.68	257.71 ± 58.25	248.41 ± 60.94	<0.001
NAFLD						<0.001
Yes	37.23 (35.13–39.39)	43.76 (39.44–48.18)	37.25 (33.21–41.48)	33.91 (29.79–38.28)	34.11 (30.10–38.37)	
MAFLD						<0.001
Yes	47.98 (45.76–50.21)	57.39 (52.98–61.68)	51.24 (46.80–55.66)	44.13 (39.65–48.71)	39.81 (35.63–44.15)	
Liver fibrosis						<0.001
Yes	21.23 (19.48–23.08)	30.58 (26.72–34.74)	20.47 (17.41–23.90)	17.15 (14.01–20.83)	16.79 (13.55–20.62)	
Liver cirrhosis						<0.001
Yes	3.08 (2.35–4.02)	6.41 (4.44–9.16)	2.26 (1.28–3.96)	1.66 (0.98–2.80)	1.93 (0.82–4.51)	

Values are weighted mean ± SD or weighted % (95% confidence interval). P-values are weighted. [a]Other races include American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, and multiracial persons.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline Phosphatase; ALB, albumin; BMI, body mass index; CRP, C reactive protein; GGT, gamma glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; MAFLD, metabolic dysfunction-associated fatty liver disease; NHANES, National Health and Nutrition Examination Survey; NAFLD, non-alcoholic fatty liver disease; TC, total cholesterol; TB, total bilirubin; VC, vitamin C.

of NAFLD and MAFLD are presented in **Tables 2**, **3**, respectively. For each model, there were statistically significant associations between VC concentrations and a reduced risk of NAFLD in Q3 [full adjustment, odds ratio (OR) = 0.675, 95% CI: 0.495–0.920], and MAFLD in Q3–Q4 [full adjustment, Q3: OR = 0.580(95% CI: 0.434–0.774); Q4: OR = 0.490(95% CI: 0.362–0.665)].

TABLE 2 | Associations between serum vitamin C level and NAFLD ($n = 4494$), NHANES 2017–2018.

	NAFLD (Yes, $n = 1802$)	Model 1 OR (95% CI), *P*	Model 2 OR (95% CI), *P*	Model 3 OR (95% CI), *P*
Quartiles of vitamin C, umol/L				
Q1 (<30.9)	491	Reference	Reference	Reference
Q2 (30.9–50.5)	485	0.763 (0.593,0.981) 0.035	0.777(0.593,1.019) 0.068	0.756 (0.557,1.027) 0.073
Q3 (50.5–67.0)	444	0.659 (0.508,0.855) 0.002	0.647(0.494,0.846) 0.002	0.675 (0.495,0.920) 0.013
Q4 (≥67.0)	382	0.665 (0.515,0.860) 0.002	0.617(0.470,0.810) 0.001	0.774 (0.556,1.076) 0.128
P trend	-	<0.001	<0.001	<0.001
Sensitivity analysis after exclusion of participants with dietary vitamin C supplement (None, $n = 2909$)				
Quartiles of vitamin C, umol/L	$N = 1136$			
Q1 (<30.9)	418	Reference	Reference	Reference
Q2 (30.9–50.5)	332	0.796 (0.600,1.055) 0.112	0.829(0.609,1.130) 0.236	0.789 (0.561,1.111) 0.175
Q3 (50.5–67.0)	247	0.575 (0.423,0.782) <0.001	0.592 (0.433,0.809) 0.001	0.578 (0.403,0.829) 0.003
Q4 (≥67.0)	139	0.510 (0.358,0.727) <0.001	0.541(0.379, 0.771) 0.001	0.683 (0.455,1.024) 0.065
P trend	-	<0.001	<0.001	<0.001

Model 1: Non-adjusted model; Model 2 adjusted for: gender; age; race; Model 3 adjusted for: gender; age; race; education; BMI; diabetes; physical activity status; serum cotinine levels; dietary vitamin C supplement, dietary vitamin C intake by food, and poverty income ratio.
NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; 95% CI, 95% confidence interval.

TABLE 3 | Associations between serum vitamin C level and MAFLD ($n = 4494$), NHANES 2017–2018.

	MAFLD (Yes, $n = 2230$)	Model 1 OR (95% CI), *P*	Model 2 OR (95% CI), *P*	Model 3 OR (95% CI), *P*
Quartiles of vitamin C, umol/L				
Q1 (<30.9)	638	Reference	Reference	Reference
Q2 (30.9–50.5)	611	0.780 (0.607,1.004) 0.053	0.790 (0.603,1.036) 0.088	0.811 (0.613,1.074) 0.144
Q3 (50.5–67.0)	535	0.587 (0.454,0.758) <0.001	0.572 (0.438,0.747) <0.001	0.580 (0.434,0.774) <0.001
Q4 (≥67.0)	446	0.491(0.382,0.632) <0.001	0.478 (0.366,0.624) <0.001	0.490 (0.362,0.665) <0.001
P trend	-	<0.001	<0.001	<0.001
Sensitivity analysis after exclusion of participants with dietary vitamin C supplement (None, $n = 2909$)				
Quartiles of vitamin C	$N = 1455$			
Q1 (<30.9)	547	Reference	Reference	Reference
Q2 (30.9–50.5)	424	0.779 (0.589,1.031) 0.081	0.788 (0.582,1.068) 0.125	0.808 (0.593,1.101) 0.177
Q3 (50.5–67.0)	314	0.589 (0.437,0.793) <0.001	0.597 (0.438,0.812) 0.001	0.602(0.434,0.833) 0.002
Q4 (≥67.0)	170	0.391 (0.279,0.549) <0.001	0.421 (0.298,0.595) <0.001	0.420 (0.291,0.607) <0.001
P trend	-	<0.001	<0.001	<0.001

Model 1: Non-adjusted model; Model 2 adjusted for: gender; age; race; Model 3 adjusted for: gender; age; race; education; alcohol; HBV infection; HCV infection; physical activity status; serum cotinine levels; dietary vitamin C supplement, dietary vitamin C intake by food, and poverty income ratio.
NHANES, National Health and Nutrition Examination Survey; MAFLD, metabolic dysfunction-associated fatty liver disease; OR, odds ratio; 95% CI, 95% confidence interval.

In fully adjusted models, participants from the Q3 group experienced a 32.5% lower risk for NAFLD and 42% lower risk for MAFLD.

Given that 38.03% of participants were VC supplement users, the significant association between blood VC levels and the risk of NAFLD or MAFLD may be explained by dietary

VC supplements. Furthermore, according to **Table 1**, serum VC concentrations were positively associated with dietary VC supplements. To verify this possibility, a sensitivity analysis was performed. Notably, the results remained largely unchanged among participants who did not take VC supplements in all models. In fully adjusted models, similar associations between VC concentrations and a reduced risk of NAFLD [full adjustment, Q3, *OR* = 0.578, 95% *CI*: 0.403–0.829], or MAFLD [full adjustment, Q3, *OR* = 0.602(95% *CI*: 0.434–0.833); Q4, *OR* = 0.420(95% *CI*: 0.291–0.607)] were still present after sensitivity analysis. The conclusions remained unchanged when we further adjusted for daily or monthly doses of VC supplements instead of a yes/no variable. These results of the sensitivity analysis were compatible with the data shown above, further confirming our findings.

As presented in **Supplementary Tables 2**, **3**, subgroup analysis revealed that participants who were among 18∼39 years old [full adjustment: NAFLD (Q3, *OR* = 0.541, 95% *CI*: 0.330–0.888; Q4, *OR* = 0.529, 95% *CI*: 0.302–0.927), MAFLD (Q3, *OR* = 0.535, 95% *CI*: 0.352–0.814; Q4, *OR* = 0.342, 95% *CI*: 0.211–0.554)], and non-Hispanic Asian [full adjustment: NAFLD (Q3, *OR* = 0.296, 95% *CI*: 0.149–0.586; Q4, *OR* = 0.197, 95% *CI*: 0.092–0.422); MAFLD (Q3, *OR* = 0.305, 95% *CI*: 0.157–0.590; Q4, *OR* = 0.194, 95% *CI*:0.095–0.397)] had significantly reduced risks of developing both NAFLD and MAFLD in Q3–Q4 groups. After stratifying data by gender, women from Q3 group had a 40.5% reduced risk of NAFLD, while there was no significant association between serum VC levels and NAFLD among men. For Q3 group, an ∼50.4% lower and 33.9% lower risk of MAFLD had been found in women and men, respectively. When analyses were stratified by BMI, findings indicated a statistical association of VC with decreased risk of both NAFLD (Q3, *OR* = 0.613, 95% *CI*: 0.399–0.941) and MAFLD (Q3, *OR* = 0.610, 95% *CI*: 0.376–0.989) among participants with BMI $\geq$ 30 kg/m^2. Moreover, participants who were among 40–59 years old [full adjustment: Q3 (*OR* = 0.464, 95% *CI*: 0.265–0.814), Q4 (*OR* = 0.516, 95% *CI*: 0.291–0.915)], and Non-Hispanic White [full adjustment: Q3 (*OR* = 0.492, 95% *CI*: 0.325–0.745), Q4 (*OR* = 0.495, 95% *CI*: 0.324–0.756)] also had a significantly reduced risk of developing MAFLD in Q3–Q4 groups.

Associations Between VC and Significant Fibrosis or LC

The associations of serum VC levels with risks of LF and LC are presented in **Tables 4**, **5**, respectively. In all models and quartiles, inverse associations of VC concentrations and the risk of LF or LC were observed. The fully adjusted *OR*s across quartiles of serum VC concentrations were 1.00 (reference), 0.606 (95% *CI*: 0.451–0.814), 0.543 (95% *CI*: 0.391–0.752), and 0.597 (95% *CI*: 0.400–0.889) for significant LF, and 1.00 (reference), 0.276 (95% *CI*: 0.142–0.534), 0.290 (95% *CI*: 0.139–0.605), and 0.312 (95% *CI*: 0.136–0.717) for LC. Notably, individuals from the Q3 group showed a 45.7% reduced risk of LF and a 71.0% reduced risk of LC ($p \leq 0.001$). Of note, the serum concentration of VC (Q2: 30.9–50.5 μmol/L) inversely associated with LF and LC was lower than the serum VC level (Q3: 50.5–67.0 μmol/L) associated with NAFLD and MAFLD. Similar and significant results were observed in the sensitivity analysis, except for LF in Q4.

In subgroup analysis (**Supplementary Table 4**), the serum VC level [full adjustment: Q2 (*OR* = 0.561, 95% *CI*: 0.381–0.826), Q3 (*OR* = 0.452, 95% *CI*: 0.291–0.703)] that was inversely associated with LF was relatively lower in men than the VC concentration [full adjustment: Q4 (*OR* = 0.515, 95% *CI*: 0.295–0.902)] in women. Similarly, the concentration of serum VC that was inversely associated with LF among participants with BMI < 30 kg/m^2 [BMI < 25 kg/m^2: (Q3, *OR* = 0.378, 95% *CI*: 0.156–0.919); BMI 25–30 kg/m^2: (Q3, *OR* = 0.386, 95% *CI*: 0.195–0.766)] was lower than participants with BMI $\geq$ 30 kg/m^2 (Q4, *OR* = 0.536, 95% *CI*: 0.323–0.887). The serum concentration of VC statistically associated with LF was lowest in the 18–39 age group [full adjustment: Q2 (*OR* = 0.458, 95% *CI*: 0.286–0.732), Q3 (*OR* = 0.338, 95% *CI*: 0.192–0.594)], intermediate in the 40–59 age group [full adjustment: Q3 (*OR* = 0.516, 95% *CI*: 0.289–0.921)], and highest in the 60–80 age group [full adjustment: Q4 (*OR* = 0.396, 95% *CI*: 0.212–0.740)]. Subgroup analysis of the association between VC levels and cirrhosis were not performed because of the small sample size in that category.

DISCUSSION

The European Association for the Study of the Liver lifestyle recommended modifications toward a healthy diet and regular exercise for people with NAFLD, while suggested pharmacotherapy should be reserved for people with NASH (27). However, biological complexity and incomplete understanding of NAFLD and MAFLD complicated evidence-based clinical recommendations for VC administration. The present study found that serum VC concentrations were statistically associated with reduced risks of NAFLD, MAFLD, LF, and LC after adjusting for the corresponding risk factors and sensitivity analysis. Of note, the serum concentration of VC inversely associated with LF and LC was lower than the serum VC level associated with NAFLD and MAFLD. Given that the newly proposed MAFLD terminology identified a cohort of individuals with a wider range of metabolic traits, our finding that participants from the Q3 group experienced a slightly lower risk of MAFLD than the risk of NAFLD has major clinical implications.

Our finding that the female sex is associated with a lower prevalence of NAFLD, MAFLD, LF, and LC is of particular interest. Given that the baseline level of VC may impact the benefits of VC administration, sensitivity analysis and gender stratification were conducted. Subsequent analysis revealed that the sex predominant might be partly due to the higher dietary VC supplement among women. Interestingly, a step-like change in the VC concentration associated with LF and LC when stratified by age, suggesting dose adjustment according to age. Another important finding in our study was that participants with obesity and diabetes tended to have lower serum VC levels, which is relevant, as prior studies have shown that NAFLD is particularly common among people with obesity and diabetes (28, 29). The most striking novel finding is the potential hepatoprotective effects of VC, especially for BMI $\geq$ 30 subjects against NAFLD,

TABLE 4 | Associations between serum vitamin C level and significant liver fibrosis ($n = 4494$), NHANES 2017–2018.

	Significant liver fibrosis (Yes, $n = 1071$)	Model 1 OR (95% CI), P	Model 2 OR (95% CI), P	Model 3 OR (95% CI), P
Quartiles of vitamin C, umol/L				
Q1 (<30.9)	357	Reference	Reference	Reference
Q2 (30.9–50.5)	280	0.584 (0.444,0.769) <0.001	0.590 (0.446,0.780) <0.001	0.606 (0.451,0.814) 0.001
Q3 (50.5–67.0)	229	0.470 (0.346,0.638) <0.001	0.468 (0.344,0.638) <0.001	0.543(0.391,0.752) <0.001
Q4 (≥67.0)	205	0.458 (0.334,0.628) <0.001	0.468 (0.333,0.657) <0.001	0.597 (0.400,0.889) 0.011
P trend	-	<0.001	<0.001	<0.001
Sensitivity analysis after exclusion of participants with dietary vitamin C supplement (None, $n = 2909$)				
Quartiles of vitamin C, umol/L	$N = 705$			
Q1 (<30.9)	304	Reference	Reference	Reference
Q2 (30.9–50.5)	184	0.494 (0.362,0.675) <0.001	0.512 (0.373,0.703) <0.001	0.531 (0.379,0.743) <0.001
Q3 (50.5–67.0)	139	0.471 (0.326,0.681) <0.001	0.485 (0.334,0.704) <0.001	0.584 (0.394,0.866) 0.007
Q4 (≥67.0)	78	0.443 (0.274,0.717) 0.001	0.499 (0.304,0.820) 0.006	0.719 (0.414,1.247) 0.240
P trend	-	<0.001	<0.001	<0.001

Model 1: Non-adjusted model; Model 2 adjusted for: gender; age; race; Model 3 adjusted for: gender; age; race; education; alcohol; diabetes; HBV infection; HCV infection; physical activity status; serum cotinine levels; dietary vitamin C supplement; dietary vitamin C intake by food; BMI, and poverty income ratio.
NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval.

TABLE 5 | Associations between serum vitamin C level and liver cirrhosis, NHANES 2017–2018.

	Liver cirrhosis (Yes, $n = 138$)	Model 1 OR (95% CI), P	Model 2 OR (95% CI), P	Model 3 OR (95% CI), P
Quartiles of vitamin C, umol/L				
Q1 (<30.9)	61	Reference	Reference	Reference
Q2 (30.9–50.5)	28	0.338 (0.169,0.678) 0.002	0.356 (0.178,0.715) 0.004	0.276 (0.142,0.534) <0.001
Q3 (50.5–67.0)	28	0.247 (0.128,0.477) <0.001	0.251 (0.130,0.483) <0.001	0.290 (0.139,0.605) 0.001
Q4 (≥67.0)	21	0.288 (0.111,0.748) 0.011	0.303 (0.103,0.892) 0.030	0.312 (0.136,0.717) 0.006
P trend	-	<0.001	<0.001	<0.001
Sensitivity analysis after exclusion of participants with dietary vitamin C supplement (None, $n = 2909$)				
Quartiles of vitamin C, umol/L	$N = 85$			
Q1 (<30.9)	51	Reference	Reference	Reference
Q2 (30.9–50.5)	14	0.203 (0.094,0.436) <0.001	0.212 (0.101,0.441) <0.001	0.206 (0.095,0.444) <0.001
Q3 (50.5–67.0)	16	0.236 (0.102,0.546) 0.001	0.246(0.108,0.564) 0.001	0.279 (0.095,0.821) 0.020
Q4 (≥67.0)	4	0.108 (0.032,0.358) <0.001	0.112(0.034,0.370) <0.001	0.146 (0.042,0.509) 0.003
P trend	-	<0.001	<0.001	<0.001

Model 1: Non-adjusted model; Model 2 adjusted for: gender; age; race; Model 3 adjusted for: gender; age; race; education; alcohol; diabetes; HBV infection; HCV infection; physical activity status; serum cotinine levels; dietary vitamin C supplement; dietary vitamin C intake by food; BMI, and poverty income ratio.
NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval.

MAFLD, and significant fibrosis. These findings are highly important as the epidemic trend of NAFLD has been rising rapidly in recent decades and is increasing in parallel with obesity and diabetes worldwide (28). With a higher burden of metabolic dysregulation, such as obesity and diabetes, it is not surprising that our study found that participants with

higher serum VC status had a lower risk of developing MAFLD compared with NAFLD.

It has been reported that the optimum plasma level is about the concentration of saturation (70 μmol/L) (30), which is consistent with our findings that serum VC concentration of 50.5–67.0 μmol/L was associated with decreased risks of NAFLD, MAFLD, LF, and LC. Surprisingly, no correlation was found between the highest quartile of VC and the risk of NAFLD. Additionally, participants in the highest quartile of VC had a slightly higher risk of MAFLD, LF, and LC than those in the 3rd quartile of VC. These data may be partly due to the dual action of VC, which tends to function as a pro-oxidant and contributes to tissue damage at higher concentrations (31, 32). Moreover, several studies have reported that only high doses of VC are associated with liver injury during chronic stress conditions in animal models (33, 34).

Our findings of the inverse association between serum VC levels and a spectrum of liver diseases ranging from MAFLD to LF and LC are in line with prior studies. A recent experimental study has shown that VC treatment decreased high-fat diet-induced NAFLD in mice and had hepatocellular protective effects evidenced by significant weight loss, ballooned hepatocytes, lobular inflammation, and ameliorative liver steatosis (35). To date, research in serum VC levels and NAFLD or MAFLD is scarce, and only two prior studies have found similar associations between VC intake and NAFLD (36, 37). Dana et al. demonstrated that dietary VC intake is inversely associated with lower risks of NAFLD and NASH among 789 subjects (37). However, this analysis might be limited due to the inadequate sample size and inaccurately ultrasonography detection of NAFLD. Furthermore, these findings of dietary VC intake based on recall questionnaires are less reliable due to the absorption obstacles in the gastrointestinal tract, which limited its promising application as a therapeutic agent. Compared with oral VC administration, studies with serum VC levels are often of high quality because circulating VC levels were rarely determined, and therefore, bioavailability and clinical practice could be verified. Notably, we further analyzed the association of serum VC concentrations with the newly proposed MAFLD.

Our novel finding is consistent with previous study findings that VC alleviates inflammation. The subsequent inflammatory environment is a vital contribution to severe NAFLD progression. Several previous studies averred that VC inhibits inflammatory responses mediated by tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) (38, 39). In addition, studies have indicated that VC potentially reduces inflammatory status through alleviation of CRP and IL-6 (40). Consistent with previous studies, findings of the current study indicated an inverse association between serum VC levels with CRP concentration. Interestingly, Seoung-Woo Lee et al. (41) proposed dual roles of VC in early stages of NAFLD and inflammatory steatohepatitis, and findings indicated that VC deficiency significantly inhibited progression of NAFLD by impairing *de novo* lipogenesis, whereas VC supplementation attenuated inflammatory injuries, including ballooning and lobular inflammation. Therefore, targeted modulation of antifibrotic activity aimed to alleviate the inflammatory environment is a potential therapeutic and preventive strategy against NASH.

Merits of the current study include serum measurement of VC (compared with dietary recall questionnaires) along with representative US civilian data in NHANES. Moreover, this relatively large sample of adults with the TE examination provided opportunities for the study of weak associations (42). The novelty of the present study includes the application of more accurately defined NAFLD using TE compared with an examination of NAFLD using non-invasive algorithms reported in previous studies (43). The current study undertook a detailed stratified analysis, sensitivity analysis, and adjusted for major potential interactions between VC and NAFLD. However, the current study has some limitations. Since the current study adopted a cross-sectional design, temporality cannot be fully clear, which limited the inferences on causes and effects. However, the indicated inverse association between VC with NAFLD is having a reasonable agreement with previous studies on the relationship of VC with fatty liver disease, metabolic syndrome, and inflammation. Another limitation of the current study was the use of TE for diagnosis of NAFLD. Although TE examination is probably the most validated non-invasive method to evaluate liver stiffness (16), the current study lacked histological confirmation. However, TE is considerably an accurate technique, which has been recommended by the World Federation for Ultrasound in Medicine and Biology to distinguish between non-significant and significant fibrosis (44).

CONCLUSION

In conclusion, the findings of the current study indicated that increased serum VC concentrations are associated with reduced risks of NAFLD, MAFLD, significant LF and LC. This implies that individuals with MAFLD may benefit from VC supplements. Further studies, including prospective cohort studies, are recommended to identify the clinical significance of VC treatment and prevention of MAFLD.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://wwwn.cdc.gov/nchs/nhanes/.

AUTHOR CONTRIBUTIONS

Z-QX and H-XL contributed to the conception and design, the acquisition, analysis, interpretation of the data, the drafting of the article, or critical revision for important intellectual content. W-LT, LY, X-WM, W-XL, and Q-BW collected data. C-ZS and Y-JC contributed to the conception and design, the reviewing of the article, or critical revision for important intellectual

content. All authors approved the final version and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.795391/full#supplementary-material

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Assessment of Sarcopenia Related Quality of Life Using SarQoL® Questionnaire in Patients With Liver Cirrhosis

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Introduction: Sarcopenia, malnutrition, physical deconditioning, and frailty contribute to a significantly altered quality of life (QoL) in patients with cirrhosis and sarcopenia.

Aim: To investigate the sarcopenia-linked alterations of QoL by SarQoL® questionnaire in patients with end-stage liver disease.

Methods: Consecutive patients with liver cirrhosis, admitted to our department between May and August 2021, completed the SarQoL® questionnaire by themselves. They were evaluated for sarcopenia according to the 2019 European Working Group on Sarcopenia in Older People (EWGSOP) definition [hand grip cut-offs and skeletal muscle index (SMI) calculation at CT scan].

Results: A total of 71 patients with liver cirrhosis were included in the study, with a median age of 54 years. Sarcopenia was present in 31.2% of patients with Child-Pugh class A, in 58.3% with class B, and in 93.5% with class C. The SarQoL® score was statistically significant and lower in Child-Pugh class C vs. class B and class A (70.2 vs. 66.5 vs. 52.5 points, $p = 0.0002$). The SarQoL® score was evaluated according to different complications of cirrhosis, with statistically significant lower scores in patients with sarcopenia ($p < 0.0001$), in patients with ascites requiring paracentesis ($p = 0.0006$), and in patients with hepatic encephalopathy ($p < 0.0001$). A cut-off level of 75.9 points for SarQoL® score can accurately detect sarcopenia in patients with end-stage liver disease [area under the receiver operating characteristic (AUROC) curve of.823, SE of 92.1%, SP of 45.5%, positive predictive value (PPV) and negative predictive value (NPV) of 66 and 83.3%, respectively, correctly classified 73.2% of cirrhotic patients with sarcopenia].

Conclusions: The use of SarQoL® questionnaire in cirrhotic patients can, at the same time, evaluate the quality of life and identify subjects with sarcopenia and altered QoL.

Keywords: quality of life, sarcopenia, cirrhosis, liver, frailty

INTRODUCTION

The prevalence of sarcopenia in cirrhosis ranges from 30–70%, depending on the diagnostic tools utilised and the severity of the underlying liver disease (1). Sarcopenia is defined as a progressive and generalised skeletal muscle disorder associated with an increased likelihood of adverse outcomes including falls, fractures, disability, and mortality (2). It is associated with higher rates of complications of cirrhosis (especially hepatic encephalopathy and infections), hospital admissions, and premature mortality (3).

However, muscle strength is better recognised as being associated with adverse outcomes compared to low muscle mass (4).

Sarcopenia proved to risk-stratify patients with cirrhosis independent of the Child-Pugh score and the model for end-stage liver disease (MELD) score and thus may serve as an independent prognostic marker (5). It is associated with poorer clinical outcomes after liver transplantation (LT) (e.g., higher incidence of postoperative sepsis, neurological complications, ventilator support, rejection, length of ICU, and hospital stay and mortality) in addition to a reduced quality of life (QoL) and lack of functional independence (6).

Sarcopenia is the central and dominant component of frailty that influences pre-and post-liver transplant survival. The inclusion of functional measures in validated frailty metrics suggests that the influence of sarcopenia may be modified by factors related to muscle function rather than purely muscle mass according to Lai et al. (7).

There is a strong interplay between sarcopenia, malnutrition, physical deconditioning, and frailty in cirrhosis. All these contribute to a significantly altered QoL in patients with cirrhosis and sarcopenia (8). The health-related QoL of patients with end-stage liver disease is significantly impaired when compared to healthy controls or patients with chronic liver disease as was recently proven in a systematic review (9).

In comparison to other end-stage organ failure or terminal illnesses, the end-stage liver disease disproportionately affects younger age groups, and the years of life lost is estimated to be around 20 years (10, 11). Although these patients are vulnerable and at high risk of death, little attention has been paid to describing their symptom prevalence and health-related QoL.

This indicates the importance of preventive and interventional management strategies for managing patients with liver diseases with compensated or decompensated liver diseases by a multidisciplinary team.

The consequences of sarcopenia on QoL, disability, and mortality are important, and it is recommended that physicians should consider screening for sarcopenia in different settings (12). Only specific domains of QoL are impacted by sarcopenia. Therefore, generic tools may not be able to detect the subtle effects of sarcopenia on QoL. Usually, generic health-related QoL tools (the Medical Outcome Study Questionnaire 36-Item Short Form Survey) were more frequently administered than disease-specific health-related QoL tools (the Chronic Liver Disease Questionnaire) in cirrhosis. Given the diversity of symptoms and significantly impaired health-related QoL, a multidisciplinary approach and timely intervention are crucial (9). In 2015, Beaudart and colleagues (13) reported the development of the first disease-specific self-administrated sarcopenia-related QoL questionnaire, the SarQoL® questionnaire. The SarQoL® is a valid, consistent, and reliable tool that can be used for clinical and research purposes as it proved to be able to discriminate sarcopenic of non-sarcopenic subjects with regard to their QoL, regardless of the definition used for diagnosis as long as the definition includes an assessment of both muscle mass and muscle function.

The aim of our study was to investigate the sarcopenia-linked alterations of QoL by SarQoL® questionnaire in patients with end-stage liver disease.

METHODS

We included consecutive patients with liver cirrhosis admitted to the Hepatology Department of Fundeni Clinical Institute between May and August 2021. Liver cirrhosis was defined based on clinical, biochemical, abdominal ultrasound, and CT scan features. Patients completed the SarQoL® questionnaire by themselves in Romanian language and they were evaluated for sarcopenia according to the 2019 European Working Group on Sarcopenia in Older People (EWGSOP) definition: hand grip cut-offs $<$26 kg for men and $<$18 kg for women (4) and skeletal muscle index (SMI) calculation at CT scan. Patients were evaluated for the severity of liver disease (Child-Pugh and MELD-Na scores) and associated complications. An SMI with $<$ 39 cm^2/m^2 in women and with $<$ 50 cm^2/m^2 in men were used to define sarcopenia in cirrhosis. The Liver Frailty Index (LFI) was calculated for all patients. The LFI has established the cut-points to define robust (LFI $<$ 3.2), prefrail (LFI 3.2–4.3), and frail (LFI $\geq$ 4.4), according to the American Association for the Study of Liver Diseases (AASLD) guidelines on malnutrition, sarcopenia, and frailty in cirrhosis (2).

The SarQoL® Questionnaire

The SarQoL® questionnaire is a patient-reported outcome measure specific to sarcopenia in aged people. The SarQoL® questionnaire consists of 22 questions incorporating 55 items that fall into seven domains of health-related quality of life (HRQoL). These domains are "Physical and Mental Health," "Locomotion," "Body Composition," "Functionality," "Activities of Daily Living," "Leisure activities," and "Fears," and it takes 10 min to complete. It is available in 16 languages, including Romanian (14). Most questions (19 out of 22) use a Likert scale of frequency or intensity, among which the respondents choose the answer most applicable to them. Each domain is scored from 0 to 100, and an overall score is calculated. We obtained the official scoring algorithm from the developers of the SarQoL® questionnaire (13). The questionnaire has demonstrated its ability to differentiate between sarcopenic and non-sarcopenic subjects (discriminative power) (15).

The authors (13) that developed this questionnaire used the definition of sarcopenia based on the handgrip strength

TABLE 1 | Characteristics of the whole analysed cohort.

Characteristics	Population ($N = 71$)
Age (years)	
Mean	54.5 ± 12.6
Median	54 (22.3–83)
Gender	
Male	48 (67.6)
Female	23 (32.4)
Aetiology of liver cirrhosis	Alcohol 32.4% Hepatitis c 26.8% Hepatitis b 7.1% Hepatitis b and d 25.3% Other 8.4% (autoimmune, cholestatic diseases, Wilson disease)
Child-Pugh classification	45.1% class A 33.8% class B 21.1% class C
Mean MELD-Na score	14.9 ± 6.1
Mean BMI (body mass index) kg/m^2	26.4 ± 4.2
Mean SarQoL® score	64.9 ± 16.9

SMI assessed by Dual-energy X-ray absorptiometry (DEXA). However, in patients with cirrhosis, DEXA has major limitations due to its inability to differentiate water from muscle. In order to fulfil all the requirements of sarcopenia evaluation, we included in the definition the calculation of SMI at the CT scan performed routinely in our clinic for all patients with liver cirrhosis.

Our patients completed the questionnaire in their ward, in a quiet atmosphere, alone, after a period of 10 min of rest.

Statistical Analysis

Statistical analysis was performed using SPSS V20.0 (SAS Institute Inc., Cary, NC, USA) software. Continuous data are expressed as mean ± SD unless otherwise indicated. Categorical data are described as frequencies of the subjects with a specific characteristic. The comparison of categorical parameters was determined by the two-tailed χ^2-test or Fisher's exact test. Normally distributed continuous parameters were compared with Student's *t*-test, and non-normally distributed continuous parameters were compared with the Mann–Whitney *U* test. Univariate analysis was carried out to identify variables that were significantly different between patients with and without sarcopenia.

The Kruskal-Wallis test was performed for global comparison of quantitative variables.

The diagnostic value of the SarQoL® score for sarcopenia was assessed by calculating the area under the receiver operating characteristic (AUROC) curve. The diagnostic accuracy was calculated using sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio (LR). The cut-off value for SarQoL® score was chosen to maximise sensitivity in order to use it as a screening tool in patients with liver cirrhosis compensated or decompensated.

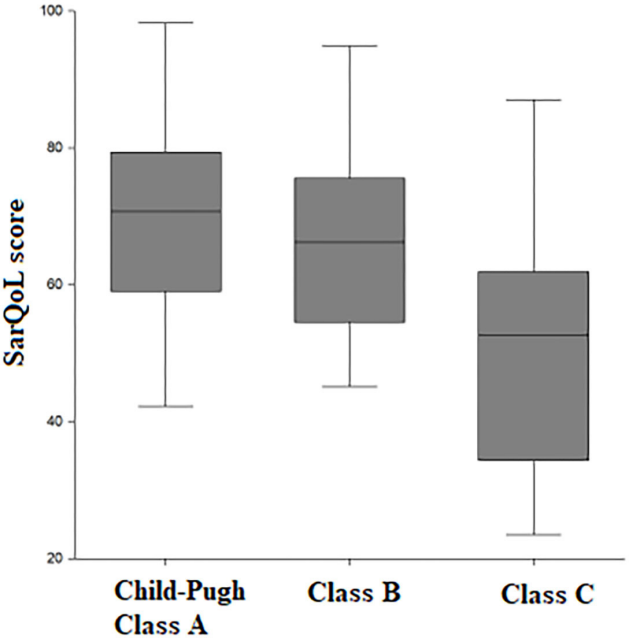

FIGURE 1 | Distribution of SarQoL score according to Child-Pugh score.

RESULTS

A total of 71 patients with liver cirrhosis were included in the study (**Table 1**); 53.5% of the included subjects fulfilled the criteria for sarcopenia according to the EWGSOP definition and measurement of skeletal muscle index (SMI) at CT scan. There was a statistically significant higher proportion of patients with sarcopenia as liver function worsened. Specifically, sarcopenia was present in 31.2% of patients with Child-Pugh class A, in 58.3% of patients with class B, and in 93.5% with class C ($p = 0.0003$). The SarQoL® score was statistically significantly lower in Child-Pugh class C vs. class B and class A (70.2 vs. 66.5 vs. 52.5 points, $p = 0.0002$) (**Figure 1**).

Patients were analysed according to the presence or absence of sarcopenia as is detailed in **Table 2**. Patients with liver cirrhosis and sarcopenia were statistically and significantly sicker compared to patients without sarcopenia, particularly, with higher MELD-Na and Child-Pugh scores, higher rate of infections, large/refractory ascites, and more episodes of hepatic encephalopathy, as well as lower values of platelets.

The SarQoL® score was evaluated according to different complications of cirrhosis, with statistically significant lower scores in patients with sarcopenia, in patients with ascites requiring paracentesis, and in patients with hepatic encephalopathy. The results are presented in **Table 3**. Even after we excluded patients with Child-Pugh C from the analysis, SarQoL® score was significantly lower in patients with sarcopenia (59.38 ± 10.95) vs. patients without sarcopenia and compensated or with early decompensation (75.58 ± 13.16 points, $p < 0.0001$). There was a statistically significant lower SarQoL® score in patients with LFI >3.2 (49.93 ± 13.44)

TABLE 2 | Summary of patients' characteristics according to presence or absence of sarcopenia.

Variable	Sarcopenia Yes ($n = 38$)	SarcopeniaNo($n = 33$)	*P* value
SarQoL® score	56.3 ± 14.7	74.9 ± 13.5	**<0.0001**
Age (years)	57.7 ± 11.8	50.9 ± 12.5	**0.02**
Weight (kg)	78.7 ± 15.9	77.8 ± 13.4	0.87
Body mass index (BMI) (kg/m^2)	26.2 ± 4.4	26.5 ± 3.9	0.75
Haemoglobin (g/dL)	10.9 ± 2.6	12.7 ± 2.1	**0.008**
MELD-Na score	17.4 ± 6.5	11.9 ± 3.7	**0.00008**
Child-Pugh score	8.6 ± 2.5	6.2 ± 1.3	**0.00001**
Haemoglobin (g/dL)	10.9 ± 2.6	12.7 ± 2.1	**0.008**
Albumin (g/dL)	3.2 ± 0.6	3.9 ± 0.6	**0.00002**
Total bilirubin (mg/dL)	3.2 ± 2.4	1.7 ± 1.2	**0.004**
Sodium (mmol/L)	134.9 ± 7.1	139 ± 3.2	**0.006**
Platelet count/mm^3	91.9 ± 47.5	127.4 ± 81.3	**0.04**
Lymphocytes/mm^3	1.07 ± 0.55	1.27 ± 0.54	0.08
LFI < 3.2	39.47%	93.94%	**<0.0001**
Hepatocellular carcinoma	36.84%	18.18%	0.08
Spontaneous bacterial peritonitis (SBP)	8.82%	3.13%	0.33
Acute kidney injury	6.06%	3.13%	0.57
Other infections (except SBP)	31.43%	3.13%	**0.002**
Portal vein thrombosis	16.67%	18.75%	0.82
Ascites-large/refractory	50%	12.12%	**0.0006**
Hepatic encephalopathy episodes	55.26%	6.06%	**0.00001**
Presence of clinically significant portal hypertension	21.62%	15.15%	0.48
Presence of diabetes mellitus	21.62%	9.38%	0.16
Included on the waiting list for liver transplantation	63.16%	45.45%	0.13
Time elapsed since diagnosis of cirrhosis (months)	83.5 + 67.2	83.9 + 67.2	0.78

Bold values are the variables that proved to be statistically significant different.

TABLE 3 | SarQoL® score distribution according to different complications of cirrhosis.

Type of complication of cirrhosis	SarQoL® score in patients with the specified complication	SarQoL® score in patients without the specified complication	*P* value
Hepatocellular carcinoma	62.32 ± 15.07	66.66 ± 17.16	0.19
Spontaneous bacterial peritonitis (SBP)	56.06 ± 30.59	66.26 ± 16.16	0.58
Acute kidney injury	63.60 ± 4.39	65.85 ± 17.21	0.77
Other infections (except SBP)	57.62 ± 18.06	67.28 ± 16.13	0.10
Portal vein thrombosis	91.9 ± 47.5	127.4 ± 81.3	0.71
Ascites-large/refractory	54.25 ± 14.87	70.53 ± 14.86	**0.0002**
Hepatic encephalopathy episodes	55.53 ± 17.23	69.48 ± 14.93	**0.002**
Presence of clinically significant portal hypertension	65.94 ± 19.07	64.99 ± 12.71	0.78

Bold values are the variables that proved to be statistically significant different.

compared to patients with LFI < 3.2 (73.13 ± 12.45, $p < 0.0001$). The AUROC curve was calculated for establishing the statistical performance of SarQoL® score for the diagnosis of sarcopenia (**Figure 2**). We found that a cut-off level of 75.9 points for SarQoL® score can accurately detect sarcopenia in patients with end-stage liver disease (AUROC of 0.823, SE of 92.1%, and SP of 45.5%, PPV and NPV of 66 and 83.3%, respectively, correctly classified 73.2% of cirrhotic patients with sarcopenia). After excluding patients with Child-Pugh class C cirrhosis, the cut-off value was ≤73.5 points for SarQoL® score with an AUROC of 0.827 with SE of 90.1%, SP of 56.1%, and PPV and NPV of 60.7 and 98.1%, respectively, correctly classified 78% of cirrhotic patients with sarcopenia and Child-Pugh class A and B.

DISCUSSION

In patients with cirrhosis evaluated for liver transplantation, sarcopenia and frailty are increasingly recognised as independent

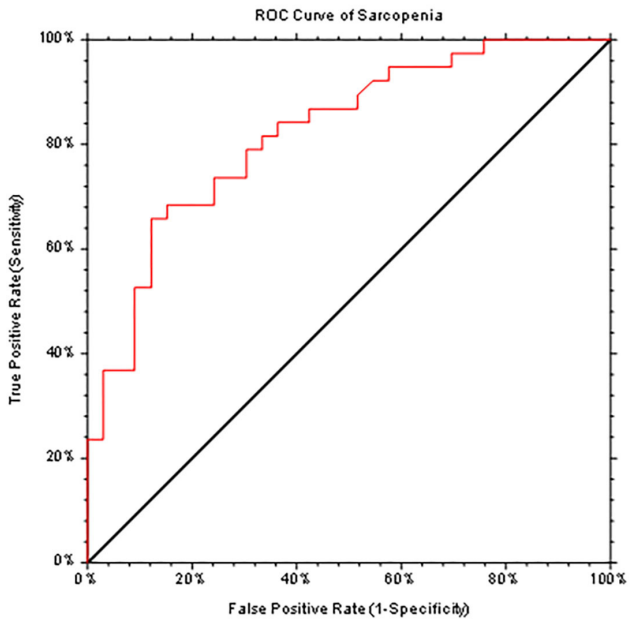

FIGURE 2 | ROC curve (AUC = 0.823) generated by SarQoL® score which accurately diagnoses sarcopenia in patients with end-stage liver diseases.

predictors of clinical outcomes including wait-list mortality and reduced survival after surgery. Evaluation of body mass composition and assessment of frailty are becoming increasingly important in the management of liver transplant candidates and recipients and their proactive treatment may improve outcomes (7). Sarcopenia represents an important economic and social burden, and improvements in QoL for people with liver cirrhosis and sarcopenia should be a priority for future interventions designed to prevent or treat sarcopenia (16). A positive frailty or sarcopenia screen should prompt evaluation for underlying etiologic risk factors and the development of an ambulatory personalised management plan as stated in the recent AASLD guideline (2).

The symptom prevalence of patients with end-stage liver disease resembles that of patients with other advanced conditions. The most frequently reported symptoms were pain, breathlessness, muscle cramps, sleep disturbance, depression, anxiety, and erectile dysfunction. Decompensation led to a significant worsening of health-related QoL as was previously demonstrated (9). In our study, patients with sarcopenia had significantly advanced liver failure and a significantly higher rate of important portal hypertension, but also a significantly lower QoL quantified by the SarQoL® score. The pathogenesis of sarcopenia is multifactorial and favouring factors are interrelated. There are several studies (5, 17–19) demonstrating that incorporating sarcopenia in the conventional prognostic factors had added value, particularly in compensated and early decompensated cirrhosis. This was confirmed by our study regarding the screening of patients with Child-Pugh A and B cirrhosis by SarQoL® questionnaire. Subclassification of prognostic factors according to sarcopenia may help to better assess the prognosis of cirrhosis and intervene through different strategies (exercise, nutrition, and psychological support).

Screening for sarcopenia by a simple easy-to-do questionnaire that reflects low QoL related to sarcopenia was never done. This is the first study that validated this SarQoL® score in end-stage liver disease and also proved to be a valuable tool for screening of patients with cirrhosis for sarcopenia (a good clinical value with an AUROC of >0.8, a SE >90%, and an NPV >90%). Gasparik et al. (20) already proved that the Romanian version of the SarQoL® questionnaire is conceptually and literally equivalent with the source instrument, qualified in terms of psychometric properties, and can be a useful tool for assessing a sarcopenia-related QoL among frail elderly individuals. A recent study (21) demonstrated that the SarQoL® questionnaire can discriminate between robust, pre-frail, and frail subjects with declining QoL scores according to the category of frailty. There was also a significant difference in our cohort between robust (LFI < 3.2) and prefrail/frail (LFI > 3.2) patients regarding the obtained SarQoL® score. In other words, the SarQoL® questionnaire is able to discriminate on more than just the physical aspects of QoL. Particularly, it brings extra precision in being able to discriminate between robust, frail, and pre-frail individuals. Despite this, it needs further investigation in this regard in patients with liver cirrhosis awaiting liver transplantation.

The strength of the study is represented by the first time use of SarQoL® score in liver cirrhosis and its correlation with advanced liver failure and complications of cirrhosis (mainly due to portal hypertension).

Our study revealed that, in patients with liver cirrhosis, even in those with low Child-Pugh and low MELD scores, QoL can be severely affected by the presence of sarcopenia. In addition, its identification and reversal are required by a combination of nutritional, physical, pharmacological, and psychological interventions in order to improve the prognosis of patients with end-stage liver disease. The SarQoL® questionnaire is an already validated tool for assessing sarcopenic individuals. It can easily and repeatedly be applied to prevent and assess response to therapy of sarcopenia in specific populations, especially in patients with liver cirrhosis who are of high priority for LT or in patients avoiding futile LT. One limitation of our study is the relatively small sample size; we intend to validate our cut-off for the SarQoL® score on another cohort evaluated in our Hepatology Department.

Hence, the SarQoL® questionnaire could establish the patients that would benefit mostly following a multidisciplinary approach and therapeutic interventions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Hospital Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent for all performed procedures was signed by patients at admittance into the hospital.

AUTHOR CONTRIBUTIONS

SI: conceptualization and Writing-original draft. SI and RV: methodology. SI and RI: statistical analysis. SI, VM, MM, VB, DB, and GI: data collection. SI, RI, CG, and LG: writing, reviewing, and editing. LG: visualization. All authors contributed to the article and approved the submitted version.

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