BMC Cancer

RESEARCH Open Access

Chitinase-3 like-protein-1, a prognostic biomarker in patients with hepatocellular carcinoma and concomitant myosteatosis

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Abstract

Background Chitinase-3 like-protein-1 (CHI3L1) is a member of the mammalian chitinase-like proteins and elevated serum CHI3L1 level has been proved to be associated with poor prognosis in hepatocellular carcinoma (HCC). This study aimed to investigate the relationship between serum CHI3L1 levels and body composition parameters in patients with HCC after liver transplantation (LT).

Methods This retrospective study enrolled 200 patients after LT for HCC. Blood samples were collected and serum concentrations of CHI3L1 were measured by enzyme-linked immunosorbent assay. Computer tomography (CT) were used to estimate skeletal muscle and adipose tissue mass. Spearman's rank correlation test was performed to assess associations between serum CHI3L1 levels and these body composition parameters. A Cox proportional-hazards regression model was performed to identify independent prognostic factors. Overall survival (OS) and recurrence-free survival (RFS) curves were constructed using the Kaplan-Meier method and compared by the log-rank test.

Results Total 71 patients (35.5%) were diagnosed with myosteatosis according to skeletal muscle radiation attenuation (SMRA). The 5-year OS rates were 66.9% in non-myosteatosis group, significantly higher than 49.5% in myosteatosis group ($p = 0.025$), while the RFS of myosteatosis group (5-year RFS: 52.6%) or non-myosteatosis group (5-year RFS: 42.0%) shown no significant difference ($p = 0.068$). The serum CHI3L1 level were significantly negative correlated with SMRA ($r = -0.3$, $p < 0.001$). Interestingly, in patients with myosteatosis, Kaplan-Meier analysis revealed that elevated serum CHI3L1 levels were associated with worse OS ($p < 0.001$) and RFS ($p = 0.047$). However, in patients

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without myosteatosis, Kaplan-Meier analysis found elevated serum CHI3L1 levels were not associated with OS ($p = 0.070$) or RFS ($p = 0.104$).

Conclusions Elevated CHI3L1 was negatively correlated with SMRA, and predicted poorer prognosis in Chinese population after LT for HCC, especially in those patients with concomitant myosteatosis. Monitoring serum CHI3L1 can predict prognosis and effectively guide individual nutrition intervention.

Keywords Myosteatosis, CHI3L1, Hepatocellular carcinoma, Liver transplantation, Computer tomography

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and one of the leading causes of cancer-related death worldwide [1–3]. Most HCC patients developed on the basis of cirrhosis, characterized by dysregulation of essential protein synthesis [4]. Many treatment options are available for patients with HCC, including liver transplantation (LT), surgical resection, percutaneous ablation, immunotherapy, transarterial and systemic therapies [5–8]. LT is the preferred treatment for unresectable HCC and it is the only treatment that can simultaneously treat HCC and underlying liver diseases [9–11]. About 25% of LTs are performed for underlying HCC in Western countries [12], and HCC accounts for 17–42% of LT in Asian [13, 14]. Progress has been made in identifying predictive factors for prognosis after LT and establishing models assessing prognosis [15–17].

Body composition, including the contents and distribution of adipose tissue and skeletal muscle, has been suggested to be associated with many cancer outcomes [18, 19]. Sarcopenia, defined as the presence of both low muscle mass and low muscle function [20], is widely recognized to be associated with the prognosis of multiple tumors. Myosteatosis, characterized by myocellular fatty infiltration, is associated with metabolic abnormalities and decreased muscle strength, which is associated with shorter survival in patients with various cancers [21]. However, the relationship between adipose mass and the prognosis of patients with cancer remains controversial [22, 23]. Body mass index (BMI), a representative indicator of body shape and the most commonly marker and the most widely used measured marker, was also shown the association with cancer prognosis [24], but it cannot distinguish between skeletal muscle and fat or independently assess their prognostic role. Computer tomography (CT) have been clinically widely used to estimate the contents and distribution of skeletal muscle and adipose tissue [25], which could also distinguish between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). Relevant parameters derived from CT image analysis have shown prognostic role to predict cancer-related outcomes [26–28].

Chitinase-3 like-protein-1 (CHI3L1) is a member of the mammalian chitinase-like proteins, which plays a key role in inflammation, tissue injury and repair, and remodeling responses and is associated with the processes of many diseases such as liver fibrosis, diabetes and asthma. In addition, CHI3L1 signal is closely related to the biological behavior of tumor including cancer cell growth, proliferation, invasion, metastasis and angiogenesis [29]. Although CHI3L1 is expressed in a variety of cells including macrophages, neutrophils, smooth muscle cells and tumor cells, it is a highly liver-enriched gene which may be a good marker of liver disease [30]. CHI3L1 may serve a serum biomarker cirrhosis and also highly expressed in HCC [31, 32]. More importantly, CHI3L1 can help to evaluate prognosis for HCC patients [33, 34] and our recent study found that CHI3L1 was up-regulated to protect skeletal muscle in sarcopenia patients with HCC [35], which indicated CHI3L1 may affect body composition.

In this setting, this study verified the prognostic role of CHI3L1 and involved a comprehensive assessment of body composition parameters according to CT image analysis to explore the association between serum CHI3L1 levels and these parameters in patients with HCC after LT.

Patients and methods

Patients

A total of 200 patients who received LT for HCC in Shulan (Hangzhou) Hospital were enrolled in this retrospective study between July 2017 and December 2020. All patients underwent LT with histo-pathologically confirmed HCC. The present study was conducted in accordance with the Declaration of Helsinki (2013) and the Declaration of Istanbul (2018). This study was approved by ethical committee of Shulan (Hangzhou) hospital. Informed consent was taken from all individual participants. No organs from executed prisoners were used.

Study design and data collection

We collected the pre-LT laboratory test result which was closest to the liver transplantation. The patients' clinical data including age, gender, drink status, smoke status, model of end-stage liver disease (MELD) score, hepatitis B virus (HBV) infection status, morphological features and relevant information were collected.

We also collected the pre-LT abdominal CT scan images closest to the transplantation date (within 1

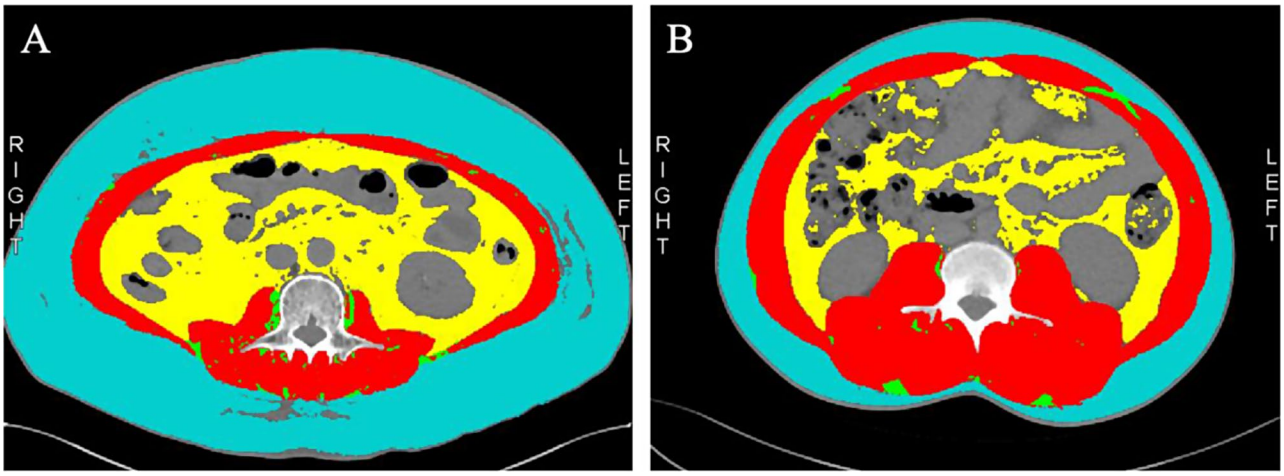

Fig. 1 Cross-section of CT-scan images at the L3 region from two typical recipients with or without sarcopenia. (**A**) sarcopenia; (**B**) non-sarcopenia. Red: skeletal muscle; Blue: subcutaneous adipose tissue; Yellow: visceral adipose tissue; Green: intermuscular adipose tissue

month). Skeletal muscle (SM), VAT, and SAT were analyzed using axial portal phase CT images at the level of the third lumbar vertebra (L3) by SliceOmatic software (version 5.0; Tomovision). Tissue Hounsfield unit (HU) thresholds were described previously [36]: -29 to +150 HU for SM, -190 to -30 for SAT, and −150 to -50 for VAT. We also recorded the mean tissue-specific radiation attenuation (RA) of SM, VAT and SAT. The L3 area of SM, VAT and SAT were also measured and each value of the cross-sectional areas (cm^2) were normalized for height squared (m^2) to calculate skeletal muscle index (SMI), visceral adipose tissue index (VATI) or subcutaneous adipose tissue index (SATI) (Fig. 1), respectively. Sarcopenia and myosteatosis were evaluated on pre-LT CT at L3 level by SMI and SMRA using predefined cut-off values. Specifically, the frequently reported cut-off values for SMI were 43.75 cm^2/m^2 in male and 41.10 cm^2/m^2 in female [37], and those for SMRA were 41 HU in patients with a BMI < 25 kg/m^2 and 33 HU in patients with a BMI ≥ 25 kg/m^2 [38].

Measurement of serum CHI3L1 levels

Before LT, blood samples from HCC patients were collected in tubes, and after being centrifuged at 3000 rpm for 10 min, the serum was obtained and immediately divided and frozen at -80 °C until analysis. The serum CHI3L1 levels were measured using the Human YKL-40 ELISA kit (ab255719, abcam) according to the manufacturer's instructions.

Follow-up

The follow-up was ended on October 31, 2022 and the median follow-up time was 2.67 years. During the first six months, screening for tumor recurrence was performed by alpha-fetoprotein (AFP) measurement and ultrasonography every month, and during the second six months these examinations were performed every two months. In subsequent years, the patients received examinations every three to six months or when necessary. Thoracoabdominal CT or magnetic resonance imaging (MRI) was performed every six months or when necessary. Recurrence-free survival (RFS) was calculated from the date of surgery to recurrence, death or last known follow-up, and recurrence was confirmed by radiological examination or AFP measurement.

Statistical analysis

Continuous variables were presented as means ± standard deviations (SDs) or medians and interquartile ranges (IQRs) as appropriate for the data type. We used the Kolmogorov-Smirnov test to evaluate the normality of the data distribution. Normally distributed data were compared using Student's t-tests, while non-normally distributed continuous variables were compared using Mann-Whitney U-tests. Categorical variables are expressed as n (%) and were compared with chi-square test. Univariate analysis was calculated by the Cox proportional hazards regression model. Variables with a p value < 0.05 were subsequently entered into a multivariate analysis using a binary logistic regression method. Overall survival (OS) and RFS rates were calculated using the Kaplan-Meier method and compared using the log-rank test. Kaplan-Meier survival analyses were conducted using the "survival" and "survminer" package in R version 4.2.2 and the optimal cut-off values were determined using the "maxstat" package. Statistical analyses were conducted using SPSS software, version 26 (IBM, Armonk, NY USA). A p value < 0.05 was considered statistically significant.

Results

Baseline characteristics of the patients

A total of 200 patients were enrolled in our study. The median age was 53.2 years, and 187 patients (93.5%) were male. 186 (93.0%) patients were HBV infected. 59 patients (29.5%) were diagnosed with sarcopenia and 71 patients (35.5%) were diagnosed with myosteatosis. The optimal cut-off value of CHI3L1 was 131.5 using "maxstat" package and we divided the study population into two groups according to this value (low group: $n=149$; high group: $n=51$). The hemoglobin was higher in low CHI3L1 group, while percentage of patients with AFP≥400ng/ml, C-reactive protein (CRP) and age were higher in high CHI3L1 group. Interestingly, the percentage of patients with sarcopenia was higher in high CHI3L1 group (high vs. low=43.1% vs. 24.8%, $p=0.022$), and the percentage of patients with myosteatosis was higher in high CHI3L1 group as well (high vs. low=56.9% vs. 28.2%, $p<0.001$) (Table 1). Baseline characteristics for the patients are shown in Table 1.

Associations between serum CHI3L1 level and body composition parameters

Spearman's correlation analyses were performed to compare the degree of relevance for the associations of ln(CHI3L1) with body composition parameters. Among the study population, the ln(CHI3L1) were significantly positively correlated with SMRA (r=-0.3, $p<0.001$), VATRA ($r=0.28$, $p<0.001$), SATRA ($r=0.21$, $p<0.001$) and SMI (r=-0.15, $p=0.035$), but were not with other parameters (Fig. 2).

Elevated serum CHI3L1 level and myosteatosis predict poor prognosis

Univariate analyses of risk factors for recurrence and death shown that serum CHI3L1 levels, sex, pathological features of tumor, AFP, platelet (PLT), CRP, SMRA and SMI were risk factors (Table 2). Furthermore, multivariate analysis identified serum CHI3L1 levels ($p=0.001$), maximum tumor diameter≥5 cm ($p=0.019$), AFP≥400ng/ml ($p=0.007$) and SMRA ($p=0.011$) as independent risk factors for OS, and serum CHI3L1 levels ($p=0.016$), multiple tumor ($p=0.017$), maximum tumor diameter≥5 cm ($p<0.001$), AFP≥400ng/ml ($p<0.001$) and SMRA were independent risk factors for RFS (Fig. 3).

Then, we analyzed the prognostic effects of serum CHI3L1 levels and myosteatosis using the Kaplan-Meier method. The 1-, 3-, and 5-year OS rates were 91.9%, 74.5%, and 67.0% in low CHI3L1 group, respectively, significantly higher than 78.4%, 40.1%, and 40.1% in high CHI3L1 group, respectively ($p<0.001$, Fig. 4A). Likewise, the RFS rates of low CHI3L1 group was better than the high CHI3L1 group ($p=0.002$, Fig. 4C). And for myosteatosis, the 1-, 3-, and 5-year OS rates were 89.1%, 70.3%, and 66.9% in non-myosteatosis group, respectively, significantly higher than 87.3%, 56.9%, and 49.5% in myosteatosis group, respectively ($p=0.025$, Fig. 4B). However, the RFS of myosteatosis group or non-myosteatosis group shown no significant difference ($p=0.068$, Fig. 4D).

Elevated serum CHI3L1 level predicts prognosis in patients with myosteatosis

Since serum CHI3L1 levels and SMRA were both independent risk factors, we further analyze the role of CHI3L1 in patients with or without myosteatosis. In patients with myosteatosis, the OS and RFS of the high CHI3L1 group were shorter than the low CHI3L1 group (OS: $p<0.001$ and RFS: $p=0.047$, Fig. 5A and C). However, in patients without myosteatosis, the OS and RFS of high CHI3L1 group or low CHI3L1 group shown no significant difference (OS: $p=0.070$ and RFS: $p=0.104$, Fig. 5B and D).

Discussion

Our study demonstrated that serum CHI3L1 levels were negative correlated with SMI and SMRA and were positive correlated with VATRA and SATRA. Elevated CHI3L1 were associated with significantly poor prognosis and we further analyze its role in patients with or without myosteatosis. To our knowledge, this study is the first to show the association between serum CHI3L1 and body composition parameters in patients with HCC after LT.

CHI3L1 is overexpressed and is regarded as a prognostic biomarker in a multitude of cancers including gastric cancer, colorectal cancer, renal carcinoma and prostate carcinoma [39–42]. Consistently, a study revealed that CHI3L1 was an independent prognostic factor for OS and RFS in 158 HCC patients who received curative resection (HR=1.968, 95%CI: 1.093–3.543, $p=0.024$; HR=1.891, 95%CI: 1.106–3.232, $p=0.020$; respectively) [34]. In 212 HCC patients treated with TACE, CHI3L1 demonstrated to be an independent prognostic biomarker as well [43]. The characteristics of their patients was different with our patients. Their patients received curative resection or TACE and the Child-Pugh class of most patients was A. However, patients enrolled in our study underwent liver transplantation for HCC, and most of them suffered from cirrhosis and were categorized as Child-Pugh C. It is worth mentioning that CHI3L1 is upregulated not only in tumors, but also in benign liver diseases [44], which may hinder it from becoming an HCC diagnostic biomarker [32] and also affect the prognostic capacity in patients with different etiology and different process of disease.

Except its prognostic role in cancers, CHI3L1 may play a role in inflammation and metabolism. The levels of CHI3L1 tends to be upregulated in a variety of diseases

Table 1 Baseline characteristics of 200 patients according to CHI3L1

	Overall (*n* = 200)	Low serum CHI3L1 (*n* = 149)	High serum CHI3L1 (*n* = 51)	*P* value
Serum CHI3L1 level, ng/mL	85.94 (46.94, 131.85)	63.30 (36.36, 92.66)	201.35 (139.24, 293.94)	**< 0.001**
Age, years	53.22 (8.96)	52.46 (9.25)	55.41 (7.74)	**0.042**
Gender				0.435
Male, n (%)	187 (93.5)	141 (94.6)	46 (90.2)	
Female, n (%)	13 (6.5)	8 (5.4)	5 (9.8)	
Height, cm	169.99 (5.78)	170.38 (5.46)	168.82 (6.54)	0.096
Weight, kg	68.62 (11.55)	69.34 (11.71)	66.51 (10.92)	0.131
BMI	23.70 (3.49)	23.83 (3.55)	23.30 (3.31)	0.342
HBV, n (%)	186 (93.0)	138 (92.6)	48 (94.1)	0.964
Alcohol drinking status, n (%)				0.442
Current or former drinker	83 (41.5)	59 (39.6)	24 (47.1)	
Never drinker	117 (58.5)	90 (60.4)	27 (52.9)	
Smoking status, n (%)				0.579
Current or former smoker	91 (45.5)	70 (47.0)	21 (41.2)	
Never smoker	109 (54.5)	79 (53.0)	30 (58.8)	
ECOG score				0.896
0	56 (28.0)	43 (28.9)	13 (25.5)	
1–2	133 (66.5)	98 (65.8)	35 (68.6)	
3–4	11 (5.5)	8 (5.4)	3 (5.9)	
MELD score	37 (30, 40)	37 (30, 40)	38 (30.5, 40)	0.694
Child-Pugh class, n (%)				0.757
A	0 (0.0)	0 (0.0)	0 (0.0)	
B	24 (12.0)	19 (12.8)	5 (9.8)	
C	176 (88.0)	130 (87.2)	46 (90.2)	
Tumor number, n (%)				0.639
Solitary	86 (43.0)	66 (44.3)	20 (39.2)	
Multiple	114 (57.0)	83 (55.7)	31 (60.8)	
Tumor size ≥ 5 cm, n (%)	70 (35.0)	52 (34.9)	18 (35.3)	1.000
Pathological grade, n (%)				0.869
Complete necrosis	19 (9.5)	15 (10.1)	4 (7.8)	
Well differentiation	33 (16.5)	26 (17.4)	7 (13.7)	
Moderate differentiation	134 (67.0)	98 (65.8)	36 (70.6)	
Poor differentiation	14 (7.0)	10 (6.7)	4 (7.8)	
Pre-LT laboratory findings				
Albumin, g/L	31.30 (28.00, 33.20)	31.80 (28.00, 33.50)	30.40 (28.00, 32.65)	0.354
Platelet, *10^9/L	82.00 (48.00, 129.25)	80.00 (46.00, 127.00)	85.00 (52.50, 130.50)	0.411
Hemoglobin, g/L	121.00 (103.00, 141.00)	125.00 (104.00, 142.00)	117.00 (98.50, 131.50)	**0.037**
CRP, mg/L	7.65 (2.88, 22.33)	6.20 (2.30, 19.50)	11.10 (5.00, 40.60)	**0.005**
AFP ≥ 400 ng/ml, n (%) [a]	57 (28.6)	36 (24.3)	21 (41.2)	**0.034**

Table 1 (continued)

	Overall ($n=200$)	Low serum CHI3L1 ($n=149$)	High serum CHI3L1 ($n=51$)	*P* value
Ln(PIVKA-II) [b]	5.99 (2.65)	5.81 (2.62)	6.53 (2.72)	0.098
TC (median [IQR])	3.18 (2.32, 4.14)	3.20 (2.32, 4.15)	3.11 (2.30, 3.94)	0.811
TG (median [IQR])	0.95 (0.72, 1.49)	1.01 (0.73, 1.52)	0.90 (0.63, 1.11)	0.095
HDL-C (median [IQR])	0.76 (0.53, 1.00)	0.75 (0.52, 0.98)	0.79 (0.56, 1.02)	0.577
LDL-C (median [IQR])	1.68 (1.18, 2.26)	1.70 (1.23, 2.33)	1.65 (1.13, 2.04)	0.314
VLDL-C (median [IQR])	0.64 (0.36, 0.94)	0.64 (0.38, 0.94)	0.64 (0.36, 0.92)	0.603
Body composition parameters				
Sarcopenia, (%)	59 (29.5)	37 (24.8)	22 (43.1)	**0.022**
Myosteatosis, (%)	71 (35.5)	42 (28.2)	29 (56.9)	**<0.001**
SMRA, HU	41.88 (37.98, 45.42)	43.04 (38.71, 45.86)	39.63 (35.17, 43.12)	**<0.001**
VATRA, HU	-84.99 (-94.64, -75.92)	-86.26 (-95.98, -76.90)	-81.26 (-87.48, -71.25)	**0.008**
SATRA, HU	-96.22 (-103.93, -85.77)	-97.11 (-104.30, -88.68)	-93.28 (-101.65, -79.93)	0.056
SMI	48.35 (8.61)	49.20 (8.45)	45.85 (8.66)	**0.016**
VATI	41.05 (21.59, 58.83)	43.93 (22.72, 59.69)	35.86 (17.52, 56.18)	0.322
SATI	36.86 (24.29, 50.96)	38.19 (25.42, 50.82)	35.07 (23.36, 51.60)	0.749

Abbreviations BMI, body mass index; HBV, hepatitis B virus; ECOG, Eastern Cooperative Oncology Group; MELD, model of end-stage liver disease; LT, liver transplantation; CRP, C-reactive protein; AFP, alpha fetoprotein; HU, Hounsfield Unit; SMRA, skeletal muscle radiation attenuation; VATRA, visceral adipose tissue radiation attenuation; SATRA, subcutaneous adipose tissue radiation attenuation; SMI, skeletal muscle index; VATI, visceral adipose tissue index; SATI, subcutaneous adipose tissue index

The bold values represent statistical significance, $P<0.05$

[a] Pre-LT AFP test result of one patient was missing

[b] Pre-LT PIVKA-II test results of 4 patients were missing

characterized by inflammation [45], and also associated with insulin resistance, diabetes and diabetic lipid profile [46, 47]. A study revealed that knockout of CHI3L1 gene enhanced hepatic insulin signal transduction and limited lipid accumulation induced by high fat diet, which suggested CHI3L1 gene overexpression may be a significant factor in the generation of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis phenotype [48]. Another study also found a significant increase expression of CHI3L1 in white adipose tissue after high fat diet. And abdominal visceral fat accumulation was diminished in CHI3L1 null mice, because of the significantly smaller adipocyte size [49]. Additionally, highly expressed CHI3L1 was found in skeletal muscle tissues of mice with sepsis, and silencing of CHI3L1 could alleviated sepsis-induced skeletal muscle stem cell injury by diminishing cell apoptosis as well as serum levels of pro-inflammatory cytokines [50]. However, our recent study found that CHI3L1 was up-regulated in skeletal muscle to protect itself from atrophy in sarcopenia patients with HCC, while it promoted HCC tumor progression in turn [35].

There are some other prognostic biomarkers in patients with HCC who have concurrent skeletal muscle disease. Choi et al. found that the serum levels of myostatin and IL-6 showed a positive and negative correlation with psoas muscle index in the HCC patients, respectively. And the high IL-6 group had a significantly poorer 5-year overall survival rate (78.4%) than that of the low IL-6 group (85.8%, $p=0.018$) [51]. Dalbeni A et al. also found that sarcopenic patients with HCC presented increased values of IL-6 [52]. Sano A et al. found that the prognosis of HCC patients with low omega-3 polyunsaturated fatty acid levels was significantly worse ($p=0.011$), and this biomarker was also correlated with skeletal muscle mass index ($r=0.15$, $p=0.003$) [53]. However, there are few previous studies on the prognostic biomarkers in patients with HCC who have concurrent myosteatosis. In our study, serum CHI3L1 levels were negative correlated with SMI and SMRA, the two parameters to diagnose sarcopenia or myosteatosis, and CHI3L1 was also regarded as an independent risk factor for OS and RFS. The underlying mechanism should be investigated to figure out the

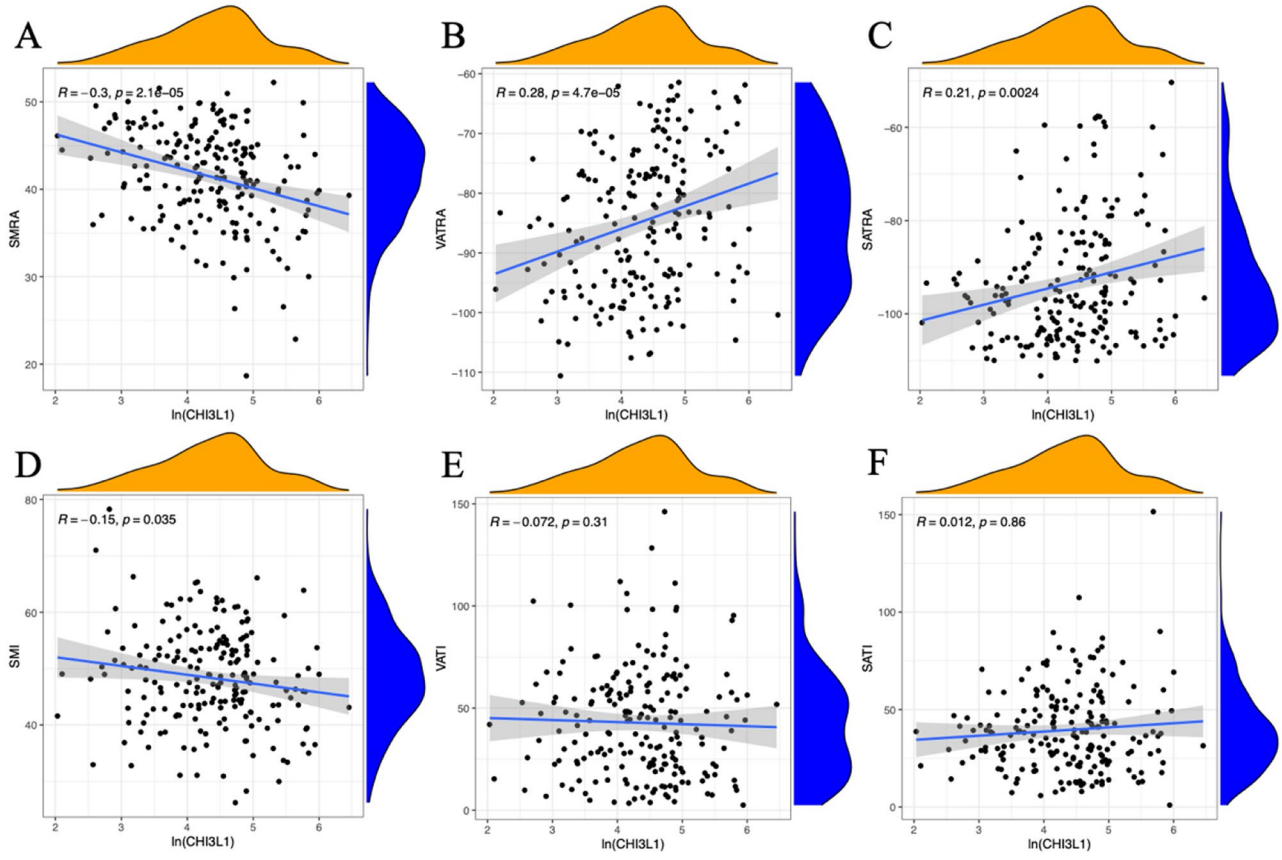

Fig. 2 Spearman's rank correlations between the serum CHI3L1 levels and body composition parameters. (**A**) CHI3L1 vs. SMRA; (**B**) CHI3L1 vs. VATRA; (**C**) CHI3L1 vs. SATRA; (**D**) CHI3L1 vs. SMI; (**E**) CHI3L1 vs. VATI; (**F**) CHI3L1 vs. SATI. Abbreviations: SMRA, skeletal muscle radiation attenuation; VATRA, visceral adipose tissue radiation attenuation; SATRA, subcutaneous adipose tissue radiation attenuation; SMI, skeletal muscle index; VATI, visceral adipose tissue index; SATI, subcutaneous adipose tissue index

Table 2 Univariate analysis of factors affecting OS and RFS

	Univariate analysis for OS		Univariate analysis for RFS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
CHI3L1	**1.004 (1.002–1.006)**	**< 0.001**	**1.003 (1.001–1.004)**	**0.002**
Sex	**2.974 (1.468–6.023)**	**0.002**	**2.675 (1.425–5.022)**	**0.002**
Age	0.996 (0.971–1.022)	0.769	1.000 (0.978–1.022)	0.998
BMI	0.963 (0.896–1.035)	0.308	0.965 (0.908–1.025)	0.251
HBV infection	0.621 (0.284–1.358)	0.232	0.686 (0.346–1.361)	0.281
MELD	1.021 (0.988–1.054)	0.213	1.024 (0.997–1.051)	0.080
Child-Pugh class	1.080 (0.516–2.259)	0.839	1.207 (0.645–2.257)	0.556
Poor or moderate differentiation	**2.012 (1.055–3.836)**	**0.034**	**2.292 (1.342–3.914)**	**0.002**
Multiple tumor	1.596 (0.972–2.621)	0.065	**1.668 (1.107–2.515)**	**0.014**
Maximum tumor diameter (≥ 5 cm)	**2.432 (1.515–3.905)**	**< 0.001**	**2.226 (1.503–3.295)**	**< 0.001**
AFP (≥ 400ng/ml)	**2.681 (1.661–4.327)**	**< 0.001**	**2.797 (1.875–4.174)**	**< 0.001**
PLT	**1.005 (1.002–1.007)**	**< 0.001**	**1.004 (1.002–1.006)**	**< 0.001**
HB	1.000 (0.991–1.009)	0.942	1.003 (0.996–1.011)	0.383
CRP	**1.008 (1.003–1.013)**	**0.001**	1.004 (1.000-1.009)	0.064
SMRA	**0.939 (0.903–0.977)**	**0.002**	**0.946 (0.913–0.979)**	**0.002**
VATRA	1.009 (0.989–1.029)	0.394	1.005 (0.988–1.021)	0.595
SATRA	1.005 (0.988–1.023)	0.580	1.006 (0.991–1.021)	0.453
SMI	**0.958 (0.931–0.987)**	**0.004**	**0.964 (0.940–0.988)**	**0.004**
VATI	0.991 (0.982–1.001)	0.072	0.995 (0.987–1.003)	0.185
SATI	0.995 (0.984–1.007)	0.449	0.996 (0.986–1.006)	0.429

Abbreviations OS, overall survival; RFS, recurrence-free survival; BMI, body mass index; HBV, hepatitis B virus; MELD, model of end-stage liver disease; AFP, alpha fetoprotein; PLT, platelet; HB, hemoglobin; CRP, C-reactive protein; SMRA, skeletal muscle radiation attenuation; VATRA, visceral adipose tissue radiation attenuation; SATRA, subcutaneous adipose tissue radiation attenuation; SMI, skeletal muscle index; VATI, visceral adipose tissue index; SATI, subcutaneous adipose tissue index

The bold values represent statistical significance, $P<0.05$

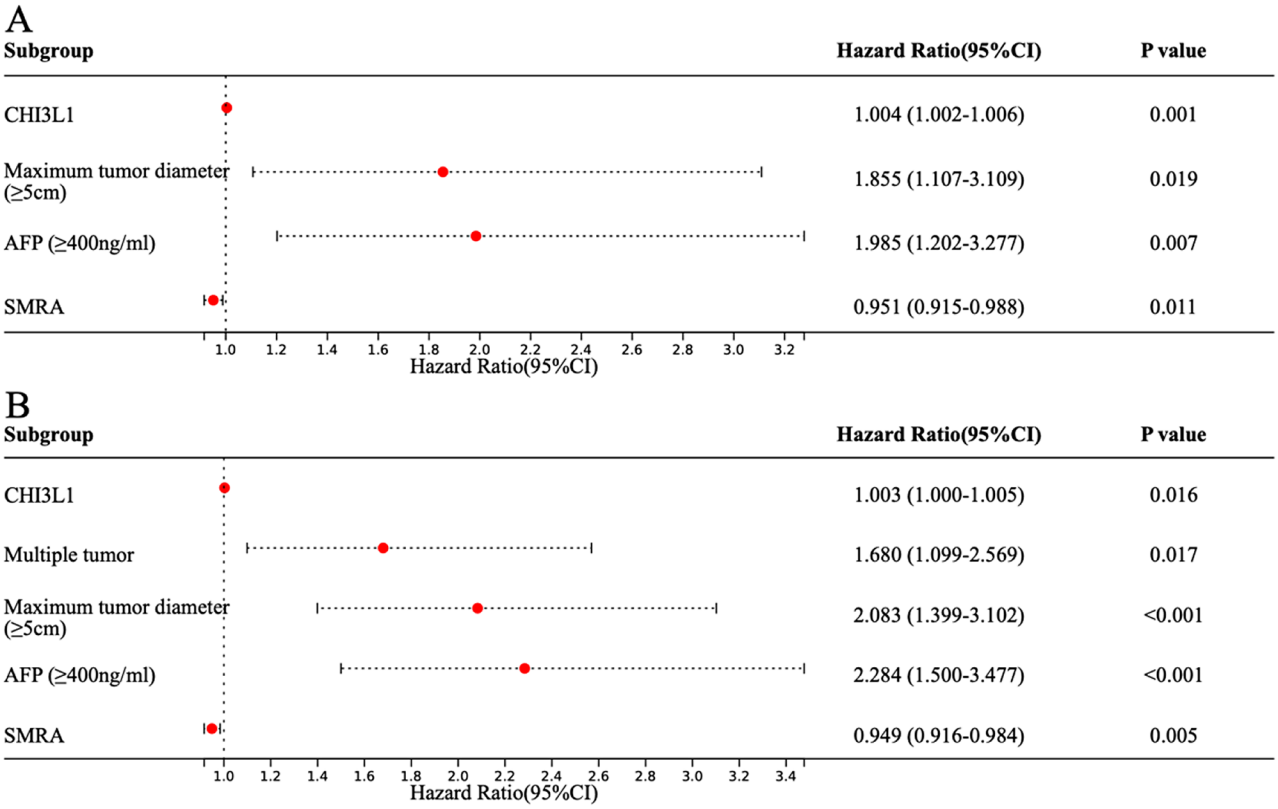

Fig. 3 Multivariate Cox regression analysis of risk factors for prognosis of recipients undergoing liver transplantation for HCC. (**A**) Overall survival; (**B**) Recurrence-free survival. Abbreviations: AFP, alpha-fetoprotein; SMRA, skeletal muscle radiation attenuation

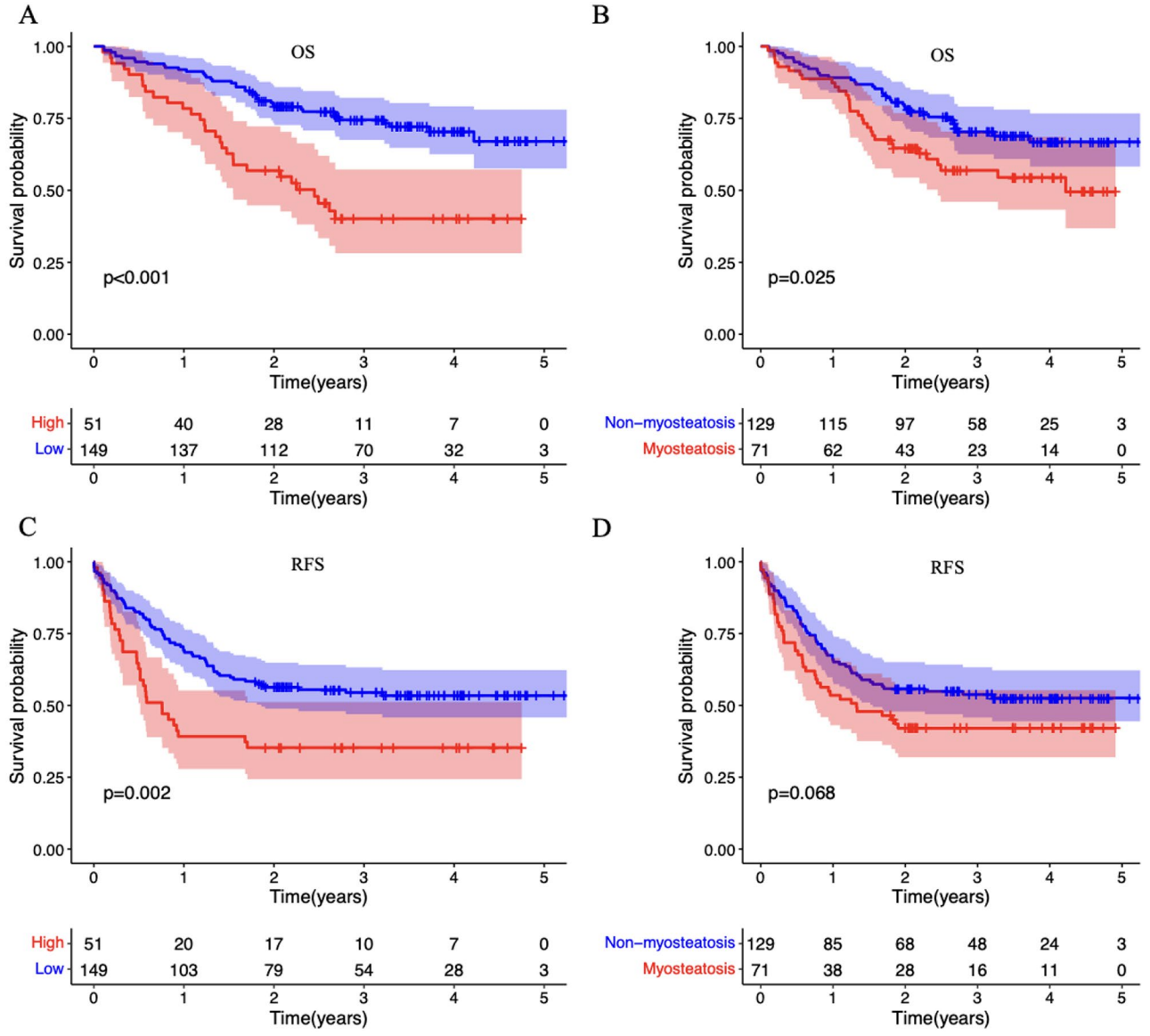

Fig. 4 Elevated serum CHI3L1 level and myosteatosis predict poor prognosis. (**A**) CHI3L1 for OS; (**B**) myosteatosis for OS; (**C**) CHI3L1 for RFS; (**D**) myosteatosis for RFS. Abbreviations: OS, overall survival; RFS, recurrence-free survival

role of CHI3L1 in patients with HCC and concomitant myosteatosis.

In addition, LT can simultaneously remove tumor and treat underlying liver diseases, and is regarded as the optimal treatment for patients with HCC. However, there are also some concerns of LT for HCC. Post-LT immunosuppression can lead to tumor recurrence. Pre-LT elevated serum CHI3L1 is an independent risk factor of recurrence in HCC patients. The immunosuppression regimen should be individualized to optimally control alloreactivity while preventing recurrence, especially in patients at high risk for tumor recurrence [54]. The potential association between immunosuppressive status and inflammatory factors such as CHI3L1 needs further investigation. The concomitant of myosteatosis or sarcopenia predicted poor prognosis in HCC patients after LT [55]. Nutritional support and improvement of muscle mass and function should be considered in long-term management of LT patients. Furthermore, inclusion of pre-LT body composition in transplant criteria is also an issue worth considering.

Admittedly, potential limitations of our study must also be considered. Firstly, we used a retrospective approach for the data analysis using limited number of center and patients. And it was difficult to assess the causal relationships between serum CHI3L1 levels and body composition parameters in our study. Secondly, we only analyzed preoperative blood samples and body composition, and

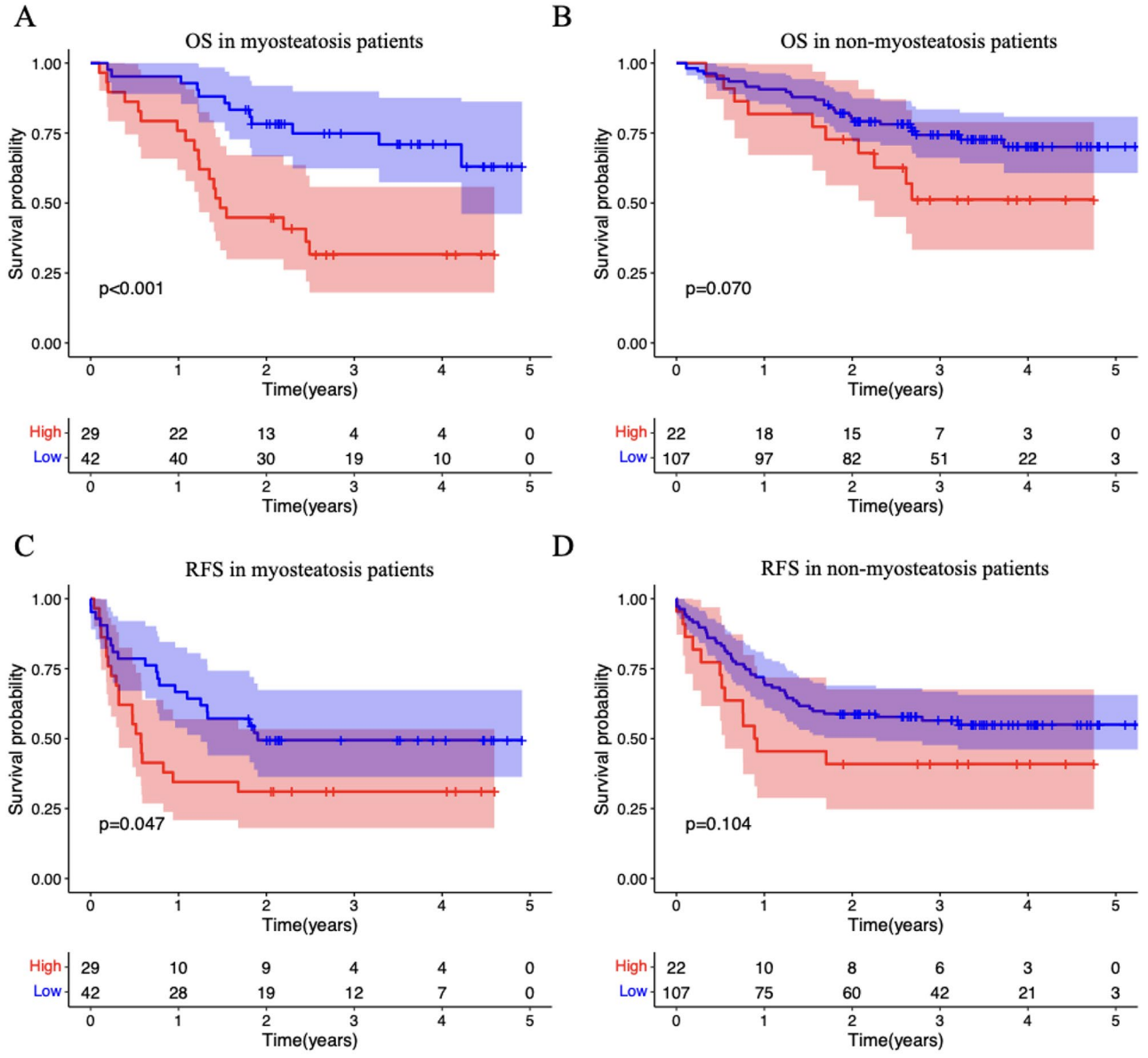

Fig. 5 The role of CHI3L1 in patients with or without myosteatosis. (**A**) OS in myosteatosis group; (**B**) OS in non-myosteatosis group; (**C**) RFS in myosteatosis group; (**D**) RFS in non-myosteatosis group; Abbreviations: OS, overall survival; RFS, recurrence-free survival

the study on dynamic changes after operation is needed in the future. Finally, the association was confirmed in our study, while the potential molecular mechanisms awaited further researches.

Conclusion

In conclusions, we found that serum CHI3L1 were a prognostic biomarker in Chinese population after LT for HCC and associated with SMI, SMRA, VATRA and SVTRA. Our findings suggested a potential mechanistic association between serum CHI3L1 and body composition in HCC patients. Monitoring serum CHI3L1 is helpful to predict prognosis and effectively guide individual nutrition intervention. And further research exploring the underlying mechanisms on the associations observed in this study is warranted.

Abbreviations

AFP	Alpha-Fetoprotein
BMI	Body Mass Index
CHI3L1	Chitinase-3 like-protein-1
CRP	C-reactive Protein
CT	Computer Tomography
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HU	Hounsfield Unit
IQR	Interquartile Range
L3	Third Lumbar Vertebra
LT	Liver Transplantation
MELD	Model of end-stage Liver Disease
MRI	Magnetic Resonance Imaging
OS	Overall Survival

PLT	Platelet
RA	Radiation Attenuation
RFS	Recurrence-free Survival
SAT	Subcutaneous Adipose Tissue
SATI	Subcutaneous Adipose Tissue Index
SD	Standard Deviation
SM	Skeletal Muscle
SMI	Skeletal Muscle Index
SMRA	Skeletal Muscle Radiation Attenuation
VAT	Visceral Adipose Tissue
VATI	Visceral Adipose Tissue Index

Acknowledgements
We thank all the researchers involved in this study for their generous assistance and cooperation.

Author contributions
Conceptualization, D.L. and C.H.; methodology, H.C., C.C. and J.C.; software, Z.H. and Z.L.; formal analysis, C.H.; resources, C.H., C.C., X.Y. H.L. W.S.; writing—original draft preparation, C.H., Z.H. and Z.L.; writing—review and editing, X.W., L.Z., S.Z., X.X. and D.L.; visualization, C.H.; supervision, S.Z.; funding acquisition, X.X. and D.L.; All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by National Key Research and Development Program of China (No. 2021YFA1100500), the Major Research Plan of the National Natural Science Foundation of China (No.92159202), the Key Research & Development Plan of Zhejiang Province (No.2024C03051) and the Scientific Research Fund of Zhejiang Provincial Education Department (Y202353201).

Data availability
The data are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
The present study was conducted in accordance with the Declaration of Helsinki (2013) and the Declaration of Istanbul (2018). This study was approved by ethical committee of Shulan (Hangzhou) hospital. Informed consent was taken from all individual participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 8 June 2024 / Accepted: 14 August 2024
Published online: 23 August 2024

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