Research Article

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Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma

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Background & Aims: Obesity defined by body mass index (BMI) significantly increases the risk of hepatocellular carcinoma (HCC). In contrast, not only obesity but also underweight is associated with poor prognosis in patients with HCC. Differences in body composition rather than BMI were suggested to be true determinants of prognosis. However, this hypothesis has not been demonstrated conclusively.

Methods: We measured skeletal muscle index (SMI), mean muscle attenuation (MA), visceral adipose tissue index, subcutaneous adipose tissue index, and visceral to subcutaneous adipose tissue area ratios (VSR) via computed tomography in a large-scale retrospective cohort of 1257 patients with different stages of HCC, and comprehensively analyzed the impact of body composition on the prognoses.

Results: Among five body composition components, low SMI (called sarcopenia), low MA (called intramuscular fat [IMF] deposition), and high VSR (called visceral adiposity) were significantly associated with mortality, independently of cancer stage or Child-Pugh class. A multivariate analysis revealed that sarcopenia (hazard ratio [HR], 1.52; 95% confidence interval [CI], 1.18–1.96; $p = 0.001$), IMF deposition (HR, 1.34; 95% CI, 1.05–1.71; $p = 0.020$), and visceral adiposity (HR, 1.35; 95% CI, 1.09–1.66; $p = 0.005$) but not BMI were significant predictors of survival. The prevalence of poor prognostic body composition components was significantly higher in underweight and obese patients than in normal weight patients.

Conclusions: Sarcopenia, IMF deposition, and visceral adiposity independently predict mortality in patients with HCC. Body composition rather than BMI is a major determinant of prognosis in patients with HCC.

Keywords: Hepatocellular carcinoma; Body composition; Prognosis; Body mass index.

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Abbreviations: HCC, hepatocellular carcinoma; BMI, body mass index; IMF, intramuscular fat; CT, computed tomography; BCLC, Barcelona Clinic Liver Cancer; HBV, hepatitis B virus; HCV, hepatitis C virus; L3, the third lumber vertebra; HU, Hounsfield units; SMI, skeletal muscle index; SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; VSR, visceral to subcutaneous adipose tissue area ratio; MA, muscle attenuation; HR, hazard ratio; CI, confidence interval; IL-6, interleukin-6; FFA, free fatty acid.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer and the third most frequent cause of cancer-related death [1]. Recent epidemiological studies have resulted in the wide recognition of obesity as a significant risk factor for HCC development [2]. We found previously that underweight patients with a BMI <18.5 kg/m^2 have the lowest risk among patients with chronic hepatitis C [3]. However, we recently conducted a nationwide survey to explore the association between BMI and mortality in patients with non-viral HCC, and unexpectedly identified not only obesity but also underweight status as a risk factor predicting poor survival [4]. Such a paradoxical relationship of underweight (high mortality despite low susceptibility) has also been found in patients with coronary heart disease [5], diabetes [6], and renal cell carcinoma [7], and our study was the first to show in patients with HCC. One possible explanation for such a relationship is that underweight patient groups may include those with more advanced disease. However, the observed trend remained after adjusting for significant factors, such as tumor stage and liver functional status, indicating that underweight patients exhibit other key features associated with a poor prognosis.

BMI is a simple anthropometric index based on individual weight and height and is widely used. However, such simplicity

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comes with a cost. BMI is limited anthropometrically, in that it does not assess individual components of body weight such as regional fat distribution or muscle volume. Regional fat distribution plays a crucial role in patients with metabolic syndrome [8]. In fact, we previously reported that visceral fat accumulation, rather than BMI, is an independent risk factor for recurrence of HCC in patients with non-viral disease [9]. Furthermore, loss of skeletal muscle, called sarcopenia, is associated with poor prognoses of several cancers including HCC [10,11]. Sarcopenia may be linked to a poor prognosis not only pathophysiologically by inducing insulin resistance, but also indirectly, by reducing activities associated with daily living [12]. Based on such findings, Ahima *et al.* proposed a new hypothesis that differences in body composition rather than BMI may be true determinants of prognosis [13]. However, most previous studies investigated only the impacts of single body composition components on prognosis [14]; thus, this hypothesis has not been proven conclusively.

In the present study, we explored BMI, skeletal muscle area, intramuscular fat (IMF) deposition, abdominal adipose tissue area and adipose tissue distribution in a large-scale retrospective cohort of 1257 patients with different stages of HCC and comprehensively analyzed the impact of body composition on the prognoses of such patients.

Patients and methods

This study was conducted according to ethical guidelines relevant to epidemiological research promulgated by the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor, and Welfare. The study design was described in a comprehensive protocol prepared by the Department of Gastroenterology, the University of Tokyo Hospital and was approved by the University of Tokyo Medical Research Center Ethics Committee (approval number 2058).

Patients

Using a prospective computerized database, we analyzed information on patients diagnosed with HCC at the Department of Gastroenterology, the University of Tokyo Hospital, a tertiary center, from January 2004 to December 2009. We excluded patients with poorly controlled ascites, because this might lead to overestimation of BMI. We included patients with undetected ascites due to diuretic agent usage, since the exclusion of such patients might constitute selection bias. HCC was diagnosed using unenhanced and dynamic computed tomography (CT) [15]. Images were obtained during the early arterial, late arterial, and equilibrium phases, thus at 28, 40, and 120 s after bolus injection of iodinated contrast material. Images were reconstructed at a section thickness of 5 mm and with a reconstruction interval of 5 mm (section thickness 2–2.5 mm, interval 1.5–2 mm, and field of view 24–35 cm for the arterial phase). A diagnosis of HCC was based on typical CT findings: hyperattenuation in the arterial and hypoattenuation in the equilibrium phase [16,17]. We assessed HCC stage using the Barcelona Clinic Liver Cancer (BCLC) staging system [18].

Clinical and anthropometric variables

We recorded the following clinical and anthropometric parameters upon first admission to our department: age; gender; BMI; hepatitis infection status (hepatitis B virus [HBV], hepatitis C virus [HCV], HBV + HCV, or none); daily alcohol consumption ($\leqslant$80 g *vs.* >80 g); smoking status (never, former or current smoker); presence of diabetes; presence of chronic kidney disease defined by estimated glomerular filtration rate <60.0 ml/min/1.73 m^2; history of cardiovascular or cerebrovascular disease; history of lung disease such as asthma and chronic obstructive pulmonary disease; Child-Pugh class; aspartate aminotransferase, alanine transaminase, total bilirubin, and albumin levels; platelet count; BCLC stage; treatment methods for patients with HCC in BCLC 0 or A; history of previous HCC treatment; and alpha-fetoprotein level (<100 *vs.* $\geqslant$100 ng/ml). We also evaluated the body composition parameters described below. We considered the definitions of underweight and obese typically applied to older adults [19] and Japanese populations [20], and we selected the following BMI categories *a priori*: <20.0, underweight; 20.0–24.9, normal; and $\geqslant$25.0, obese. Diabetes was diagnosed based on medical history or a 75-g oral glucose tolerance test [21].

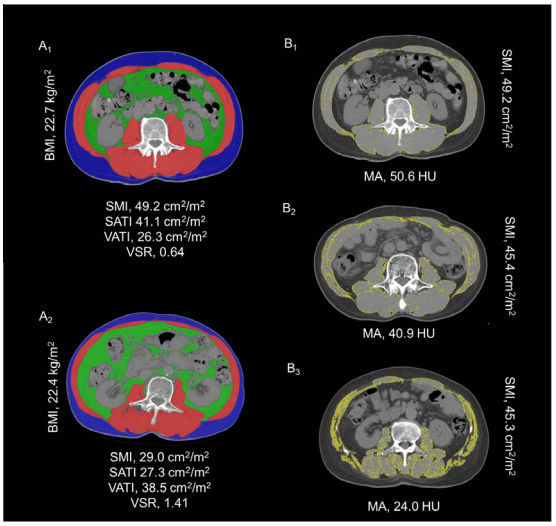

Fig. 1. Cross-sectional computed tomography (CT) images of the third lumbar vertebra used to quantify body composition variables. (A_{1-2}) illustrates the findings of two patients with hepatocellular carcinoma. The BMIs of the two patients were almost identical. The red shadows, the green shadows, and the blue shadows show the skeletal muscle areas, the visceral adipose tissue areas, and the subcutaneous adipose tissue areas, respectively. B_{1-3} illustrates the skeletal muscle area in the three patients, which were nearly identical. The yellow shadows indicate muscle attenuation from −29 to 29 HU. BMI, body mass index; SMI, skeletal muscle index; SATI, subcutaneous adipose tissue index; VATI, visceral adipose tissue index; VSR, visceral to subcutaneous adipose tissue area ratio; MA, muscle attenuation; HU, Hounsfield units.

CT analyses of body composition variables

We quantified the data from a cross-sectional unenhanced CT image (Aquilion 4/16/64, ONE; Toshiba, Tokyo, Japan; LightSpeed Qx/I, LightSpeed Ultra, LightSpeed VCT, Discovery CT 750 HD; GE Healthcare, Milwaukee, WI, USA) taken solely for the purpose of diagnosing and staging HCC, as described below. We evaluated CT scans performed within 1 month before, or soon after, the first admission to our department. The following measurements were validated by the anatomical radiologists.

We analyzed the cross-sectional CT images at the third lumbar vertebra (L3) using Slice-O-Matic software (version 5.0; Tomovision, Montreal, Canada) to determine skeletal muscle and abdominal adipose tissue area. Muscle areas included the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles. Tissue Hounsfield unit (HU) thresholds were employed as follows: −29 to 150 HU for skeletal muscle, −190 to −30 for subcutaneous adipose tissue and −150 to −50 for visceral adipose tissue [22]. As in previous reports, these body composition variables were normalized for height in meters squared and are expressed as cm^2/m^2. We termed the parameters for skeletal muscle, subcutaneous and visceral adipose tissue as skeletal muscle index (SMI), subcutaneous adipose tissue index (SATI), and visceral adipose tissue index (VATI), respectively. We also calculated visceral to subcutaneous adipose tissue area ratios (VSRs) to explore abdominal adipose tissue distributions. In addition, we calculated mean MA using the same CT images to assess skeletal muscle quality. Low MA indicates increased IMF content that contributes to muscle weakness independent of the age-associated loss in muscle mass [10,23]. Representative images used for analyses are shown in Fig. 1.

We evaluated reproducibility by analyzing data from 70 subjects randomly selected from this cohort to test the reliability of body composition determined by CT. Two trained observers (N.F. and R.N.) measured skeletal muscle area, the corresponding muscle mean CT attenuation, and subcutaneous and visceral adipose tissue area to assess inter-observer reproducibility. One observer repeated the measurements at two time points at least 1 month apart to assess intra-observer reproducibility.

Table 1. Baseline characteristics.

Characteristic	N = 1,257
Age, mean ± SD	68.8 ± 9.2
Gender male, N (%)	828 (65.9)
Body mass index (kg/m^2), median (IQR)	23.2 (21.2-25.5)
<20.0 (underweight), N (%)	186 (14.8)
20.0-24.9 (normal weight), N (%)	700 (55.7)
≥25.0 (obesity), N (%)	371 (29.5)
Viral status, N (%)	
HCV/HBV/HCV + HBV/none	895/142/13/207 (71.2/11.3/1.0/16.5)
Alcohol consumption, N (%)	
>80 g per day	191 (15.2)
Smoking status, N (%)*	
Never/former/current	645/328/239 (53.2/27.1/19.7)
Comorbidity, N (%)	
Diabetes† (yes/no)	310/848 (26.8/73.2)
Cardio/cerebrovascular disease‡ (yes/no)	142/1080 (11.6/88.4)
Pulmonary disease‡ (yes/no)	46/1176 (3.8/96.2)
Chronic kidney disease§ (yes/no)	281/973 (22.4/77.6)
Child-Pugh class, N (%)	
A/B/C	958/286/13 (76.2/22.8/1.0)
AST (IU/L), median (IQR)	53 (36-74)
ALT (IU/L), median (IQR)	44 (28-69)
Total bilirubin (mg/dl), median (IQR)	0.8 (0.6-1.1)
Albumin (g/dl), median (IQR)	3.7 (3.3-4.0)
Prothrombin time (%), median (IQR)	83 (71-99)
Platelet count (× 1000/μl) , median (IQR)	110 (79-152)
BCLC stage, N (%)	
0/A/B/C/D	181/588/427/47/14 (14.4/46.8/34.0/3.7/1.1)
Previous treatment (yes/no), N (%)	471/786 (37.5/62.5)
α-fetoprotein (<100/≥100 ng/ml) , N (%)	979/278 (77.9/22.1)
Body composition variable	
SMI (cm^2/m^2), median (IQR)	42.4 (36.6-48.7)
Male/female‖	45.8 (40.3-50.9)/36.7 (32.9-40.7)
MA (HU), median (IQR)	35.2 (29.8-40.9)
Male/female‖	38.0 (32.7-42.8)/30.9 (25.8-35.5)
VATI (cm^2/m^2), median (IQR)	31.8 (17.4-51.9)
Male/female‖	36.4 (20.3-56.3)/25.5 (13.9-40.5)
SATI (cm^2/m^2), median (IQR)	41.1 (29.7-56.4)
Male/female‖	37.7 (27.0-50.0)/51.1 (34.4-72.5)
VSR, median (IQR)	0.75 (0.46-1.11)
Male/female‖	0.93 (0.64-1.28)/0.49 (0.33-0.66)

SD, standard deviation; IQR, interquartile range; HCV, hepatitis s C virus; HBV hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine transaminase; BCLC, Barcelona Clinic Liver Cancer; SMI, skeletal muscle index; VATI, visceral adipose tissue index; SATI, subcutaneous adipose tissue index; VSR, visceral to subcutaneous fat area ratio; MA, muscle attenuation.
*Data of 45 patients were missing.
†Data of 99 patients were missing.
‡Data of 35 patients were missing.
§Data of three patients were missing.
‖Significant differences were observed in each of the body composition variables between males and females.

Statistical analyses

Quantitative variables are expressed as medians with interquartile ranges, unless otherwise indicated. Numbers and percentages are used to express the qualitative variables. Differences between groups were analyzed using Wilcoxon's test for continuous variables and Pearson's χ^2 test or Fisher's exact test for categorical data. A survival analysis was performed on a per-patient basis. Survival time was defined as the interval between the first admission to our department for HCC and death or December 31, 2012, whichever came first. Cumulative mortality curves were constructed using the Kaplan–Meier procedure and compared with log-rank test results. In addition, we explored liver-related mortality, which included death caused directly by HCC progression, including rupture of tumors or gastroesophageal varices due to HCC invasion of the portal vein; advanced hepatic failure (massive ascites, jaundice, or overt hepatic encephalopathy); or bleeding varices. We censored liver-unrelated death at the time of the cumulative liver-related mortality evaluation. We also assessed differences in cumulative recurrence rates in patients with HCC in BCLC stage 0 or A, who had undergone percutaneous therapy as the first treatment between those with or without unfavorable body composition.

Univariate and multivariate analyses of overall survival were performed using Cox's regression models, and the results are presented as HRs with 95% CIs. *p* values were derived using the Wald test. Variables exhibiting significant associations after univariate analyses were included in the multivariate analysis.

First, we assessed the HRs of SMI, MA, VATI, SATI, and VSR for mortality as continuous numbers using the restricted cubic spline with three knots at the 5th, 50th, and 95th centiles after adjusting for gender [24]. Then, we determined

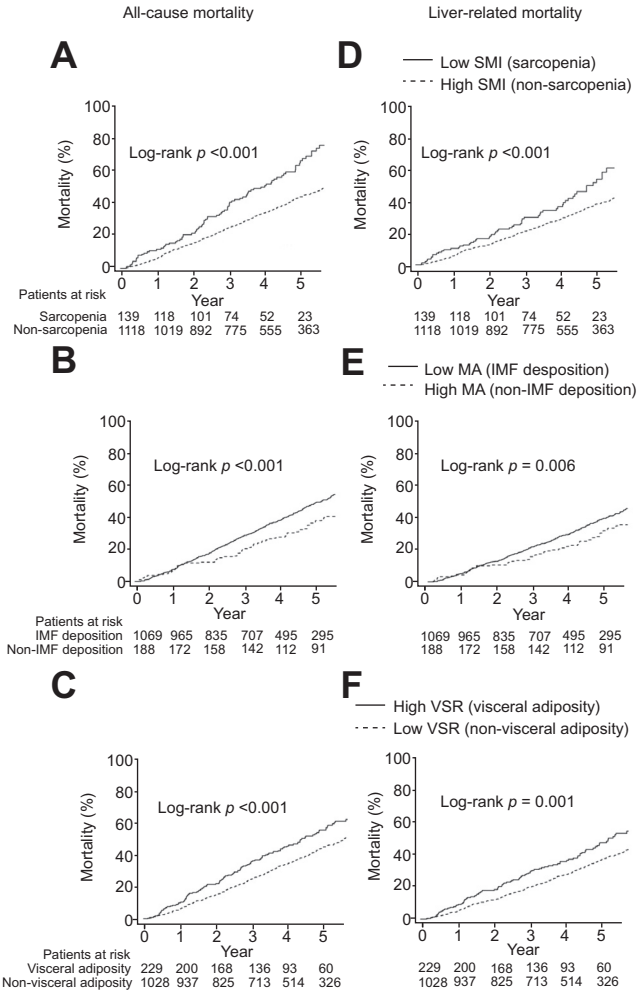

Fig. 2. Overall survival curves according to body composition variables. (A–C) All-cause mortality curves in patients with or without each body composition component. (D–F) Liver-related mortality curves in patients with or without each body composition component. SMI, skeletal muscle index; MA, muscle attenuation; IMF, intramuscular fat; VSR, visceral to subcutaneous adipose tissue area ratio.

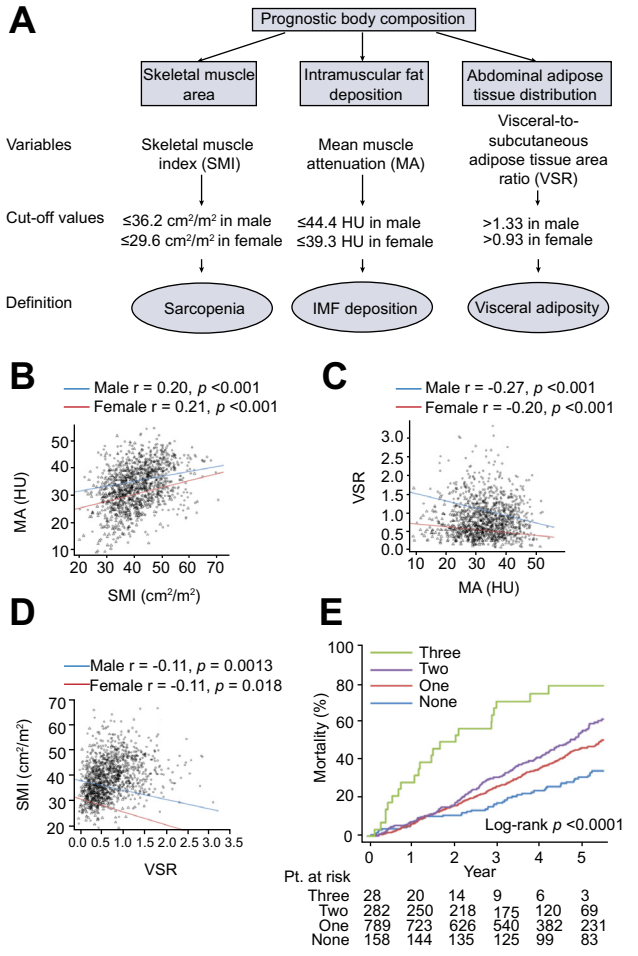

Fig. 3. Definitions of sarcopenia, intramuscular fat deposition and visceral adiposity and associations between body composition components. (A) Definitions of the prognostic body composition components. (B–D) The correlations between SMI, MA, and VSR were weak, although statistically significant. (E) Mortality risk increased based on the increase in the number of prognostic body composition components, suggesting that they were complementary predictors for a poor prognosis in patients with hepatocellular carcinoma. SMI, skeletal muscle index; MA, muscle attenuation; IMF, intramuscular fat; VSR, visceral to subcutaneous adipose tissue area ratio.

optimal cut-off values for the body composition variables used to predict mortality, with the aid of maximally selected rank statistics, as described by Lausen [25]. Such cut-off values optimally separate patients into good and poor prognosis groups. Statistically significant differences in prognoses derived using these cut-off values were examined after adjusting the *p* values, reflecting the fact that multiple testing was in play. As body composition varies by ethnicity and patient population, we re-estimated the optimal cut-off values of variables in our study cohort. We assessed the influences of body composition variables on survival using these new values.

Propensity scoring was used to control of selection bias and potential confounding [26]. The propensity scores for those with and without each prognostic body composition were evaluated non-parametrically using the following variables [27]: age; gender; viral status; BCLC stage; treatment methods for BCLC stage 0/A; prothrombin time; presence of previous treatment; and presence of chronic kidney disease. Propensity score-matching was performed with a caliper width of 0.1 multiplied by the standard deviation for the linearly transformed propensity scores. After amending these confounding factors, the all-cause and liver-related mortality analysis was repeated.

We also evaluated the associations between BMI and elements of body composition. Furthermore, we investigated the influences of BMI and body composition components on survival.

Statistical analyses were performed using R software (version 3.1.2; http://www.R-project.org) using the "survival", "maxstat", "rms" and "MatchIt" packages. All tests were two-sided, and a *p* value <0.05 was considered significant.

Results

Patient characteristics

Of the 1490 consecutive patients with HCC admitted to our department between January 2004 and December 2009, 188 (12.6%) were excluded because they lacked CT imaging data and/or notes on anthropometric parameters. We excluded 45 patients with uncontrolled ascites (3.0%). Accordingly, we assessed 1257 patients (84.4%) retrospectively.

Mean patient age was 68.8 ± 9.2 years, and HCV infection was most prevalent in this cohort (71.2%). The BCLC stage distribution was as follows: stage 0, 181 (14.4%); stage A, 588 (46.8%); stage B,

427 (34.0%); stage C, 47 (3.7%); and stage D, 14 patients (1.1%). Other baseline characteristics are shown in Table 1. The treatment methods for the 769 patients with HCC in BCLC stage 0/A were shown in Supplementary Table 1.

First, we analyzed the impact of BMI on mortality. Consistent with the data of a nationwide survey of patients with non-viral HCC [4], both obesity (BMI >25 kg/m^2) and underweight status (BMI <20 kg/m^2) were associated with higher mortality in our cohort, composed principally of patients with HCV-related HCC. This result suggests that the U-shaped relationship between BMI and mortality is common, regardless of underlying liver disease (Supplementary Fig. 1).

Association between body composition and mortality in patients with hepatocellular carcinoma

Next, we analyzed five indicators of body composition, SMI, MA, VATI, SATI and VSR, in this cohort using CT scans taken at the first admission to our department. Measurements of these indicators were consistent and highly reproducible among observers (Supplementary Fig. 2). SATI was strongly correlated, and SMI and VATI were moderately correlated with BMI (Supplementary Fig. 3). However, MA and VSR had only weak correlations with BMI, indicating that BMI cannot be used to accurately assess individual body composition components. In fact, two patients

Table 2. Clinicopathological characteristics in the patients with sarcopenia, intramuscular fat deposition and visceral adiposity.

	Sarcopenia			Intramuscular fat deposition			Visceral adiposity		
	Yes (N = 139)	No (N = 1118)	*p* value	Yes (N = 1069)	No (N = 188)	*p* value	Yes (N = 229)	No (N = 1028)	*p* value
Age, mean ± SD	73.9 ± 7.3	68.1 ± 9.2	<0.001	69.8 ± 8.7	62.8 ± 9.9	<0.001	70.5 ± 7.9	68.4 ± 9.4	<0.001
Gender, N (%)			0.45			0.022			<0.001
Male	96 (69.1)	732 (65.5)		690 (64.5)	138 (73.4)		186 (81.2)	642 (62.5)	
Female	43 (30.9)	386 (34.5)		379 (35.5)	50 (26.6)		43 (18.8)	386 (37.5)	
Body mass index (kg/m^2), median (IQR)	19.7 (17.9-21.9)	23.6 (21.7-25.7)	<0.001	23.3 (21.3-25.6)	22.6 (20.7-25.0)	0.018	24.4 (22.3-26.6)	23.0 (20.9-25.2)	<0.001
Viral status, N (%)			<0.001			<0.001			<0.001
HCV	119 (85.6)	776 (69.4)		772 (72.2)	123 (65.4)		125 (54.6)	770 (74.9)	
HBV	4 (2.9)	138 (12.3)		99 (9.3)	43 (22.9)		19 (8.3)	123 (12.0)	
HCV + HBV	0 (0)	13 (1.2)		10 (0.9)	3 (1.6)		2 (0.9)	11 (1.0)	
None	16 (11.5)	191 (17.1)		188 (17.6)	19 (10.1)		83 (36.2)	124 (12.1)	
Alcohol consumption, N (%)			0.25			0.81			0.048
>80 g per day	16 (11.5)	175 (15.7)		164 (15.3)	27 (14.4)		45 (19.7)	146 (14.2)	
Smoking status, N (%)*			0.47			0.45			0.003
Never	72 (53.3)	573 (53.2)		545 (52.6)	100 (56.8)		94 (42.7)	551 (55.5)	
Former	41 (30.4)	287 (26.6)		287 (27.7)	41 (23.3)		74 (33.6)	254 (25.6)	
Current	22 (16.3)	217 (20.2)		204 (19.7)	35 (19.9)		52 (23.6)	187 (18.9)	
Comorbidity, N (%)									
Diabetes† (yes/no)	24/101 (19.2/80.8)	286/747 (27.7/72.3)	0.08	270/717 (27.4/72.6)	40/131 (23.4/76.6)	0.32	83/128 (39.3/60.7)	227/720 (24.0/76.0)	<0.001
Cardio/cerebrovascular disease‡ (yes/no)	20/116 (14.7/85.3)	122/964 (11.2/88.8)	0.29	126/918 (12.1/87.9)	16/162 (9.0/91.0)	0.29	29/193 (13.1/86.9)	113/887 (11.3/88.7)	0.53
Pulmonary disease‡ (yes/no)	9/127 (6.6/93.4)	37/1,049 (3.4/96.6)	0.09	38/1,006 (3.6/96.4)	8/170 (4.5/95.5)	0.73	7/215 (3.2/96.8)	39/961 (3.9/96.1)	0.74
Chronic kidney disease§ (yes/no)	37/102 (26.6/73.4)	244/871 (21.9/78.1)	0.25	250/817 (23.4/76.6)	31/156 (16.6/83.4)	0.048	73/155 (32.0/68.0)	208/818 (20.3/79.7)	<0.001
Child-Pugh class, N (%)			0.57			0.46			0.13
A	109 (78.4)	849 (75.9)		820 (76.7)	138 (73.4)		186 (81.2)	772 (75.1)	
B	28 (20.2)	258 (23.1)		237 (22.2)	49 (26.1)		42 (18.3)	244 (23.7)	
C	2 (1.4)	11 (1.0)		12 (1.1)	1 (0.5)		1 (0.5)	12 (1.2)	
AST (IU/L), median (IQR)	55 (38-74)	52 (36-74)	0.53	52 (36-74)	53 (38-70)	0.69	47 (31-69)	54 (38-74)	<0.001
ALT (IU/L), median (IQR)	44 (26-61)	44 (28-70)	0.25	43 (28-69)	49 (29-69)	0.10	38 (25-64)	44 (29-69)	0.027
Total bilirubin (mg/dl), median (IQR)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.12	0.8 (0.6-1.1)	0. 9(0.6-1.2)	0.28	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.029
Albumin (g/dl), median (IQR)	3.7 (3.3-3.9)	3.7 (3.4-4.0)	0.47	3.7 (3.3-4.0)	3.7 (3.4-4.0)	0.29	3.8 (3.5-4.1)	3.7 (3.3-4.0)	0.004
Prothrombin time (%), median (IQR)	91 (75-100)	82 (71-97)	<0.001	84 (72-100)	77 (67-86)	<0.001	86 (75-100)	82 (71-98)	0.004

(*continued on next page*)

Table 2 (*continued*)

	Sarcopenia			Intramuscular fat deposition			Visceral adiposity		
	Yes (N = 139)	No (N= 1118)	*p* value	Yes (N = 1069)	No (N= 188)	*p* value	Yes (N = 229)	No (N = 1028)	*p* value
Platelet count (× 1000/μl), median (IQR)	116 (87-159)	111 (78-150)	0.09	110 (79-153)	114 (76-144)	0.43	126 (93-175)	107 (77-146)	<0.001
BCLC stage, N (%)			0.41			0.71			0.076
0	16 (11.5)	165 (14.8)		156 (14.6)	25 (13.3)		31 (13.5)	150 (14.6)	
A	63 (45.3)	525 (47.0)		495 (46.3)	93 (49.5)		94 (41.0)	494 (48.1)	
B	50 (36.0)	377 (33.7)		364 (34.1)	63 (33.5)		89 (38.9)	338 (32.9)	
C	8 (5.8)	39 (3.5)		41 (3.8)	6 (3.2)		13 (5.7)	34 (3.3)	
D	2 (1.4)	12 (1.1)		13 (1.2)	1 (0.5)		2 (0.9)	12 (1.2)	
Previous treatment, N (%)			0.31			0.51			0.005
Yes/no	58/81 (41.7/58.3)	413/705 (36.9/63.1)		396/673 (37.0/63.0)	75/113 (39.9/60.1)		105/124 (45.9/54.1)	366/662 (35.6/64.4)	
α-fetoprotein, N (%)			0.14			0.35			0.28
<100 ng/ml/≥100 ng/ml	101/38 (72.7/27.3)	878/240 (78.5/21.5)		838/231 (78.4/21.6)	141/47 (75.0/25.0)		185/44 (80.8/19.2)	794/234 (77.2/22.8)	

SD, standard deviation; IQR, interquartile range; HCV, hepatitis s C virus; HBV hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine transaminase; BCLC, Barcelona Clinic Liver Cancer.
*Data of 45 patients were missing.
†Data of 99 patients were missing.
‡Data of 35 patients were missing.
§Data of three patients were missing.

shown in Fig. 1A_1 and A_2 had a similar BMI, whereas their body components were quite different. Moreover, as shown in Fig. 1B_{1-3}, the areas of lower muscle attenuation were different among three patients despite a similar SMI.

We assessed the impacts of these indicators on all-cause mortality as a continuous variable using the restricted cubic spline estimation (Supplementary Fig. 4). SMI ($p = 0.009$) and MA ($p = 0.024$) were significant factors for mortality, and higher VSR had a tendency towards poor prognosis ($p = 0.11$). In contrast, VATI and SATI were not associated with mortality. Therefore, we focused on SMI, MA, and VSR in this study.

We performed exploratory analyses to establish the optimal cut-off values of SMI, MA, and VSR to distinguish patients with poor prognostic body composition. As significant gender differences in those variables were evident (Table 1), cut-off values were calculated by gender. Although those were also correlated with age, we did not set different cut-off values by age to investigate whether functional age or chronological age was more important for the prognosis of patients with HCC. Actually, Hubbard *et al.* proposed that sarcopenia determined by CT might be a functional age biomarker [28]. Using the maximally selected rank statistics, the cut-off values for SMI were 36.2 cm^2/m^2 in male and 29.6 cm^2/m^2 in female, those for MA were 44.4 HU in male and 39.3 HU in female, and those for VSR were 1.33 in male and 0.93 in female, respectively. To confirm the validities of these cut-off values, we analyzed the survival rates of groups stratified by each cut-off point using a Kaplan–Meier analysis. As shown in Fig. 2, patients with low SMI (n = 139, 11.1%), low MA (n = 1069, 85.0%) and high VSR (n = 229, 18.2%) showed significantly higher all-cause mortality rates than those of the others (Fig. 2A–C). In addition, patients with low SMI, low MA and high VSR also exhibited higher liver-related mortality rates (Fig. 2D–F), suggesting that these body composition factors may be associated with the progression of liver disease.

To render these descriptors intelligible, we termed low SMI, low MA, and high VSR as sarcopenia, IMF deposition, and visceral adiposity, respectively (Fig. 3A). Scatterplots between SMI, MA, and VSR revealed weak relationships, although they were statistically significant (Fig. 3B–D). In addition, these three factors contributed to increase mortality risk in an additive manner (Fig. 3E), suggesting that they are complementary predictors for a poor prognosis in patients with HCC.

The clinicopathological characteristics of the patients with and without sarcopenia, IMF deposition, and visceral adiposity are shown in Table 2. Patients with sarcopenia, IMF deposition, and visceral adiposity were significantly older than those without. BMI was significantly lower in patients with sarcopenia, and higher in patients with IMF deposition and visceral adiposity. Current smoker, diabetes and chronic kidney disease were more frequently seen in patients with visceral adiposity. Notably, none of these three factors was significantly associated with BCLC stage or Child-Pugh class.

Impacts of body composition on recurrence and mortality in patients with very early/early stage HCC who underwent curative treatment

To further elucidate the potential effect of body composition on HCC prognosis, we performed a subgroup analysis composed of 515 patients with very early/early stage HCC (BCLC stage 0/A) who underwent curative treatment by percutaneous radiofrequency ablation as a first treatment. As shown in Supplementary Fig. 5, only sarcopenia was associated with a higher risk for HCC recurrence (Supplementary Fig. 5D–F), but all three factors were significantly associated with higher mortality (Supplementary Fig. 5A–C).

Multivariate risk and propensity score-matching analyses

We performed a multivariate analysis to identify factors independently associated with all-cause mortality (Table 3). Although both underweight status and obesity were significantly associated with higher mortality in the univariate analysis, they were no longer significant prognostic factors after the multivariate analysis. In contrast, sarcopenia (HR, 1.52; 95% CI, 1.18–1.96; $p = 0.001$), IMF deposition (HR, 1.34; 95% CI, 1.05–1.71; $p = 0.020$), and visceral adiposity (HR, 1.35; 95% CI, 1.09–1.66; $p = 0.005$) were independently associated with a poor prognosis. Importantly, these body composition factors were more strongly associated with a poor prognosis than older age ($\geqslant$70 years) (HR, 1.16; 95% CI, 0.97–1.38; $p = 0.10$). The propensity score-matching analysis confirmed that all three factors were associated with all-cause and liver-related mortality (Supplementary Table 2 and Supplementary Fig. 6).

Impact of prognostic body composition on mortality according to BMI

We divided the patients into three groups in terms of the number of prognostic body composition factors; i.e., 0, 1 and 2, or 3, and assessed the impact of body composition on all-cause mortality according to BMI category. As shown in Fig. 4A, mortality risk tended to increase in accordance with increasing number of prognostic body composition components in all categories, and this tendency was most apparent in underweight patients. Furthermore, the proportion of patients who had two or three poor prognostic body composition components was significantly higher in underweight and obese patients than that in normal weight patients (Fig. 4B). This finding was consistent with the U-shaped relationship between BMI and mortality shown in Supplementary Fig. 1.

Table 3. Univariate and multivariate analyses for all-cause mortality.

Variable	No. at risk	No. of events	5-year mortality (%)	5-year mortality (95% CI)	Univariate HR	Univariate 95% CI	Univariate *p* value	Multivariate analysis HR	Multivariate analysis 95% CI	Multivariate analysis *p* value
BMI										
Underweight	186	96	57.4	(48.0-64.9)	1.35	(1.08-1.70)	0.010	1.08	(0.84-1.39)	0.55
Normal weight	700	328	42.8	(38.6-46.7)	1.00	(referent)		1.00	(referent)	
Obesity	371	183	48.6	(42.5-54.1)	1.22	(1.02-1.46)	0.032	1.00	(0.83-1.22)	0.97
Sarcopenia										
No	1118	518	44.0	(40.7-47.2)	1.00	(referent)		1.00	(referent)	
Yes	139	89	67.4	(56.6-75.5)	1.83	(1.46-2.30)	<0.001	1.52	(1.18-1.96)	0.001
IMF deposition										
No	188	83	37.1	(29.3-44.1)	1.00	(referent)		1.00	(referent)	
Yes	1069	524	48.2	(44.8-51.6)	1.52	(1.21-1.92)	<0.001	1.34	(1.05-1.71)	0.020
Visceral adiposity										
No	1028	478	54.5	(41.2-48.0)	1.00	(referent)		1.00	(referent)	
Yes	229	129	44.7	(46.7-61.1)	1.46	(1.21-1.78)	<0.001	1.35	(1.09-1.66)	0.005
Age										
<70 years old	597	267	40.8	(36.3-45.0)	1.00	(referent)		1.00	(referent)	
≥70 years old	660	340	52.0	(47.4-56.2)	1.36	(1.16-1.60)	<0.001	1.16	(0.97-1.38)	0.10
Gender, n (%)										
Male	828	407	47.3	(43.3-50.9)	1.00	(referent)				
Female	429	200	45.0	(39.5-50.0)	0.95	(0.81-1.13)	0.59			
Viral status, n (%)										
HBV	142	42	29.5	(20.9-37.2)	1.00	(referent)		1.00	(referent)	
HCV	895	448	47.5	(43.7-51.1)	2.00	(1.46-2.75)	<0.001	1.65	(1.18-2.31)	0.003
HCV + HBV	13	8	72.7	(16.5-91.1)	3.24	(1.52-6.91)	0.002	3.51	(1.63-7.57)	0.001
None	207	109	53.1	(44.5-60.3)	2.56	(1.79-3.66)	<0.001	2.13	(1.46-3.10)	<0.001
Alcohol consumption										
≤80 g per day	1066	508	45.6	(42.1-48.9)	1.00	(referent)				
>80 g per day	191	99	51.5	(43.0-58.8)	1.14	(0.92-1.42)	0.22			
Smoking status*										
Never	645	306	44.8	(40.4-48.9)	1.00	(referent)				
Former	328	145	46.5	(38.9-51.4)	0.99	(0.81-1.21)	0.92			
Current	239	127	48.6	(41.1-55.1)	1.12	(0.92-1.38)	0.27			
Diabetes†										
No	848	411	45.8	(41.9-49.4)	1.00	(referent)				
Yes	310	145	45.1	(38.5-51.0)	1.08	(0.90-1.31)	0.41			

(*continued on next page*)

Table 3 (*continued*)

Variable	No. at risk	No. of events	5-year mortality (%)	5-year mortality (95% CI)	Univariate HR	Univariate 95% CI	Univariate *p* value	Multivariate analysis HR	Multivariate analysis 95% CI	Multivariate analysis *p* value
Chronic kidney disease‡										
No	973	459	44.3	(40.7-47.7)	1.00	(referent)		1.00	(referent)	
Yes	281	146	54.8	(47.6-61.0)	1.36	(1.13-1.64)	0.001	1.15	(0.95-1.39)	0.16
Platelet count										
≥100,000/μl	732	326	42.9	(38.7-46.8)	1.00	(referent)		1.00	(referent)	
<100,000/μl	525	281	51.4	(46.4-56.0)	1.27	(1.08-1.49)	0.003	1.44	(1.22-1.70)	<0.001
BCLC										
0	181	56	28.9	(20.9-36.1)	1.00	(referent)		1.00	(referent)	
A	588	264	40.8	(36.2-45.0)	1.49	(1.12-1.99)	0.007	1.41	(1.05-1.88)	0.021
B	427	234	55.6	(50.0-60.7)	2.35	(1.76-3.15)	<0.001	2.11	(1.57-2.83)	<0.001
C	47	41	96.3	(78.4-99.4)	16.41	(10.88-24.75)	<0.001	13.6	(8.80-20.9)	<0.001
D	14	12	91.1	(43.9-98.6)	11.16	(5.97-20.85)	<0.001	13.0	(6.86-24.7)	<0.001
Previous treatment										
No	786	346	40.5	(36.5-44.2)	1.00	(referent)		1.00	(referent)	
Yes	471	261	56.9	(51.5-61.7)	1.61	(1.37-1.89)	<0.001	1.32	(1.12-1.57)	0.001
α-fetoprotein										
<100 ng/ml	979	440	42.8	(39.1-46.1)	1.00	(referent)		1.00	(referent)	
≥100 ng/ml	278	167	59.6	(52.7-65.4)	1.65	(1.38-1.98)	<0.001	1.51	(1.26-1.82)	<0.001

HR, hazard ratio; CI, confidence interval; BMI, body mass index; IMF, intramuscular fat; HCV, hepatitis C virus; HBV, hepatitis B virus; BCLC, Barcelona Clinic Liver Cancer.
*Data of 45 patients were missing.
†Data of 99 patients were missing.
‡Data of 35 patients were missing.

Discussion

In this study, we comprehensively analyzed the impact of body composition on survival in a large-scale cohort of patients in various stages of HCC and determined that sarcopenia, IMF deposition, and visceral adiposity rather than BMI were independent risk factors for a poor prognosis. In addition, we found that the proportion of patients who had two or three poor prognostic body composition components was significantly higher in underweight and obese patients than that in normal weight patients. Thus, the high prevalence of poor prognostic body composition factors in underweight and obese patients may be an explanation for the U-shaped relationship between BMI and mortality in patients with HCC.

We found that quantity (defined by low SMI) and quality (defined by low MA) of muscle were indicators of a poor prognosis in patients with HCC. This is the first report showing the negative effect of low MA on survival in patients with HCC. Low MA indicates IMF deposition, which contributes to muscle weakness independent of the age-associated loss in muscle mass [23]. Muscle weakness caused by sarcopenia and/or IMF deposition reduces activities associated with daily living. In addition, sarcopenia and IMF deposition are associated with insulin resistance [29], vitamin D deficiency [30], and increased inflammatory cytokine levels, such as interleukin-6 (IL-6) [31], all of which are associated with progression of liver fibrosis and HCC [32–34]. Therefore, the quantity and quality of skeletal muscle may be linked to the prognosis of patients with HCC through various mechanisms. Importantly, sarcopenia and IMF deposition were more strongly associated with a poor prognosis than was older age in our study, indicating that functional age rather than chronological age is a critical factor for HCC patients.

Although several reports have revealed associations between muscle depletion and survival in HCC patients of Western countries [14] and Japan [35], the cut-off values associated with poor survival and development of sarcopenia vary widely by ethnicity and/or region. Since no consensus value for CT-based sarcopenia has been established in Asian populations, we determined the cut-off values associated with poor survival in the present study (36.2 cm^2/m^2 in males and 29.6 cm^2/m^2 in females). Using these values, we found that the proportion of patients with sarcopenia was 11.1%. These cut-off values and prevalence levels were relatively lower than those in Western countries. However, using the cut-off value defined by an international consensus as the appropriate value to use to identify cancer cachexia [36], 80.5% of patients had cachexia, but this cutoff did not predict survival (data not shown). In contrast, the cut-off values derived in a very recent study from Japan were very similar to ours (36.0 cm^2/m^2 in males and 29.0 cm^2/m^2 in females) [11]. Accordingly, the values we have calculated are likely to be adequate, at least for Japanese patients. Our results will be useful for establishing diagnostic criteria for Asians. Of note, contrary to SMI, the MA thresholds were similar to those of Western countries [10].

A higher VSR was associated with a poor prognosis in HCC patients, whereas BMI, VATI, and SATI were not. This finding suggests that the distribution of adipose tissue rather than the absolute value is a major determinant for the prognosis of HCC patients. Adipose tissue controls the function of other organs by secreting adipokines. Visceral fat accumulation increases the levels of pro-inflammatory adipokines such as tumor necrosis factor-α, IL-6, and monocyte chemoattractant protein-1, and decreases that of the anti-inflammatory adipokine adiponectin [37]. In addition, excess visceral fat leads to impaired suppression of FFA release in response to insulin [38]. As FFAs and adipokines released from visceral fat flow directly into the liver though the portal vein, the liver can be influenced markedly by such changes. In contrast, subcutaneous fat effectively stores excess lipids and

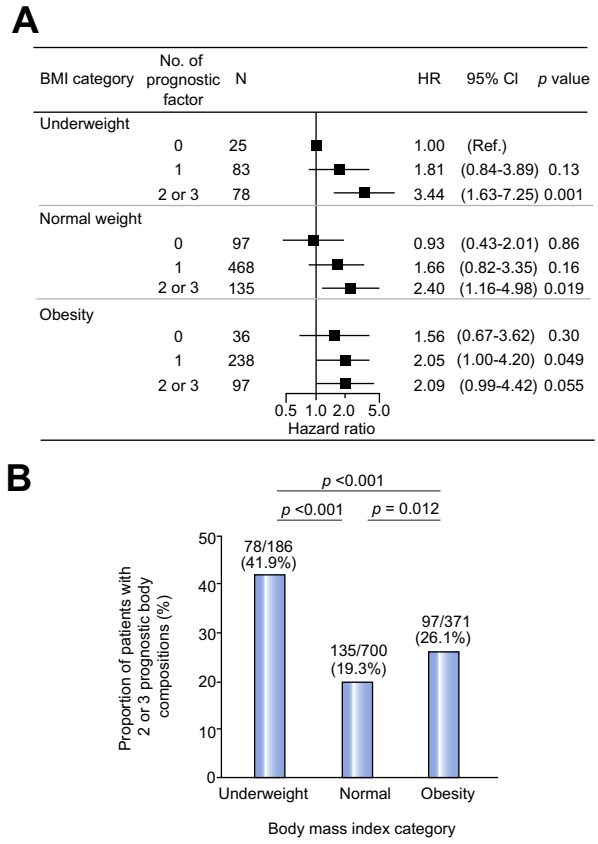

Fig. 4. Impacts of prognostic body compositions on survival stratified by BMI categories. (A) Underweight patients without any of the three negative prognostic variables exhibited a long survival time, whereas those who were underweight and had two or three prognostic factors had the shortest survival time. (B) Patients with two or three prognostic body composition factors were more prevalent among the underweight and obesity categories, which may lead to a poor prognosis among underweight and obese patients, as shown in Supplementary Fig. 1.

prevents their leakage into other organs. Furthermore, Tran *et al.* showed that transplanting subcutaneous fat into the visceral fat cavity improves glucose metabolism in mice, suggesting that subcutaneous fat cell itself can exert a metabolically advantageous function [39]. Thus, subcutaneous fat-dominant obesity may be a "metabolically healthy" status. In fact, diabetes was more prevalent among patients exhibiting visceral adiposity in this study. However, we could not address questions of causality in the present work. Thus, further basic and clinical studies are required.

The strength of our study was its large sample size of patients at different stages of HCC. However, some limitations are apparent. First, because the study was retrospective, CT data were not available for some patients, and this may have caused selection bias. Second, the study was observational; therefore, we could not infer causality between the body composition components and mortality. Finally, our cohort may not be representative of patients with HCC in general because of potential referral bias.

In conclusion, body composition rather than BMI is important in terms of survival of HCC patients, independently of liver function reserve and cancer stage. Variations in the impact of BMI on survival were explained in part by differences in body composition. We plan to conduct a prospective study investigating whether interventions targeting body composition, such as nutritional support, exercise therapy, and the use of drugs to prevent muscle depletion, improve the survival of HCC patients.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2015.02.031.

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