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ORIGINAL SCIENTIFIC REPORT

Sarcopenia Affects Systemic and Local Immune System and Impacts Postoperative Outcome in Patients with Extrahepatic Cholangiocarcinoma

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Abstract

Background A decrease in skeletal muscle mass and function, defined as sarcopenia, is associated with poor postoperative outcome in patients with cancers. Although systemic or local immune status impacts cancer progression, the relationship between sarcopenia and these statuses remains unclear. The aim of this study is to investigate the clinical impact of sarcopenia and its relationship to immune systems in patients with extrahepatic cholangiocarcinoma (ECC).

Methods A total of 110 consecutive ECC patients with curative resection between 2005 and 2014 were enrolled. Sarcopenia was determined from skeletal muscle index, assessed by a L3 skeletal muscle mass on axial computed tomography images, and their relationships with patients' clinicopathological characteristics and survival were evaluated. Systemic immune status was calculated using preoperative laboratory data, and tumor-infiltrating (TI) immune cells ($CD8^+$ T cells, $CD66b^+$ neutrophils, $CD163^+$ M2 macrophages) assayed by immunohistochemistry, and their relationship to sarcopenia were evaluated.

Results Sarcopenia was present in 31 patients (28.2%). Patients with sarcopenia had a worse recurrence-free survival (HR 1.87, $p = 0.009$) and overall survival (OS) (HR 2.47, $p = 0.0004$) than patients without sarcopenia. Moreover, patients with sarcopenia had a higher level of platelet–lymphocyte ratio (159 vs. 119; $p = 0.003$) and lower number of TI $CD8^+$ T cells (47 vs. 66 cells/spot; $p = 0.03$) than patients without sarcopenia. On multivariate analysis, the presence of sarcopenia (HR 2.60, $p = 0.0008$) was an independent predictor of poor OS.

Conclusions Our data showed that sarcopenia and systemic or local immune cells may interact with each other and play a pivotal role in clinical outcomes of patients with ECC.

Introduction

Extrahepatic cholangiocarcinoma (ECC), including perihilar and distal cholangiocarcinoma, is one of the most unfavorable cancer diagnoses because of its aggressiveness [1–3]. Although a biliary cancer was common in eastern Asia and less common in Europe and North America previously, its incidence is also increasing in these countries [4–6]. Although surgical techniques, perioperative management, and postoperative treatments have advanced, oncological outcomes remain unsatisfactory, even after curative resection, presumably because of a lack of both

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effective additional treatment and predictive biomarkers of treatment response [3].

Sarcopenia was described initially in 1989 as an age-related decrease in muscle mass [7]. Currently, sarcopenia is considered a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength. So, sarcopenia is not only a condition of older adults; it can be observed at any age as a result of several diseases, such as cancers, inflammatory diseases, malnutrition, disuse, or endocrine disorders [8–10]. Various reports show sarcopenia impacts clinical outcomes in patients with cancers [11], and with respect to hepato-biliary-pancreatic surgery, the association between preoperative sarcopenia and short-/long-term outcomes has been reported [12–16].

In the tumor microenvironment, inflammation and immune reactions play key roles in carcinogenesis and cancer progression [17]. The occurrence of a systemic inflammatory and immune response has been reportedly associated with tumor development. A host's inflammatory and immune response to a tumor leads to the up- or down-regulation of tumor proliferation and metastasis [18]. Recently, studies have shown that preoperative inflammation-based prognostic scores, such as the neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), monocyte–lymphocyte ratio (MLR), and C-reactive protein (CRP)/albumin, are predictive markers of survival in patients with cancers [19–23]. As a local immune system, tumor infiltration of immune cells can inhibit or induce tumor progression and metastasis, i.e., as tumor-infiltrating (TI) $CD8^+$ T cells, tumor-associated neutrophils (TANs), tumor-associated macrophages (TAMs) [24, 25]. In particular, TI $CD8^+$ T cells could be representative of host immune reactions against cancer cell growth, and large numbers of TI $CD8^+$ T cells indicate a favorable prognosis in patients with colorectal and ovarian cancer [26, 27].

Systematic analysis of the relationship between sarcopenia and the systemic or local immune systems would add greatly to our understanding of their role in tumor progression. Thus, the aim of this study was to investigate the role of sarcopenia as a prognostic factor and the relationship between sarcopenia and systemic or local immune systems in patients with ECC.

Materials and methods

Study group

We recruited 114 ECC patients with surgical resection at Kumamoto University Hospital between April 2005 and December 2014. From our database of 114 ECC patients with surgical resection, four patients were excluded from this analysis because their preoperative computed tomography (CT) scan was not available for a calculation of skeletal muscle mass. Thus, we finally analyzed 110 ECC patients, including 55 perihilar and 55 distal cholangiocarcinoma. Surgical procedures were pancreaticoduodenectomy, 52 patients (47.3%); extrahepatic bile duct resections only, two patients (1.8%); hepato-pancreaticoduodenectomy, one patient (0.9%) for patients with perihilar cholangiocarcinoma, and hemihepatectomy, 38 patients (34.5%); extrahepatic bile duct resections only, 12 patients (10.9%); trisectionectomy, four patients (3.6%); and hepato-pancreaticoduodenectomy, one patients (0.9%) for patients with distal cholangiocarcinoma. For patients with obstructive jaundice, we performed biliary drainage to relieve obstruction, treat segmental cholangitis, and avoid cholangitis after a detailed observation of biliary tree. Although before 2010, we primary chose percutaneous transhepatic biliary drainage (PTBD), after 2010, we gradually changed to utilize endoscopic biliary drainage to avoid seeding metastases associated with PTBD. Pathological findings were prospectively evaluated following the Japanese classification of biliary tract cancers [28]. The TNM classifications were reclassified following the American Joint Committee on Cancer system, seventh edition [29]. Patients were followed at 3- to 6-month intervals until death. Recurrence-free survival (RFS) was defined as the time between the operation date and the date of recurrence or death. Overall survival (OS) was defined as the time between the operation date and the date of death. Postoperative morbidity and mortality were evaluated according to the Clavien–Dindo Classification. Adverse events of grade IIIa or more were immediately considered to be positive for postoperative complications [30, 31]. Written informed consent was obtained from each patient, and the study procedures were approved by the Institutional Review Board.

Measurement of skeletal muscle area, visceral adiposity, and subcutaneous adiposity

Skeletal muscle area was measured retrospectively on CT scans performed before surgery at the level of the third lumbar vertebra in the inferior direction, with the patient in the supine position. Briefly, we measured pixels using a window width of −30 to 150 HU to delineate the muscle compartments and to compute their cross-sectional areas in cm^2 using the Volume Analyzer Synapse Vincent 3D image analysis system (Fujifilm Medical, Tokyo, Japan). The cross-sectional area of muscle (cm^2) at the L3 level computed from each image was normalized by the square of the height (m^2) to obtain the skeletal muscle index (SMI) (cm^2/m^2) [32]. The visceral and subcutaneous adiposity was measured retrospectively on CT scans at the level of the umbilicus with the patient in the supine position. As

well as the measurement of skeletal muscle area, we used the same system to measure pixels using a window width of −190 to −30 HU to delineate the subcutaneous and visceral compartments and to compute the cross-sectional area of each in cm^2.

Calculation of systemic immune score

Blood samples were obtained preoperatively. Because obstructive jaundice or cholangitis could affect the nutritional status and the immune state, for patients who suffered from them preoperatively, blood samples were obtained after total bilirubin levels reached a nadir and inflammatory reaction was calmed down by treatment to reduce jaundice. NLR was defined as neutrophil count (/μl) divided by lymphocyte count (/μl), PLR was defined as platelet count (/μl) divided by the lymphocyte count (/μl), MLR was defined as monocyte count (/μl) divided by the lymphocyte count (/μl), and CRP/albumin was defined as CRP value (mg/dl) divided by albumin value (g/dl).

Immunohistochemistry and evaluation

Paraffin-embedded tumor sections were dewaxed in xylene and ethanol and autoclaved for 15 min in an antigen retrieval solution to retrieve their antigen epitopes; endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Tissue sections were incubated overnight at 4 °C with primary antibodies, including rabbit polyclonal anti-CD8 (1:200 dilution; ab4055, Abcam, Cambridge, UK) as $CD8^+$ T lymphocyte, mouse monoclonal anti-CD66b (1:300 dilution; clone G10F5, BD Pharmigen, San Diego, CA) as TAN, and anti-CD163 (1:300 dilution; clone 10D6, Novocastra, Newcastle, UK) as TAM. The secondary antibody was incubated in a ready-for-use EnVision–Peroxidase system (Dako Japan, Tokyo, Japan). Sections were incubated with horseradish peroxidase-labeled polymer (EnVision1kit, Dako, Carpinteria, CA) for 30 min at 25 °C and incubated in 3,30-diaminobenzidine tetrahydrochloride (applied as a 0.02% solution containing 0.005% H_2O_2 in 0.05 M Tris–HCl; pH 7.6) at 25 °C for 5–15 min and counterstained with hematoxylin. Stained slides were evaluated by light microscopy at 200× by two researchers (Y.K. and Y.S.) blinded to patients' clinicopathological data. For CD8, CD66b, and CD163 staining, positive cells in each 1-mm-diameter field were counted and expressed as the mean (cells/field) of triplicate counts [33].

Statistical analysis

Continuous variables were expressed as mean ± SD or median (interquartile range) according to data type (parametric or nonparametric, respectively); differences were assessed for significance using Student's t test or the Mann–Whitney test. Categorical variables were evaluated using Chi-squared or Fisher exact tests, as appropriate. Cox proportional hazard regression analyses were performed to identify predictors of prognosis. RFS and OS rates were estimated by the Kaplan–Meier method, and survival curves were compared using the log-rank test. $p < 0.05$ was considered significant. All tests were performed on JMP software version 10.0.2 (SAS Institute Inc., Cary, NC, USA).

Results

Relationships between sarcopenia and patients' clinicopathological characteristics

According to established cutoff value, we identified an optimal cutoff value of SMI for sarcopenia as <41 cm^2/m^2 for women and <43 cm^2/m^2 for men with a body mass index (BMI) <25, and <53 cm^2/m^2 for men with a BMI ≥ 25 [34]. The presence of sarcopenia was 28.2% (31/110 cases) in our study population. Patients' clinicopathological characteristics are categorized as patients with and without sarcopenia and are summarized in Table 1. Patients with sarcopenia had a significantly lower body mass index (BMI) (22.2 vs. 23.6 kg/m^2, $p = 0.03$) and lower level of albumin (3.6 vs. 3.9 g/dl, $p = 0.006$) than patients without sarcopenia. In terms of a short-term clinical outcome, patients with sarcopenia experienced a significantly higher rate of postoperative complications (Clavien–Dindo ≥ IIIa) (71.0 vs. 50.6%, $p = 0.04$).

Relationships between sarcopenia and systemic or local immune systems

To investigate the impact of sarcopenia on immune systems against cancer progression, we analyzed the relationship between sarcopenia and several systemic immune scores or TI immune cells. We regarded NLR, PLR, MLR, and CRP/albumin as systemic immune status and TI $CD8^+$ T lymphocytes, TANs, and TAMs as local immune status. These local immune cells were evaluated by immunohistochemistry and are shown in Fig. 1. In our analysis, patients with sarcopenia had a significantly higher level of PLR (159 vs. 119; $p = 0.003$) and lower number of TI $CD8^+$ cells (47 vs. 66 cells/spot; $p = 0.03$), as shown in Table 2.

Table 1 Baseline clinicopathological characteristics in patients with sarcopenia

	Sarcopenia (n = 31)	Non-sarcopenia (n = 79)	p value
Age			0.32
Median (range)	71 (48–81)	69 (19–88)	
Sex			0.06
Male	17 (54.8%)	58 (73.4%)	
Female	14 (45.2%)	21 (26.6%)	
Tumor location			0.83
Perihilar	15 (48.4%)	40 (50.6%)	
Distal	16 (51.6%)	39 (49.4%)	
Macroscopic growth patterns			0.82
Periductal-infiltrating	25 (80.7%)	60 (76.0%)	
Intraductal-growing	4 (12.9%)	14 (17.7%)	
Others or unknown	2 (6.4%)	5 (6.3%)	
DM			0.10
Present	11 (35.5%)	16 (20.3%)	
Absent	20 (64.5%)	63 (79.7%)	
BMI			**0.03**
Median (range)	22.2 (17.0–31.9)	23.6 (18.0–33.2)	
Visceral adiposity			0.13
Median (range)	114.8 (13.4–225.4)	126.6 (22.9–272.4)	
Subcutaneous adiposity			0.88
Median (range)	117.8 (25.0–328.0)	113.8 (33.5–368.8)	
Albumin (g/dl)			**0.006**
Median (range)	3.6 (2.7–4.5)	3.9 (3.0–4.9)	
CRP (mg/dl)			0.79
Median (range)	0.4 (0.07–4.1)	0.3 (0.01–4.5)	
CEA (ng/ml)			0.19
Median (range)	1.6 (0.3–81.7)	1.6 (0.5–16.2)	
CA19-9 (U/ml)			0.99
Median (range)	57.8 (1.2–6230)	35.0 (6.3–7810)	
Operation time (min)			0.70
Median (range)	610 (384–974)	615 (223–1645)	
Blood loss (ml)			0.47
Median (range)	889 (300–1696)	1169 (130–574)	
Postoperative complication			**0.04**
Present	22 (71.0%)	40 (50.6%)	
Absent	9 (29.0%)	39 (49.4%)	
Tumor size (mm)			0.90
Median (range)	23.5 (12–70)	25 (10–100)	
Histologic grade			0.63
Papillary	1 (3.2%)	2 (2.5%)	
Well	11 (35.5%)	38 (48.1%)	
Moderately	15 (48.4%)	30 (38.0%)	
Poorly	4 (12.9%)	7 (8.9%)	
Others or unknown	0	2 (2.5%)	
pT			0.68
T1	3 (9.7%)	5 (6.4%)	
T2	11 (35.5%)	37 (47.4%)	
T3	16 (51.6%)	33 (42.3%)	
T4	1 (3.2%)	3 (3.9%)	

Table 1 continued

	Sarcopenia (n = 31)	Non-sarcopenia (n = 79)	p value
pN			0.37
N0	18 (58.1%)	53 (67.1%)	
N1	13 (41.9%)	26 (32.9%)	
Adjuvant therapy			0.70
Present	15 (48.4%)	35 (44.3%)	
Chemotherapy	12 (38.7%)	29 (36.7%)	
Radiotherapy	3 (9.7%)	4 (5.1%)	
Chemoradiotherapy	0	2 (2.5%)	
Absent	16 (51.6%)	44 (55.7%)	

SD standard deviation, *DM* diabetes mellitus, *BMI* body mass index, *CRP* C-reactive protein, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *pT* pathological tumor, *pN* pathological node

Bold values are statistically significant at $p < 0.05$

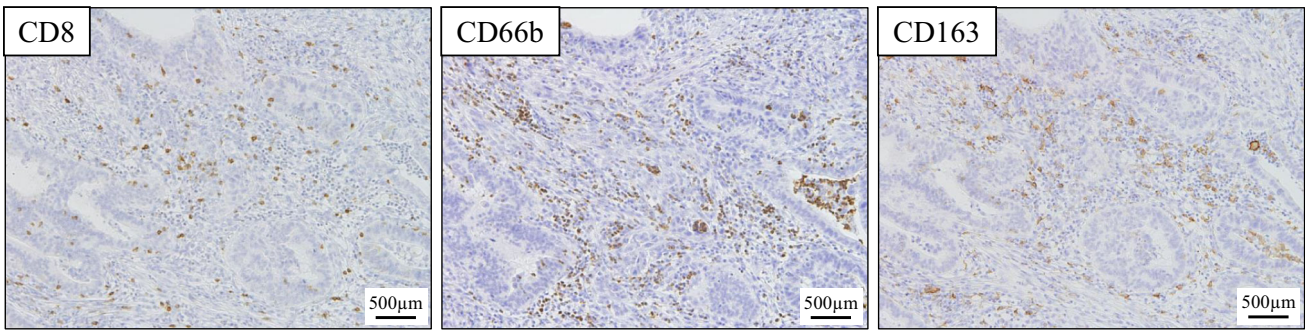

Fig. 1 Immunohistochemistry on tumor-filtrating $CD8^+$, $CD66b^+$, and $CD163^+$ cells

Table 2 The relationship between sarcopenia and systemic or local immune cells

	Sarcopenia (n = 31)	Non-sarcopenia (n = 79)	p value
WBC (/μl)	5400 (2700–6800)	5300 (2600–9700)	0.42
Lymphocyte (%)	26.3 (16.3–46.7)	30.7 (14.0–56.4)	0.26
Neutrophil (%)	60.0 (36.9–76.9)	58.0 (34.9–79.9)	0.50
Monocyte (%)	7.1 (2.0–11.6)	6.2 (2.8–18.0)	0.75
NLR	2.38 (0.81–4.49)	1.92 (0.62–5.28)	0.52
PLR	159 (77–433)	119 (61–341)	**0.003**
MLR	0.23 (0.1–0.48)	0.20 (0.07–0.77)	0.50
CRP/albumin	0.21 (0.02–1.20)	0.18 (0.003–1.36)	0.69
TI $CD8^+$ cells (cell/spot)	47 (2–143)	66 (3–256)	**0.03**
TI $CD66^+$ cells (cell/spot)	18 (0–291)	36 (0–277)	0.76
TI $CD163^+$ cells (cell/spot)	48 (19–109)	40 (3–101)	0.41

WBC white blood cell, *NLR* neutrophil–lymphocyte ratio, *PLR* platelet–lymphocyte ratio, *MLR* monocyte–lymphocyte ratio, *CRP* C-reactive protein, *TI* tumor-infiltrating

Bold values are statistically significant at $p < 0.05$

Prognostic value of sarcopenia and systemic or local immune system in patients with ECC

The median patient follow-up was 62.5 months (95% confidence limits: 51.4–94.1), measured by reverse Kaplan–Meier. Patients with sarcopenia had significantly worse RFS (HR 1.87, $p = 0.009$; Fig. 2a) and OS (HR 2.47, $p = 0.0004$; Fig. 2b). Univariate and multivariate analyses of OS are summarized in Table 3. Univariate analysis found that the presence of sarcopenia, a low number of TI $CD8^+$ and high number of TI $CD66^+$ cells, a high value of CA19-9, and the presence of lymph node metastasis were significantly associated with poor OS. And the presence of sarcopenia (HR 2.60, $p = 0.0008$), a low number of TI $CD8^+$ (HR 2.39, $p = 0.02$) and high number of TI $CD66^+$ cells (HR 2.14, $p = 0.02$), and the presence of lymph node metastasis (HR 2.09, $p = 0.007$) were independent predictors of poor OS in multivariate analysis. We next determined whether the influence of sarcopenia on OS was affected by any of the clinical, pathological, or epidemiological variables. The effect of sarcopenia was not significantly modified by age, sex, tumor location, diabetes mellitus, postoperative complication, tumor stage, or histologic grade ($p > 0.05$ for all interactions) (Fig. 3). Notably, we did not observe a modifying effect of adjuvant therapy on the relationship between sarcopenia and OS (p for interaction = 0.12). For further analysis, to investigate the impacts of sarcopenia related immune systems on patients' prognosis comprehensively, we selected PLR and TI $CD8^+$ cells as the representative prognostic marker of sarcopenia related systemic and local immune systems, respectively (Table 2). We determined optimal cutoff quartiles that fit the current outcome for prognostic analyses of PLR and TI $CD8^+$ cells. Patients with high level of PLR (HR 1.71, $p = 0.04$; Fig. 4a) and a low number of TI $CD8^+$ cells (HR 2.99, $p = 0.004$; Fig. 4b) had significantly worse OS.

Discussion

In this study, we comprehensively analyzed the impact of sarcopenia and immune systems of patients with ECC who underwent surgical resection. To the best of our knowledge, this is the first report that showed sarcopenia correlated with both the level of PLR and the count of TI $CD8^+$ cells, and that sarcopenia was an independent poor prognostic factor in patients with ECC.

In previous reports, although sarcopenia was associated with morbidity and mortality, the mechanisms were not fully understood. Some reports explained that skeletal muscle and adipose tissue are considered secretory organs with several different cytokines and peptides. Thus, they affect the immune system, especially natural killer cells, innate immune cells that help to control intracellular infectious agents and cancers [35, 36]. However, there have been few reports that showed this relationship in clinical samples. We comprehensively analyzed systemic immune cells from clinical data and assayed TI immune cells by immunohistochemistry. We particularly focused on lymphocytes, neutrophils, and monocytes and presented the positive relationships between sarcopenia and the systemic/local immune statuses.

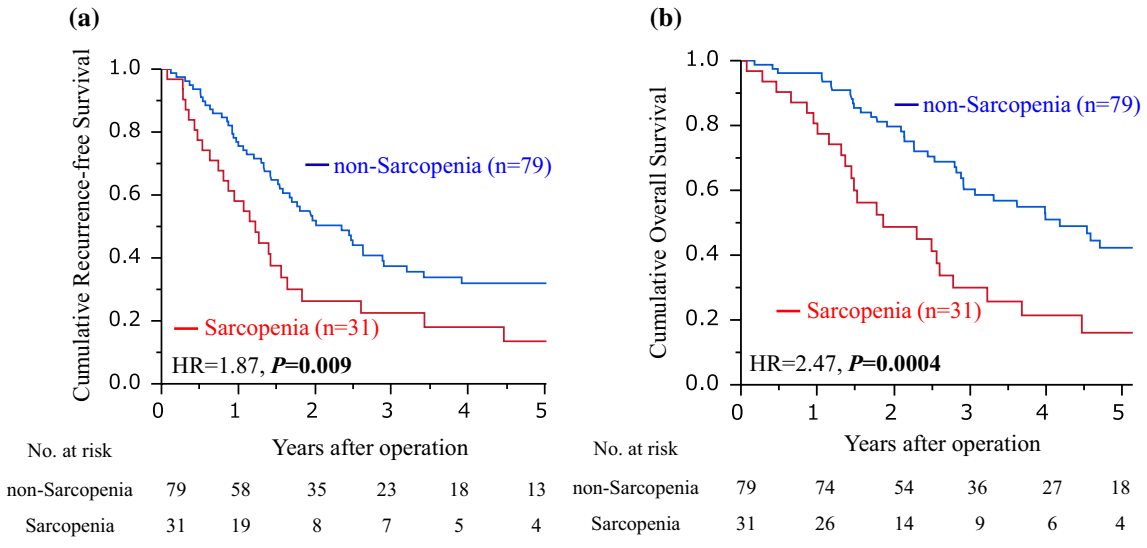

Fig. 2 Kaplan–Meier curves of recurrence-free survival (**a**) and overall survival (**b**) in patients with extrahepatic cholangiocarcinoma according to the presence of sarcopenia

Table 3 Univariate and multivariate analysis for overall survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age > 70	1.15	0.69–1.92	0.58			
Female	1.43	0.86–2.36	0.17			
BMI > 25.0	0.78	0.42–1.37	0.41			
Sarcopenia	2.47	1.46–4.11	**0.001**	2.60	1.51–4.38	**0.0008**
Visceral fat < 153 cm^2	1.39	0.83–2.41	0.22			
Subcutaneous fat < 82 cm^2	1.38	0.75–2.41	0.29			
NLR > 2.93	1.44	0.77–2.57	0.25			
PLR > 185	1.71	0.97–2.89	0.06			
MLR > 0.24	0.87	0.51–1.44	0.59			
CRP/albumin > 0.08	1.33	0.81–2.21	0.26			
TI $CD8^+$ cells low	2.99	1.45–7.22	**0.002**	2.39	1.13–5.86	**0.02**
TI $CD66^+$ cells high	2.23	1.18–4.61	**0.01**	2.14	1.12–4.50	**0.02**
TI $CD163^+$ cells high	1.47	0.84–2.70	0.18			
CEA > 3.5 ng/ml	0.75	0.26–1.71	0.53			
CA19-9 > 75 U/ml	1.85	1.11–3.04	**0.02**	1.68	0.99–2.82	0.06
Tumor size > 23 mm	1.36	0.81–2.34	0.21			
Postoperative complication	1.02	0.62–1.70	0.93			
Histologic grade, mod/poorly	1.63	0.98–2.79	0.06			
T3, 4	1.19	0.72–1.96	0.49			
N1	2.15	1.29–3.57	**0.004**	2.09	1.23–3.52	**0.007**
Adjuvant therapy (−)	0.89	0.54–1.48	0.64			

BMI body mass index, *SMI* skeletal muscles index, *NLR* neutrophil-to-lymphocyte ratio, *PLR* platelet-to-lymphocyte ratio, *MLR* monocyte–lymphocyte ratio, *CRP* C-reactive protein, *TI* tumor-infiltrating, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *HR* hazard ratio, *CI* confidence interval

Bold values are statistically significant at $p < 0.05$

First, we showed that patients with sarcopenia had a high level of PLR. Liaw et al. also revealed that a high level of PLR was associated with a greater risk of sarcopenia in geriatric populations from a large cohort study. They discussed that an elevated PLR level was a novel immune marker not only in various oncologic disorders, but also in non-oncologic disorders, such as atherosclerosis, and diabetes mellitus, polymyositis, and osteoporosis. As well as such diseases, a high platelet count indicates ongoing inflammation and may affect the development of sarcopenia because of inflammatory substances secreted by platelets [37]. Second, we showed that patients with sarcopenia had a small number of TI $CD8^+$ cells and systemic lymphocyte ratio. Lutz et al. revealed that interleukin-15 (IL-15) was highly expressed in skeletal muscle tissue and declined in aging rodent models and was required for the development and survival of natural killer lymphocytes. They posit that decreased IL-15 levels during aging constitute a common mechanism for sarcopenia and immune senescence [35]. Another report showed that immune senescence was the result of an imbalance between inflammatory and anti-inflammatory mechanisms, and cancer was one such situation as well as aging. In such an inflammatory situation, pro-inflammatory cytokines IL-6 are elevated and lead to one of the most important components of sarcopenia [38].

Recently, some studies showed the relationship between sarcopenia and obesity, such as BMI and visceral adiposity and visceral to subcutaneous adipose tissue area ratio as a prognostic factor [14, 39, 40]. However, Table 3 shows that there was not a significant correlation between such factors, and obesity was not associated with ECC patients' worse prognosis.

We acknowledge that there are several limitations in the current study, and they are major issues to be addressed in the future work. (1) As the definition of sarcopenia, various cutoff values have existed in previous reports, and the authors of those reports decided the cutoff value by sex. However, we decided our cutoff values irrespective of sex because there were too few patients in our study. For

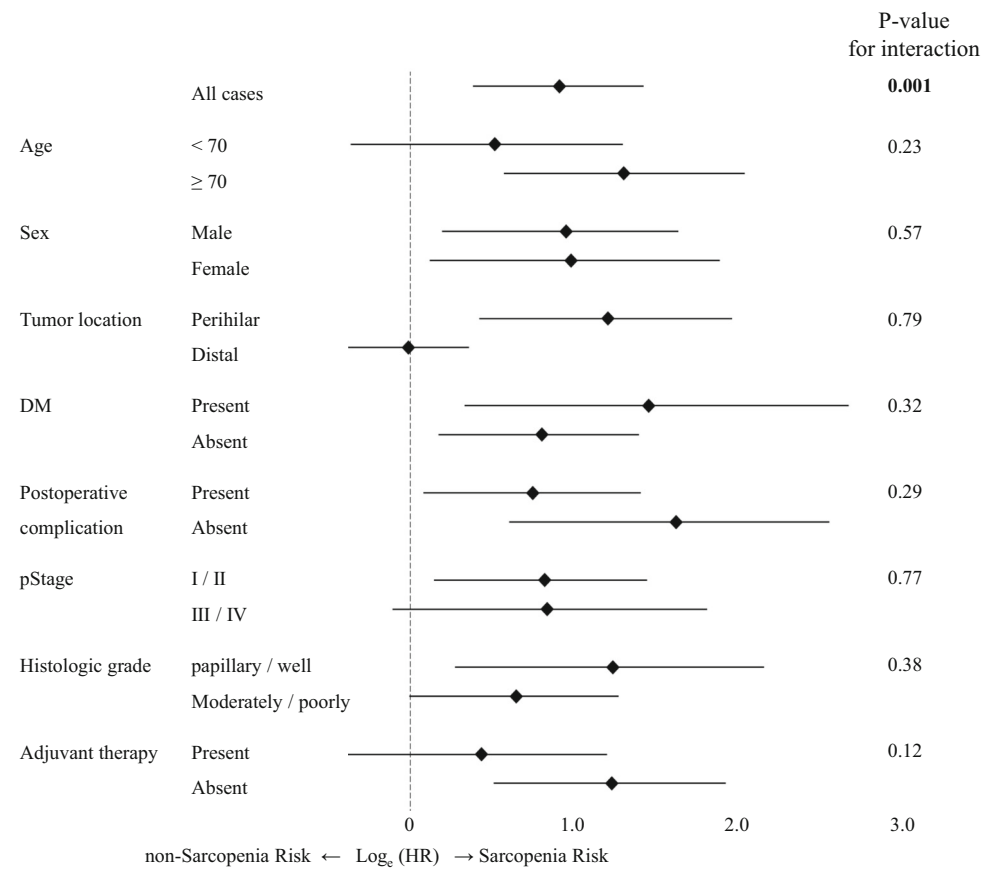

Fig. 3 Relationship between sarcopenia and overall survival in patients with cholangiocarcinoma. Log$_e$ (HRs) plots of overall survival rate in sarcopenia and non-sarcopenia groups are shown

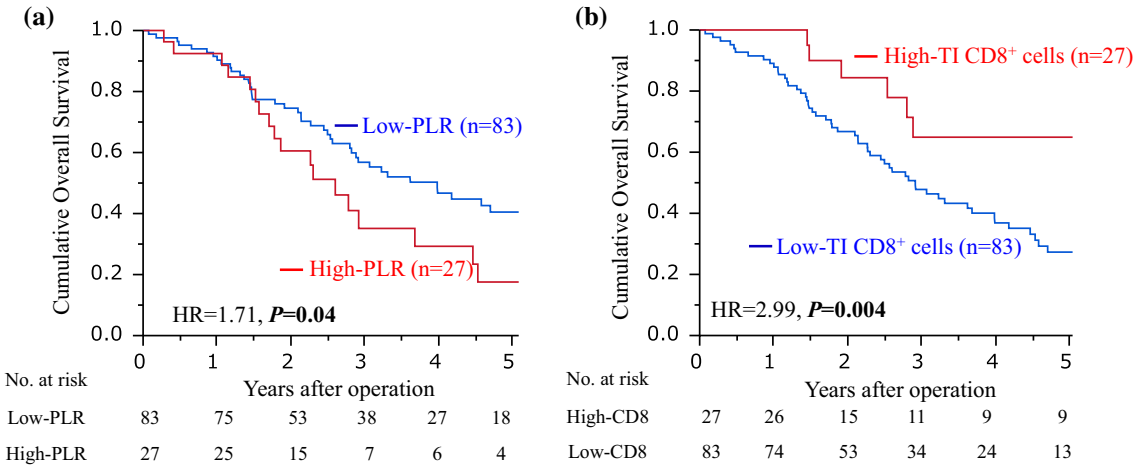

Fig. 4 Kaplan–Meier curves of overall survival in patients with extrahepatic cholangiocarcinoma according to **a** the level of PLR, **b** the number of tumor-infiltrating CD8$^+$ cells

validation of the outcome, multicenter analysis with different cohorts could confirm the impact of sarcopenia and immune systems on ECC patients' outcomes. (2) In this study, although we revealed that sarcopenia has a relationship with immune systems, it remains unclear whether sarcopenia is a cause or result, and whether another confounding factor existed or not. Therefore, further analysis, such as with an in vivo model, is our next concern.

In conclusion, sarcopenia and systemic or local immune cells may interact with each other and play a pivotal role in

clinical outcomes of patients with ECC who underwent surgical resection. These mechanisms may be exploited in cancer therapeutics, such as immune therapy or preoperative rehabilitation intervention. Future studies are needed to confirm our findings and to examine other potential mechanisms by which sarcopenia affects tumor behavior.

Compliance with ethical standards

Conflict of interest No conflict of interest exists related to this manuscript.

Ethical approval All procedures complied with the ethical standards of the relevant local and national committees on human experimentation and with the latest version of the Helsinki Declaration of 1964 Informed consent or an acceptable substitute was obtained from all patients before study inclusion.

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