Prognostic significance of sarcopenia in microsatellite-stable gastric cancer patients treated with programmed death-1 inhibitors

Yeun-Yoon Kim^{1,5} · Jeeyun Lee² · Woo Kyoung Jeong¹ · Seung Tae Kim² · Jae-Hun Kim¹ · Jung Yong Hong² · Won Ki Kang² · Kyoung-Mee Kim³ · Insuk Sohn⁴ · Dongil Choi¹

Received: 26 July 2020 / Accepted: 13 September 2020 / Published online: 24 September 2020
© The International Gastric Cancer Association and The Japanese Gastric Cancer Association 2020

Abstract

Background Sarcopenia has been underscored as a significant predictor of poor prognosis in cancer patients undergoing immunotherapy with programmed death-1 (PD-1) inhibitors. We aimed to investigate the prognostic significance of computed tomography (CT)-determined sarcopenia in patients with microsatellite-stable (MSS) gastric cancer (GC) treated with PD-1 inhibitors.

Methods We retrospectively assessed patients with MSS GC who had been treated with PD-1 inhibitors from March 2016 to June 2019. Pre-treatment sarcopenic status was determined by analyzing L3 skeletal muscle index with abdominal CT. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method, and the differences in survival probability according to sarcopenic status were compared using the log-rank test. Cox proportional hazards regression analyses were performed to identify predictors of PFS and OS.

Results Of 149 patients with MSS GC (mean age, 57.0 ± 12.3 years; 93 men), 79 (53.0%) had sarcopenia. Patients with sarcopenia had significantly shorter PFS than patients without sarcopenia (median, 1.4 months vs. 2.6 months; $P=0.026$). Sarcopenia was independently associated with shorter PFS (adjusted hazard ratio [HR], 1.79; 95% confidence interval [CI], 1.10–2.93; $P=0.020$). Patients with sarcopenia had shorter OS than patients without sarcopenia (median, 3.6 months vs. 4.9 months; $P=0.052$), but sarcopenia itself was not a significant prognostic factor for OS (adjusted HR, 1.01; 95% CI, 0.58–1.75; $P=0.974$).

Conclusions CT-determined sarcopenia is an independent prognostic factor for PFS in patients with MSS GC treated with PD-1 inhibitors.

Keywords Gastric cancer · Pembrolizumab · Nivolumab · Sarcopenia

Yeun-Yoon Kim and Jeeyun Lee have contributed equally to this work.

✉ Woo Kyoung Jeong
jeongwk@gmail.com

¹ Department of Radiology and Center for Imaging Sciences, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea

² Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

³ Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

⁴ Statistics and Data Center, Research Institute for Future Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

⁵ Present Address: Department of Radiology and Research Institute of Radiological Science, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

Introduction

Gastric cancer is the fifth most common cancer worldwide and the third leading cause of cancer-related death [1]. Although the prognosis of metastatic gastric cancer (GC) remains poor, immunotherapy with programmed death-1 (PD-1) inhibitor, such as pembrolizumab or nivolumab, has shown promising outcomes in recent clinical trials [2–5]. The PD-1 inhibitor blocks the PD-1/programmed death-ligand 1 (PD-L1) pathway by which cancer cells escape immune recognition [6]. We and others have recently shown that microsatellite instability-high (MSI-H) GC tumors respond substantially to PD-1 inhibitor [2, 3, 7]. Unfortunately, the proportion of MSI-H tumors in metastatic GC patient population is only 2–4% [8], and a better predictive tool to optimize anti-PD-1 treatment in patients with microsatellite-stable (MSS) GC is urgently needed. Although PD-L1 immunohistochemistry is a widely accepted biomarker in patients with GC, especially for pembrolizumab [4], additional and accurate biomarker in MSS GC is crucial to optimize immunotherapy strategies.

Sarcopenia, defined as low muscle strength and low muscle quantity or quality [9], is prevalent in patients with GC, with up to 62% of patients undergoing surgery being sarcopenic [10]. One of the plausible reasons for high prevalence of sarcopenia in GC is the likelihood of having poor nutritional status due to the natural course of the disease [11]. Sarcopenia before surgery or at the time of first-line chemotherapy is a significantly poor prognostic factor for survival [12–15]. Moreover, sarcopenia has been underscored as a significant predictor for poor response to PD-1 inhibitors, especially in non-small cell lung cancer (NSCLC) [16–19]. Therefore, we hypothesized that sarcopenia could be a significant prognostic factor in GC patients after PD-1 inhibition. Among several methods to determine sarcopenia [9], abdominal computed tomography (CT) may be a clinically feasible option for patients with GC. Hence, we undertook this study to determine the prognostic implication of CT-determined sarcopenia in patients with MSS GC receiving PD-1 inhibitors.

Methods

Patients

The Institutional Review Board approved our study and waived the requirement for patient consent given that this is a retrospective review of medical records and images. This study was carried out in accordance with the Declaration of Helsinki. The potentially eligible subjects of

the study were 231 patients who underwent anti-PD-1 treatment (pembrolizumab or nivolumab) for metastatic or recurrent GC at our institution from March 2016 to June 2019. The inclusion criteria were: (a) MSS or mismatch repair (MMR)-proficient GC and (b) abdominal CT examination obtained within 3 months before treatment initiation. The exclusion criteria were: (a) tumors with unknown MSI status, (b) generalized edema on pre-treatment abdominal CT hampering skeletal muscle index (SMI) measurement, and (c) unavailable tumor assessment following anti-PD-1 treatment.

Definition of sarcopenia

Using an open-source semi-automated software (BMI measurement tools, version 1.0; <https://sourceforge.net/projects/muscle-fat-area-measurement/>) [20, 21], cross-sectional areas of the rectus, transverse and oblique abdominal muscles, psoas muscles, and paraspinal muscles were measured at mid-L3 vertebra level by setting a threshold of –29 to 150 Hounsfield units. A board-certified radiologist with 6 years of experience in abdominal imaging performed the analysis using de-identified Digital Imaging and Communications in Medicine files, blinded to patient information. L3 SMI was normalized for patient stature by dividing the muscle area by height squared as follows: lumbar total muscle cross-sectional area (cm²)/height (m²). Sex-specific L3 SMI cutoffs for sarcopenia were defined as ≤ 49 cm²/m² for men and ≤ 31 cm²/m² for women, which were used in a previous study recruiting GC patients from the same geographic region [15].

Data collection

Baseline demographics, Eastern Cooperative Oncology Group performance status (ECOG PS), previous treatment history for gastric cancer, laboratory results, and histopathologic report, including histopathologic grade, MSI status, PD-L1 positivity, Epstein–Barr virus (EBV) positivity, and HER2 positivity, were collected by a retrospective review of medical records. The response to pembrolizumab or nivolumab was evaluated using Response Evaluation Criteria in Solid Tumors version 1.1 [22]. Objective response rate (ORR) was defined as proportion of patients showing complete response (CR) or partial response (PR) as best of response, and disease control rate (DCR) was defined as proportion of patients showing CR, PR, or stable disease (SD) as best of response. We also collected relevant dates: date of disease progression, date of death, or date of last follow-up. The last date of follow-up data collection was October 10, 2019.

Molecular profile analysis

MSI status of tumor tissue was determined by immunohistochemistry (IHC) for MLH1 (antibody: ES05 clone; 1:100 dilution; Novocastra, UK) and MSH2 (clone G219-1129; 1:500 dilution; CELL Marque; Rocklin, CA, USA) in formalin-fixed paraffin-embedded tissue sections. Preservation of MLH1 and MSH2 protein expression was determined by the presence of nuclear staining in the tumor cells; loss of MMR protein expression (i.e., MLH1- or MSH2-) was determined by absence of nuclear staining in the tumor cells. In the present study, MSI-H tumors were indicative of the loss of MLH1 and/or MSH2 expression.

PD-L1 expression was assessed by using PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies; Santa Clara, CA, USA) [3]. Combined positive score (i.e., the number of PD-L1 staining cells divided by the total number of viable tumor cells) $\geq 1\%$ indicated PD-L1 positivity.

For determination of EBV status, in situ hybridization for EBV-encoded small RNA was performed by using Leica BOND-MAX system (Leica Biosystems; Melbourne, Australia) [23]. Strong signals within most tumor cells were considered EBV positive.

Statistical analysis

Continuous variables were compared by using the Student's *t* test or Mann–Whitney *U* test, and categorical variables were compared by using the Chi-squared test or Fisher's exact test, according to normality. The ORR and DCR according to sarcopenic status were compared by using the Chi-squared test. The primary and secondary end points were progression-free survival (PFS) and overall survival (OS), respectively, according to sarcopenic status. PFS and OS were estimated using the Kaplan–Meier method, and the differences in survival probability according to sarcopenic status were

compared using the log-rank test. Cox proportional hazards regression analyses were performed to identify predictors of PFS and OS in 89 patients without any missing values for the variables. For variables with $P < 0.2$ in univariable analysis, variable selection was performed using backward elimination method, with sarcopenia, number of previous chemotherapy regimen, and ECOG PS being fixed for the variable selection. SAS version 9.4 (SAS Institute, Cary, NC) was used for the analyses. A two-sided *P* value of less than 0.05 indicated statistical significance.

Results

Baseline characteristics

Of the 231 potentially eligible patients with GC received anti-PD-1 monotherapy at our institution from March 2016 to June 2019, 180 patients with MSS GC were eligible. After excluding 25 patients with inadequate SMI measurement on baseline CT, and 6 patients with unavailable data following the treatment, 149 patients were identified to have MSS tumor and adequate baseline CT for evaluation of sarcopenia (Fig. 1). All patients received pembrolizumab ($n = 83$) or nivolumab ($n = 66$) as $\geq$ second-line treatment.

The median interval between the dates of abdominal CT and anti-PD-1 treatment initiation was 6.6 days (interquartile range [IQR], 2.6, 13.2). Of 149 patients with MSS GC (mean body mass index [BMI], 21.1 ± 3.4 kg/m²), 79 (53.0%) had sarcopenia based on the L3 SMI cutoffs. According to the cutoffs, 69 (74.2%) of 93 men and 10 (17.9%) of 56 women were sarcopenic (Fig. 2). Men with sarcopenia had a median SMI of 40.9 (IQR, 35.4, 44.3), whereas men without sarcopenia had a median SMI of 55.1 (IQR, 52.2, 57.7) ($P < 0.001$). Women with sarcopenia had a median SMI of

Fig. 1 Flow diagram of patients. GC gastric cancer, CT computed tomography, MSI microsatellite instability, MSI-H microsatellite instability-high, MSS microsatellite-stable, PD-1 programmed death-1, SMI skeletal muscle index

Fig. 2 Sarcopenic status according to sex-specific L3 skeletal muscle index (SMI) cutoffs for sarcopenia ($\leq 49 \text{ cm}^2/\text{m}^2$ for men and $\leq 31 \text{ cm}^2/\text{m}^2$ for women)

29.2 (IQR, 25.7, 29.7), whereas women without sarcopenia had a median SMI of 38.7 (IQR, 35.1, 41.9) ($P < 0.001$).

As summarized in Table 1, patients with sarcopenia had lower BMI than those without sarcopenia ($P < 0.001$), and were more frequently underweight (30.4% vs. 12.9%; $P = 0.004$). Previous treatment histories were not different according to sarcopenic status; 38.9% and 40.9% of patients had received two lines of prior chemotherapy regimens, and three or more lines of prior chemotherapy regimens, respectively. The number of metastatic sites and baseline serum albumin level were also comparable according to sarcopenic status. The majority (89.9%) of patients were in ECOG PS of 1. The neutrophil-to-lymphocyte ratio (NLR) was higher in patients with sarcopenia than in those without (median, 2.8 vs. 2.2; $P = 0.046$), but a similar proportion of patients had NLR higher than 2.5 ($P = 0.082$). Histopathologic characteristics were not different according to sarcopenic status.

Treatment response

Of 147 patients whose treatment response was evaluable, CR was achieved in 1 (0.7%) patient, PR in 19 (12.9%) patients, and SD in 36 (24.5%) patients, resulting in ORR and DCR values of 13.6% (95% CI, 8.5–20.2) and 38.1% (95% CI, 30.2–46.5), respectively (Table 2). CT-determined sarcopenia did not differentiate responders from non-responders to anti-PD-1 treatment (ORR, 11.7% [95% CI, 5.5–21.0] vs. 15.7% [95% CI, 8.1–26.4], respectively; $P = 0.477$). Nevertheless, MSS GC patients with sarcopenia had significantly lower DCR values (29.9% [95% CI, 20.0–41.4]) to anti-PD-1 treatment when compared to those without sarcopenia (47.1% [95% CI, 35.1–59.4]) ($P = 0.031$).

Progression-free survival

In all patients, median PFS, calculated from the date of anti-PD-1 treatment to progression or death, was 1.9 months (95% CI, 1.5–2.3). Of note, GC patients with sarcopenia had shorter PFS than patients without sarcopenia (median, 1.4 months vs. 2.6 months; $P = 0.026$) (Fig. 3). In univariable Cox regression analysis, younger patients (< 60 years), sarcopenia, three or more lines of previous chemotherapy regimens, three or more metastatic sites, and baseline serum albumin $< 3.5 \text{ g/dL}$ were significant prognostic factors to predict poor PFS (Table 3). In multivariable analysis, CT-determined sarcopenia (hazard ratio [HR], 1.79; 95% CI, 1.10–2.93; $P = 0.020$), and EBV negativity (HR, 2.18; 95% CI, 1.01–4.70; $P = 0.047$) were independently associated with shorter PFS.

Overall survival

Over a median follow-up duration of 20.3 months, 119 (79.9%) patients died. The median OS was 4.9 months (95% CI, 3.1–6.7) in all patients. Patients with sarcopenia had shorter OS than patients without sarcopenia, showing a marginal trend toward significance (median, 3.6 months vs. 4.9 months; $P = 0.052$) (Fig. 4). In univariable Cox regression analysis, younger patients (< 60 years), three or more lines of previous chemotherapy regimens, three or more metastatic sites, ECOG PS of 2 or 3, NLR > 2.5 , baseline serum albumin $< 3.5 \text{ g/dL}$, and poorly differentiated grade or signet ring cell carcinoma (SRC) type were significant prognostic factors for OS (Table 4). In multivariable analysis, younger patients (< 60 years) (HR, 2.33; 95% CI, 1.34–4.08; $P = 0.003$), male sex (HR, 2.24; 95% CI, 1.25–4.03; $P = 0.007$), three or more metastatic sites (HR, 3.20; 95% CI, 1.81–5.65; $P < 0.001$), and baseline serum albumin $< 3.5 \text{ g/dL}$ (HR, 4.27; 95% CI, 2.32–7.85; $P < 0.001$) were independently associated with shorter OS. Sarcopenia was not a significant prognostic factor for OS, with the adjusted HR of 1.01 (95% CI, 0.58–1.75; $P = 0.974$).

Discussion

In this study, we demonstrated that CT-determined sarcopenia may identify GC patients who are likely to respond to anti-PD-1 treatment, especially in patients with MSS tumors. Anti-PD-1 treatment is not available to GC patients in several countries, largely owing to the medical expense; hence, an optimized algorithm to predict response to anti-PD-1 treatment may expand the patient population that receives anti-PD-1 treatment with reduced medical expenditure. In the USA, pembrolizumab is approved for treatment of patients with MSI-H tumors, including GC patients. Herein,

Table 1 Baseline clinicopathologic characteristics

Variable	Sarcopenia (n = 79)	No sarcopenia (n = 70)	P	Total (n = 149)
Age (years), mean $\pm$ SD	58.8 $\pm$ 12.2	55.0 $\pm$ 12.2	0.061	57.0 $\pm$ 12.3
Male sex, n (%)	69 (87.3)	24 (34.3)	< 0.001	93 (62.4)
BMI (kg/m ²), mean $\pm$ SD	20.2 $\pm$ 2.8	22.2 $\pm$ 3.7	< 0.001	21.1 $\pm$ 3.4
BMI, n (%)			0.004	
Underweight (< 18.5 kg/m ²)	24 (30.4)	9 (12.9)		33 (22.2)
Normal (18.5–22.9 kg/m ²)	43 (54.4)	36 (51.4)		79 (53.0)
Overweight (23.0–24.9 kg/m ²)	8 (10.1)	10 (14.3)		18 (12.1)
Obesity ($\geq$ 25.0 kg/m ²)	4 (5.1)	15 (21.4)		19 (12.8)
L3 SMI (cm ² /m ²), median (IQR)	39.9 (33.3, 44.1)	42.3 (36.7, 53.2)	< 0.001	41 (34.9, 45.9)
Previous gastrectomy, n (%)	31 (39.2)	28 (40.0)	0.925	59 (39.6)
No. of previous chemotherapy regimens, n (%)			0.892	
1	16 (20.3)	14 (20.0)		30 (20.1)
2	32 (40.5)	26 (37.1)		58 (38.9)
3 or more	31 (39.2)	30 (42.9)		61 (40.9)
Previous radiation therapy, n (%)	11 (13.9)	5 (7.1)	0.182	16 (10.7)
Previous immunotherapy, n (%)	2 (2.5)	0 (0)	0.499	2 (1.3)
No. of metastatic sites			0.528	
1–2	40 (50.6)	40 (57.1)		80 (53.7)
3 or more	39 (49.4)	30 (42.9)		69 (46.3)
ECOG PS, n (%)			0.185	
0	4 (5.1)	0 (0)		4 (2.7)
1	70 (88.6)	64 (91.4)		134 (89.9)
2–3	5 (6.3)	6 (8.6)		11 (7.4)
NLR, median (IQR)	2.8 (1.8, 4.5)	2.2 (1.4, 3.5)	0.046	2.5 (1.6, 4.0)
NLR, n (%)			0.082	
$\leq$ 2.5	35 (44.3)	41 (58.6)		76 (51.0)
> 2.5	44 (55.7)	29 (41.4)		73 (49.0)
Serum albumin, median (IQR)	3.9 (3.4, 4.1)	3.9 (3.6, 4.2)	0.505	3.9 (3.4, 4.2)
Serum albumin, n (%)			0.611	
$\geq$ 3.5 g/dL	57 (72.2)	54 (77.1)		111 (74.5)
< 3.5 g/dL	22 (27.9)	16 (22.9)		38 (25.5)
Histopathologic grade, n (%)			0.130	n = 146*
WD/MD	26 (34.2)	16 (22.9)		42 (28.8)
PD/SRC type	50 (65.8)	54 (77.1)		104 (71.2)
PD-L1 status, n (%)			0.942	n = 112*
Positive	24 (38.1)	19 (38.8)		43 (38.4)
Negative	39 (61.9)	30 (61.2)		69 (61.6)
EBV status, n (%)			0.329	n = 120*
Positive	8 (12.5)	4 (7.1)		12 (10.0)
Negative	56 (87.5)	52 (92.9)		108 (90.0)
HER2 status, n (%)			0.129	n = 138*
Positive	6 (7.9)	1 (1.6)		7 (5.1)
Negative	70 (92.1)	61 (98.4)		131 (94.9)

BMI body mass index, EBV Epstein–Barr virus, ECOG PS, Eastern Cooperative Oncology Group performance status, IQR interquartile range, MD moderately differentiated, NLR neutrophil-to-lymphocyte ratio, PD poorly differentiated, PD-L1 programmed death-ligand 1, SD standard deviation, SMI skeletal muscle index, SRC signet ring cell carcinoma, WD well differentiated

*Histopathologic characteristics are described in patients with available data

Table 2 Response to treatment according to sarcopenic status

Best of response	Sarcopenia (<i>n</i> = 77)	No sarcopenia (<i>n</i> = 70)	<i>P</i>	Total (<i>n</i> = 147)
Objective response	9 (11.7; 5.5–21.0)	11 (15.7; 8.1–26.4)	0.477	20 (13.6; 8.5–20.2)
Disease control	23 (29.9; 20.0–41.4)	33 (47.1; 35.1–59.4)	0.031	56 (38.1; 30.2–46.5)
Complete response	1 (1.3)	0 (0)		1 (0.7)
Partial response	8 (10.4)	11 (15.7)		19 (12.9)
Stable disease	14 (18.2)	22 (31.4)		36 (24.5)

Data are represented as number of patients with percentages and 95% confidence intervals in parentheses

Objective response was defined as complete response or partial response as best of response, and disease control was defined as complete response, partial response, or stable disease as best of response. Response rates were compared by using the Chi-squared test

Fig. 3 Kaplan–Meier estimates of progression-free survival in patients with microsatellite-stable (MSS) gastric cancer (GC) treated by programmed death-1 (PD-1) inhibitor according to sarcopenic status

a powerful selective tool for patients with MSS GC is essential to better optimize immunotherapy strategies, as less than 10% of patients with MSS GC actually benefit from anti-PD-1 treatment.

We showed that baseline CT-determined sarcopenia predicted poorer response to anti-PD-1 therapy with statistical significance, which translated into shorter PFS in the sarcopenia subset. Although ORR was not significantly different according to sarcopenic status, sarcopenic patients did show substantially lower DCR (29.9%) than non-sarcopenic patients (47.1%; $P = 0.031$). Moreover, sarcopenia was an independent predictor of poor PFS. Therefore, CT-determined sarcopenia would predict an unfavorable treatment response and poor prognosis following PD-1 blockade in patients with MSS GC.

To the best of our knowledge, this is the first study in which the prognostic value of CT-determined sarcopenia in GC patients treated by PD-1 inhibitors was investigated. Similar to our findings, sarcopenia was associated with shorter PFS after treatment by PD-1/PD-L1 inhibitors in patients with tumors, such as NSCLC, melanoma, or renal

cell carcinoma [17, 24]. Skeletal muscle cells are thought to modulate the immune system by producing myokines, such as interleukin (IL)-15 and IL-6, and by interacting with immune cells [25, 26]. Sarcopenia, with impaired myokine signaling, is thought to induce immune dysregulation and create a proinflammatory environment [25]. Furthermore, chronic inflammation in the presence of cancer not only contributes to sarcopenia, but also causes immunosuppression, such as T cell exhaustion, and subsequent resistance to immunotherapy [27–30]. For example, increased serum level of IL-6, a proinflammatory cytokine contributing to muscle catabolism, following PD-1 blockade was associated with poor treatment response in patients with melanoma [31–33]. In addition, hindered T cell infiltration in the tumor due to transforming growth factor- β signaling, an immunosuppressive cytokine that also contributes to sarcopenia, was associated with poor response to PD-L1 blockade in urothelial cancer [34–36]. The higher NLR, which reflects systemic inflammation (i.e., neutrophil infiltration and lymphocyte depletion), and lower DCR in sarcopenic patients in this study may also mirror this phenomenon [19, 37]. Therefore,

Table 3 Results of Cox regression analysis for progression-free survival

Variable [reference]	Univariable		Multivariable	
	HR (95% CI)	P	HR (95% CI)	P
Age < 60 years [≥ 60 years]	1.66 (1.06–2.59)	0.028		
Sex [female]	1.55 (0.96–2.48)	0.072		
Sarcopenia [no sarcopenia]	1.60 (1.02–2.52)	0.041	1.79 (1.10–2.93)	0.020
No. of previous chemotherapy regimens [1]		0.071		
2	1.78 (0.97–3.27)	0.061	1.73 (0.94–3.20)	0.081
3 or more	2.07 (1.10–3.88)	0.024	1.76 (0.89–3.45)	0.102
No. of metastatic sites, 3 or more [1–2]	1.76 (1.12–2.78)	0.015	1.49 (0.88–2.54)	0.142
ECOG PS 2–3 [0–1]	1.67 (0.41–6.84)	0.479	0.91 (0.20–4.22)	0.905
NLR > 2.5 [≤ 2.5]	1.40 (0.90–2.17)	0.136		
Albumin < 3.5 g/dL [≥ 3.5 g/dL]	1.84 (1.09–3.10)	0.022	1.66 (0.96–2.87)	0.067
PD/SRC type [WD/MD]	1.58 (0.94–2.64)	0.084	1.55 (0.89–2.68)	0.120
PD-L1 negativity [positivity]	1.00 (0.64–1.57)	0.990		
EBV negativity [positivity]	1.83 (0.87–3.84)	0.112	2.18 (1.01–4.70)	0.047
HER2 negativity [positivity]	2.65 (0.65–10.86)	0.175	3.47 (0.76–15.75)	0.108

Analysis was performed in 89 patients without any missing values for the variables. Variables with $P < 0.2$ in univariable analysis were included in the backward elimination, with number of previous chemotherapy regimens and ECOG PS being fixed for the variable selection

CI confidence interval, EBV Epstein–Barr virus, ECOG PS Eastern Cooperative Oncology Group performance status, HR hazard ratio, MD moderately differentiated, NLR neutrophil-to-lymphocyte ratio, PD poorly differentiated, PD-L1 programmed death-ligand 1, SRC signet ring cell carcinoma, WD well differentiated

Fig. 4 Kaplan–Meier estimates of overall survival in patients with MSS GC treated by PD-1 inhibitor according to sarcopenic status

management of sarcopenia in patients undergoing immunotherapy would be beneficial to enhance treatment response and survival outcomes [38].

In our statistical analysis, sarcopenia was not a significant prognostic factor for OS, although sarcopenic patients had a trend toward poorer OS (log-rank test, $P = 0.052$) as in a prior study [19]. Although previous studies on immunotherapy for other solid tumors reported that sarcopenia was an independent prognostic factor for OS [18, 24, 39], this

might not entirely be applicable in gastric cancer patients. Surprisingly, male sex was shown to predict poor OS in the multivariable analysis. Previous analyses revealed that Asian patients with metastatic GC did not show survival differences according to sex, but female sex was a poor prognostic factor due to the association with younger age and SRC type in a Korean surgical cohort [40, 41]. Among the covariates in our analysis, only sarcopenia, but not age or histopathologic grade, showed a significant association with male sex

Table 4 Results of Cox regression analysis for overall survival

Variable [reference]	Univariable		Multivariable	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Age < 60 years [≥ 60 years]	2.01 (1.21–3.35)	0.007	2.33 (1.34–4.08)	0.003
Sex [female]	1.46 (0.87–2.42)	0.150	2.24 (1.25–4.03)	0.007
Sarcopenia [no sarcopenia]	1.55 (0.95–2.52)	0.080	1.01 (0.58–1.75)	0.974
No. of previous chemotherapy regimen [1]		0.124		
2	1.4 (0.74–2.64)	0.296	1.47 (0.78–2.80)	0.236
3 or more	1.98 (1.02–3.86)	0.045	1.32 (0.66–2.66)	0.432
No. of metastatic sites, 3 or more [1–2]	3.22 (1.95–5.31)	< 0.001	3.20 (1.81–5.65)	< 0.001
ECOG PS 2–3 [0–1]	4.54 (1.07–19.24)	0.040	2.90 (0.57–14.68)	0.197
NLR > 2.5 [≤ 2.5]	1.97 (1.23–3.15)	0.005		
Albumin < 3.5 g/dL [≥ 3.5 g/dL]	3.64 (2.10–6.29)	< 0.001	4.27 (2.32–7.85)	< 0.001
PD/SRC type [WD/MD]	1.85 (1.07–3.18)	0.027		
PD-L1 negativity [positivity]	0.89 (0.55–1.44)	0.635		
EBV negativity [positivity]	1.68 (0.73–3.91)	0.226		
HER2 negativity [positivity]	1.45 (0.36–5.95)	0.604		

Analysis was performed in 89 patients without any missing values for the variables. Variables with $P < 0.2$ in univariable analysis were included in the backward elimination, with sarcopenia, Number of previous chemotherapy regimens and ECOG PS being fixed for the variable selection

CI confidence interval, EBV Epstein–Barr virus, ECOG PS Eastern Cooperative Oncology Group performance status, HR hazard ratio, MD moderately differentiated, NLR neutrophil-to-lymphocyte ratio, PD poorly differentiated, PD-L1 programmed death-ligand 1, SRC signet ring cell carcinoma, WD well differentiated

(Chi-squared test, $P < 0.001$). Thus, the results may be partly attributable to the discrepant distribution of sarcopenia depending on sex and to the tendency toward poor prognosis in the presence of sarcopenia. Meanwhile, younger age as a poor prognostic factor could be explained by association of younger age with poorly differentiated tumor grade or SRC type (Chi-squared test, $P = 0.004$) [42, 43]. Three or more metastatic sites, representing high tumor burden, and baseline hypoalbuminemia, reflecting poor nutritional status, were independent predictors of OS, which confirms the well-defined prognostic factors in cancer patients [18, 44–47].

Our study has limitations. First, selection bias may have been introduced by excluding patients with MSI-H tumors. However, we aimed to investigate the prognostic significance of sarcopenia in patients with MSS tumors because MSI-H subtype alone could be a strong predictor of good prognosis [48]. Second, sarcopenic status could not be determined with CT in patients with generalized edema. A profound water component in the body wall in some patients increased CT attenuation of subcutaneous fat and hampered differentiation of muscle and subcutaneous fat based on Hounsfield units [20, 49]. Strategies to overcome this technical limitation are needed to better determine pre-treatment body composition. Lastly, generalizability of our results may be somewhat limited to the Western population as L3 SMI cut-offs used in our study were specific to geographic region. Future multinational studies are warranted to further validate our study results.

In conclusion, CT-determined sarcopenia is an independent prognostic factor for PFS in patients with MSS GC treated by PD-1 inhibitors. Management of sarcopenia in patients with MSS GC undergoing PD-1 blockade would be beneficial to enhance survival outcomes.

Compliance with ethical standards

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The Institutional Review Board approved our study and waived the requirement for informed consent given that this is a retrospective review of medical records and images.

References

1. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol*. 2019;14(1):26–38.
2. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. *JAMA Oncol*. 2018;4(5):e180013.
3. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med*. 2018;24(9):1449–588.

4. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol*. 2016;17(6):717–26.
5. Kang Y-K, Boku N, Satoh T, Ryu M-H, Chao Y, Kato K, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;390(10111):2461–71.
6. Kruger S, Ilmer M, Kobold S, Cadilha BL, Endres S, Ormanns S, et al. Advances in cancer immunotherapy 2019 - latest trends. *J Exp Clin Cancer Res*. 2019;38(1):268.
7. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–20.
8. Lee J, Kim ST, Kim K, Lee H, Kozarewa I, Mortimer PGS, et al. Tumor genomic profiling guides patients with metastatic gastric cancer to targeted treatment: the VIKTORY umbrella trial. *Cancer Discov*. 2019;9(10):1388–405.
9. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. 2019;48(1):16–311.
10. Kamarajah SK, Bundred J, Tan BHJGC. Body composition assessment and sarcopenia in patients with gastric cancer: a systematic review and meta-analysis. *Gastric Cancer*. 2019;22(1):10–22.
11. Kuwada K, Kuroda S, Kikuchi S, Yoshida R, Nishizaki M, Kagawa S, et al. Clinical impact of sarcopenia on gastric cancer. *Anticancer Res*. 2019;39(5):2241–9.
12. Zhuang CL, Huang DD, Pang WY, Zhou CJ, Wang SL, Lou N, et al. Sarcopenia is an independent predictor of severe postoperative complications and long-term survival after radical gastrectomy for gastric cancer: analysis from a large-scale cohort. *Medicine (Baltimore)*. 2016;95(13):e3164.
13. Wang SL, Zhuang CL, Huang DD, Pang WY, Lou N, Chen FF, et al. Sarcopenia adversely impacts postoperative clinical outcomes following gastrectomy in patients with gastric cancer: a prospective study. *Ann Surg Oncol*. 2016;23(2):556–64.
14. Tegels JJ, van Vugt JL, Reisinger KW, Hulsewe KW, Hoofwijk AG, Derikx JP, et al. Sarcopenia is highly prevalent in patients undergoing surgery for gastric cancer but not associated with worse outcomes. *J Surg Oncol*. 2015;112(4):403–7.
15. Lee JS, Kim YS, Kim EY, Jin W. Prognostic significance of CT-determined sarcopenia in patients with advanced gastric cancer. *PLoS ONE*. 2018;13(8):e0202700.
16. Nishioka N, Uchino J, Hirai S, Katayama Y, Yoshimura A, Okura N, et al. Association of sarcopenia with and efficacy of anti-PD-1/PD-L1 therapy in non-small-cell lung cancer. *J Clin Med*. 2019;8(4):450.
17. Shiroyama T, Nagatomo I, Koyama S, Hirata H, Nishida S, Miyake K, et al. Impact of sarcopenia in patients with advanced non-small cell lung cancer treated with PD-1 inhibitors: a preliminary retrospective study. *Sci Rep*. 2019;9(1):2447.
18. Derclé L, Ammari S, Champiat S, Massard C, Ferte C, Taihi L, et al. Rapid and objective CT scan prognostic scoring identifies metastatic patients with long-term clinical benefit on anti-PD-1/L1 therapy. *Eur J Cancer*. 2016;65:33–42.
19. Roch B, Coffy A, Jean-Baptiste S, Palaysi E, Daures JP, Pujol JL, et al. Cachexia - sarcopenia as a determinant of disease control rate and survival in non-small lung cancer patients receiving immune-checkpoint inhibitors. *Lung Cancer*. 2020;143:19–26.
20. Kim SS, Kim JH, Jeong WK, Lee J, Kim YK, Choi D, et al. Semi-automatic software for measurement of abdominal muscle and adipose areas using computed tomography: a STROBE-compliant article. *Medicine (Baltimore)*. 2019;98(22):e15867.
21. Kang SH, Jeong WK, Baik SK, Cha SH, Kim MY. Impact of sarcopenia on prognostic value of cirrhosis: going beyond the hepatic venous pressure gradient and MELD score. *J Cachexia Sarcopenia Muscle*. 2018;9(5):860–70.
22. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228–47.
23. Song HJ, Srivastava A, Lee J, Kim YS, Kim KM, Ki Kang W, et al. Host inflammatory response predicts survival of patients with Epstein-Barr virus-associated gastric carcinoma. *Gastroenterology*. 2010;139(1):84–92e2.
24. Cortellini A, Bozzetti F, Palumbo P, Brocco D, Di Marino P, Tinari N, et al. Weighing the role of skeletal muscle mass and muscle density in cancer patients receiving PD-1/PD-L1 checkpoint inhibitors: a multicenter real-life study. *Sci Rep*. 2020;10(1):1456.
25. Nelke C, Dziewas R, Minnerup J, Meuth SG, Ruck T. Skeletal muscle as potential central link between sarcopenia and immune senescence. *EBioMedicine*. 2019;49:381–8.
26. Pillon NJ, Bilan PJ, Fink LN, Klip A. Cross-talk between skeletal muscle and immune cells: muscle-derived mediators and metabolic implications. *Am J Physiol Endocrinol Metab*. 2013;304(5):E453–E465465.
27. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30(7):1073–81.
28. Bano G, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, et al. Inflammation and sarcopenia: a systematic review and meta-analysis. *Maturitas*. 2017;96:10–5.
29. Bilen MA, Martini DJ, Liu Y, Shabto JM, Brown JT, Williams M, et al. Combined effect of sarcopenia and systemic inflammation on survival in patients with advanced stage cancer treated with immunotherapy. *Oncologist*. 2019. <https://doi.org/10.1634/theoncologist.2019-0751>.
30. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. 2015;15(8):486–99.
31. Londhe P, Guttridge DC. Inflammation induced loss of skeletal muscle. *Bone*. 2015;80:131–42.
32. Schaap LA, Pluijm SM, Deeg DJ, Visser M. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 2006;119(6):526.e9–17.
33. Tsukamoto H, Fujieda K, Miyashita A, Fukushima S, Ikeda T, Kubo Y, et al. Combined blockade of IL6 and PD-1/PD-L1 signaling abrogates mutual regulation of their immunosuppressive effects in the tumor microenvironment. *Cancer Res*. 2018;78(17):5011–22.
34. Ábrigo J, Campos F, Simon F, Riedel C, Cabrera D, Vilos C, et al. TGF- β requires the activation of canonical and non-canonical signalling pathways to induce skeletal muscle atrophy. *Biol Chem*. 2018;399(3):253–64.
35. Lima J, Simoes E, de Castro G, Morais M, de Matos-Neto EM, Alves MJ, et al. Tumour-derived transforming growth factor-beta signalling contributes to fibrosis in patients with cancer cachexia. *J Cachexia Sarcopenia Muscle*. 2019;10(5):1045–59.
36. Mariathasan S, Turley SJ, Nickles D, Castiglioni A, Yuen K, Wang Y, et al. TGF β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554(7693):544–8.
37. Takada K, Yoneshima Y, Tanaka K, Okamoto I, Shimokawa M, Wakasu S, et al. Clinical impact of skeletal muscle area in patients with non-small cell lung cancer treated with anti-PD-1 inhibitors. *J Cancer Res Clin Oncol*. 2020;146(5):1217–25.
38. Morley JE. Treatment of sarcopenia: the road to the future. *J Cachexia Sarcopenia Muscle*. 2018;9(7):1196–9.

39. Daly LE, Power DG, O'Reilly A, Donnellan P, Cushen SJ, O'Sullivan K, et al. The impact of body composition parameters on ipilimumab toxicity and survival in patients with metastatic melanoma. *Br J Cancer*. 2017;116(3):310–7.
40. Yang D, Hendifar A, Lenz C, Togawa K, Lenz F, Lurje G, et al. Survival of metastatic gastric cancer: significance of age, sex and race/ethnicity. *J Gastrointest Oncol*. 2011;2(2):77–84.
41. Kim HW, Kim JH, Lim BJ, Kim H, Kim H, Park JJ, et al. Sex disparity in gastric cancer: Female sex is a poor prognostic factor for advanced gastric cancer. *Ann Surg Oncol*. 2016;23(13):4344–51.
42. Park JC, Lee YC, Kim JH, Kim YJ, Lee SK, Hyung WJ, et al. Clinicopathological aspects and prognostic value with respect to age: an analysis of 3,362 consecutive gastric cancer patients. *J Surg Oncol*. 2009;99(7):395–401.
43. Li X, Wang W, Ruan C, Wang Y, Wang H, Liang X, et al. Age-specific impact on the survival of gastric cancer patients with distant metastasis: an analysis of SEER database. *Oncotarget*. 2017;8(57):97090–100.
44. Arkenau HT, Olmos D, Ang JE, de Bono J, Judson I, Kaye S. Clinical outcome and prognostic factors for patients treated within the context of a phase I study: the Royal Marsden Hospital experience. *Br J Cancer*. 2008;98(6):1029–33.
45. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J*. 2010;9:69.
46. Lee J, Lim T, Uhm JE, Park KW, Park SH, Lee SC, et al. Prognostic model to predict survival following first-line chemotherapy in patients with metastatic gastric adenocarcinoma. *Ann Oncol*. 2007;18(5):886–91.
47. Oñate-Ocaña LF, Aiello-Crocifoglio V, Gallardo-Rincón D, Herrera-Goepfert R, Brom-Valladares R, Carrillo JF, et al. Serum albumin as a significant prognostic factor for patients with gastric carcinoma. *Ann Surg Oncol*. 2007;14(2):381–9.
48. Mishima S, Kawazoe A, Nakamura Y, Sasaki A, Kotani D, Kuboki Y, et al. Clinicopathological and molecular features of responders to nivolumab for patients with advanced gastric cancer. *J Immunother Cancer*. 2019;7(1):24.
49. Park HJ, Shin Y, Park J, Kim H, Lee IS, Seo DW, et al. Development and validation of a deep learning system for segmentation of abdominal muscle and fat on computed tomography. *Korean J Radiol*. 2020;21(1):88–100.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.