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Abstract. *Background/Aim: This study aimed to investigate the effect of preoperative skeletal muscle mass and muscle mass loss after surgery on overall survival in patients with gastric cancer who underwent radical resection. We also examined factors involved in postoperative skeletal muscle loss. Patients and Methods: One hundred fifty gastric cancer patients who underwent radical resection were retrospectively examined. Skeletal muscle index (SMI) was measured using computed tomography before surgery and 1 year after. Degree of muscle reduction (MR) was calculated. Patients were stratified according to preoperative SMI (high/low) and MR (high/low) for analysis. In addition, patients were grouped according to SMI and MR stratification as follows: group A, low SMI/high MR; group B, low SMI/low MR; group C, high SMI/high MR; and group D, high SMI/low MR. Results: In multivariate analysis, preoperative SMI and MR were independent predictors of overall survival. Overall survival significantly differed among groups A, B, C, and D (p<0.0001). The list of groups in order of worsening overall survival was as follows: group D, group C, group B, and group A. In multivariate analysis, patient group according to SMI and MR stratification was an independent predictor of overall survival. MR was affected by operation time (>430 min) and surgical procedure (total gastrectomy). Conclusion: Preoperative SMI and reduction in skeletal muscle mass after gastric cancer surgery were significantly associated with overall survival. Long-term management of these patients should focus on maintenance of postoperative skeletal muscle mass.*

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Key Words: Gastric cancer, sarcopenia, skeletal muscle reduction, surgical resection, survival outcome.

Gastric cancer is the third-most common cancer worldwide and second leading cause of cancer-related death (1). Despite advances in diagnosis and treatment, patients with gastric cancer frequently experience malnourishment and loss of skeletal muscle mass owing to tumor-induced gastrointestinal obstruction or nutritional disorders associated with surgery and perioperative management (2). Previous studies have suggested that body composition of patients with gastric cancer is associated with postoperative complications and long-term outcomes (3-6). Sarcopenia, which is characterized by loss of skeletal muscle mass and strength, has been associated with worse survival in healthy older people as well as patients with gastric cancer (7, 8).

Myokines secreted by muscle tissue are physiologically active substances that affect muscle anabolism and catabolism (7, 9, 10). In cancer patients, preoperative cachexia is suspected to alter myokine production and influence cancer growth and response to therapy (11). Low skeletal muscle mass before surgery in patients with gastric cancer increases incidence of postoperative complications and is associated with worse long-term outcomes (2).

However, the relationship between postoperative skeletal muscle mass reduction and survival in patients with gastric cancer remains poorly understood. Postoperative skeletal muscle mass in these patients is influenced by the extent of gastric resection, method of gastrointestinal reconstruction, and chemotherapy. We hypothesized that both preoperative skeletal muscle mass and muscle mass loss after surgery have a strong effect on long-term survival in patients with gastric cancer undergoing radical resection. This study aimed to investigate our hypothesis and clarify factors involved in postoperative skeletal muscle loss.

Patients and Methods

Patients. This retrospective study reviewed consecutive patients who underwent radical resection of gastric cancer between January 2012 and December 2013 at Kurume University Hospital. Among the 202 patients who underwent primary gastric cancer surgery during this period, we excluded 20 patients with unresectable disease, nine with other active concurrent cancers, and 23 who underwent preoperative

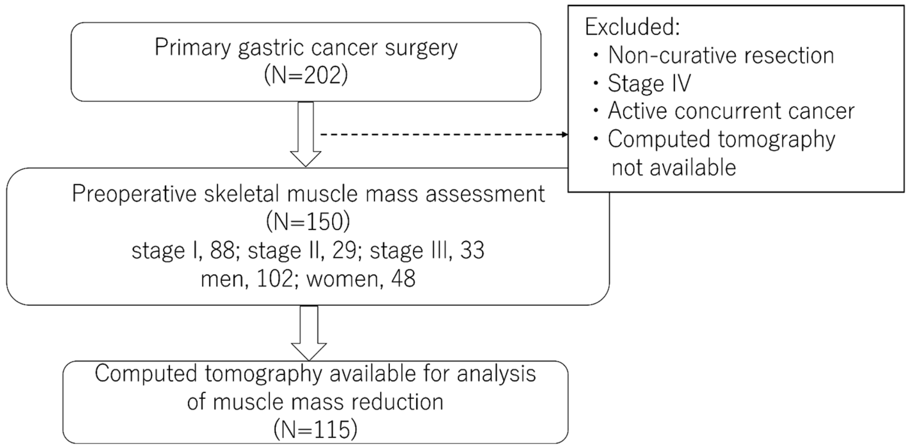


Figure 1. *The flowchart for the process for inclusion of patients.*

whole-body computed tomography (CT) at another hospital. Finally, 150 patients were included for analysis. Skeletal muscle mass reduction was examined in 115 patients whose CT was available for analysis one year after surgery (Figure 1). Patients were diagnosed before surgery using upper gastrointestinal endoscopy, upper gastrointestinal series, and CT according to the Japanese classification of gastric carcinoma, third English edition (12). Selection of surgical method was based on tumor location within the stomach. D1+ and D2 lymph node dissection were performed for early-stage and advanced cancer, respectively, according to the 2010 Japanese gastric cancer treatment guidelines (13). This study was approved by the Kurume University Hospital ethics committee (approval number, 20098).

Skeletal muscle mass assessment. Skeletal muscle mass was assessed as total skeletal muscle area on CT at the level of the inferior margin of the third lumbar vertebra. Measurements were performed using Image J software (National Institutes of Health, Bethesda, MD, USA) before surgery and 1 year after (14). Skeletal muscle index (SMI) was calculated by dividing the measured total skeletal muscle area by the square of the height. Muscle reduction (MR) was calculated as follows: (preoperative SMI – SMI 1 year after surgery)/preoperative SMI×100%. The 25th percentile of the preoperative SMI value and MR for each sex was set as the cutoff. For each preoperative SMI and MR, patients with higher values than the cutoff values were classified as the high group, and patients with lower values were classified as the low group. Patients were stratified according to preoperative SMI (low/high) and MR (low/high) for analysis. In addition, patients were grouped according to SMI and MR stratification as follows: group A, low SMI/high MR; group B, low SMI/low MR; group C, high SMI/high MR; and group D, high SMI/low MR.

Assessment of clinical parameters. All patients followed the Enhanced Recovery After Surgery (ERAS) program (15). Fluid intake was initiated on postoperative day 1 and oral intake on postoperative day 2. Length of hospitalization was defined as the time from the date of surgery to the date of discharge. Post-operative adjuvant therapy was administered according to the 2010 Japanese gastric cancer treatment guidelines (13). Postoperative adverse events were graded according to the Clavien-Dindo classification; those classified as grade II and higher were recorded and reported as complications (16).

Table I. *Patient characteristics.*

Age†	69 (34-88)
Sex (male/female)	102 (68.0%)/48 (32.0%)
Upper stomach location	39 (25.3%)
Size of tumor (mm)†	44 (5-240)
T stage (T1-2/T3-4)	101 (67.3%)/49 (32.7%)
N stage (N0/N+)	93 (63.4%)/56(37.6%)
Pathological stage (I/II/III)	88 (58.7%)/29 (19.3%)/33 (22.0%)
Preoperative comorbidity	47 (31.3%)
Circulatory	26 (17.3%)
Dialysis	3 (2.0%)
Diabetes mellitus	13 (8.7%)
Respiratory	13 (8.7%)
Operative method	
TG/DG/PG/Other	41 (27.3%)/91 (60.7%)/11 (7.3%)/7 (4.7%)
Preoperative SMI (cm^2/m^2)‡	Male, 41.7±0.8; Female, 34.2±0.7
Muscle reduction (%)‡	7.5±0.8
Postoperative complications*	36 (24.0%)
Bleeding	3 (2.0%)
Anastomotic leakage	8 (5.3%)
Pancreatic fistula	2 (1.3%)
Abscess	6 (4.0%)
Pneumonia	5 (3.3%)
Other	13 (8.7%)
Recurrence	24 (16.0%)
Death	41 (27.3%)

†Median (range). ‡Mean±standard deviation. *Clavien-Dindo classification ≥2. T1: Tumor confined to the mucosa or submucosa; T2: tumor invading the muscularis propria; T3: tumor invading the subserosa; T4: tumor invasion beyond the serosa; N0: no regional lymph node metastasis; N+: metastasis in regional lymph nodes; TG: total gastrectomy; DG: distal gastrectomy; PG: proximal gastrectomy; SMI: skeletal muscle index.

Nutritional parameters. Body mass index, prognostic nutrition index (PNI), and neutrophil/lymphocyte ratio (NLR) were used to assess nutrition. PNI was calculated as follows: [10×albumin concentration (g/dl)]+[0.005×total lymphocyte count (/mm^3)].

Table II. *Univariate and multivariate analysis for overall survival.*

	Univariate analysis		Multivariate analysis	
Variable	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (years)	1.052 (1.019-1.086)	0.0020	1.031 (0.975-1.090)	0.2825
Sex				
Male	1.664 (0.815-3.394)	0.1617		
Upper location	2.265 (1.209-4.245)	0.0107	2.227 (0.117-0.812)	0.1101
T stage				
T1-T2	2.823 (1.527-5.221)	0.0009	1.600 (0.536-5.956)	0.4328
T3-T4				
N stage				
N0	3.018 (1.602-5.686)	0.0006	2.297 (0.812-6.689)	0.1194
N1-3				
Pathological stage				
Stage I	3.342 (1.779-6.559)	0.0002	1.148 (0.242-4.908)	0.8544
Stage II/III				
Lymphatic invasion	2.694 (1.349-5.383)	0.0050	2.280 (0.656-8.492)	0.1944
Venous invasion	2.242 (1.208-4.162)	0.0105	0.682 (0.269-1.823)	0.4263
Preoperative comorbidity	2.127 (1.150-3.933)	0.0161	1.622 (0.661-3.995)	0.2878
Postoperative complication	1.831 (0.959-3.495)	0.0667	0.954 (0.354-2.336)	0.9217
Preoperative SMI	0.903 (0.863-0.945)	<0.0001	0.927 (0.877-0.979)	0.0066
Postoperative SMI	0.911 (0.867-0.957)	0.0002		
Muscle reduction	0.953 (0.917-0.990)	0.0133	0.954 (0.917-0.992)	0.0177
PNI	0.919 (0.874-0.966)	0.0008	1.043 (0.953-1.149)	0.3712
NLR	1.250 (1.031-1.515)	0.0230	1.370 (0.877-0.979)	0.0802

HR: Hazard ratio; CI: confidence interval; T1: tumor confined to the mucosa or submucosa; T2: tumor invading the muscularis propria; T3: tumor invading the subserosa; T4: tumor invasion beyond the serosa; N0: no regional lymph node metastasis; N1: metastasis in 1-2 regional lymph nodes; N2: metastasis in 3-6 regional lymph nodes; N3: metastasis in 7 or more regional lymph nodes; SMI: skeletal muscle index; PNI: prognostic nutrition index; NLR: neutrophil/lymphocyte ratio.

Decision tree algorithm. Factors involved in MR were analyzed using a decision tree algorithm that included surgical factors (operation time, intraoperative bleeding, surgical method, and postoperative complications), oncological factors (tumor markers, tumor size, depth of tumor invasion, and lymph node metastasis), and patient factors (preoperative SMI, preoperative comorbidity, PNI, and NLR).

Statistical analysis. Statistical analyses were performed using JMP software version 15 (SAS Institute, Cary, NC, USA). Groups were compared using the Student's *t*-test or Fisher's exact test as appropriate. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional hazards regression was performed to calculate hazard ratios (HRs) with 95% confidence intervals (CIs). $p<0.05$ was considered significant.

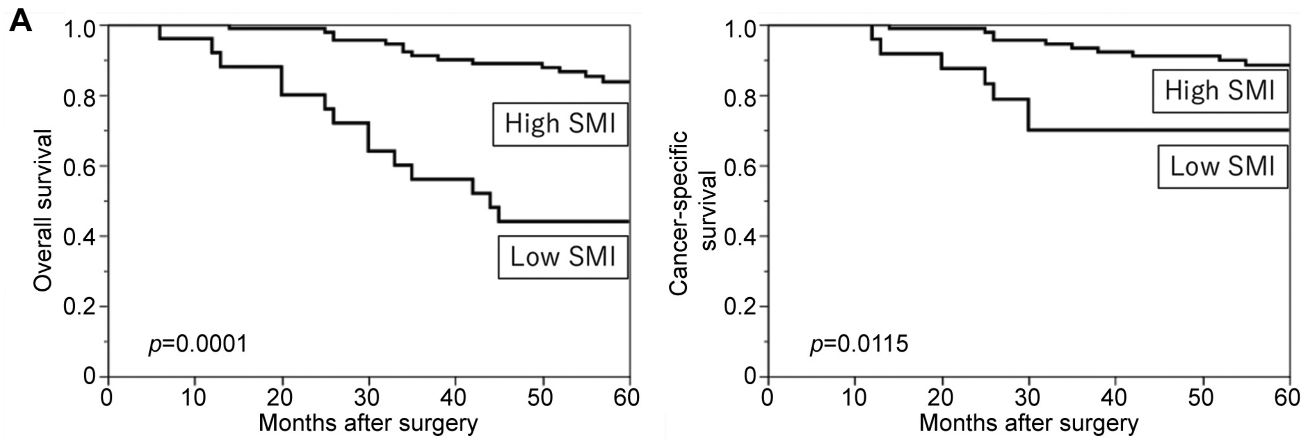
Terms. Clinicopathological terms used in this study were defined according to the Japanese classification of gastric carcinoma, third English edition (12).

Results

Patient characteristics. Patient characteristics are shown in Table I. Median follow up was 57 months. Median age was 69 years. One hundred two patients were men and 48 were women. Tumor location was upper third of the stomach in 39% of patients. Distal gastrectomy (DG) was performed in 91 patients (60.7%), total gastrectomy (TG) in 41 (27.3%), and proximal gastrectomy (PG) in 11 (7.3%).

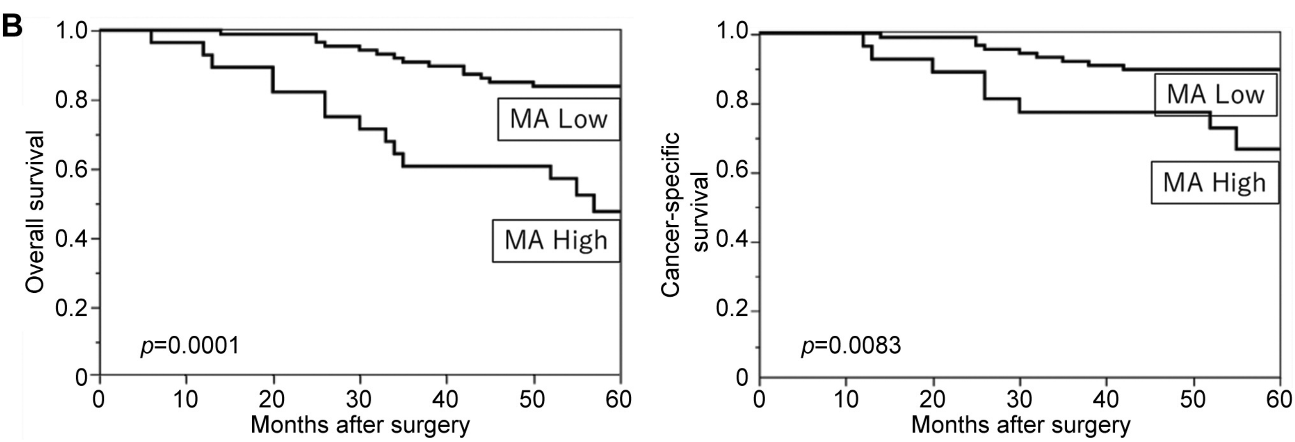
Preoperative SMI and MR. Mean SMI was 41.7±0.8 cm^2/m^2 in men and 34.2±0.7 cm^2/m^2 in women; the cut-off value in men and women was 36.4 cm^2/m^2 and 31.2 cm^2/m^2, respectively. SMI was classified as low in 35 patients (23.3%). Mean SMI 1 year after surgery was 39.8±0.9 cm^2/m^2 in men and 31.3±1.1 cm^2/m^2 in women. Mean MR was 7.5%±0.8%. The MR cut-off value was 14% in both men and women. MR was classified as high in 28 patients (24.3%).

Survival outcome. The univariate and multivariate overall survival analyses are shown in Table II. Factors evaluated included age, sex, tumor location, depth of invasion (T stage), lymph node metastasis (N stage), pathological stage (I, II, or III), lymphatic invasion, venous invasion, and preoperative comorbidity, SMI, MR, PNI, and NLR. All factors except sex were significantly associated with overall survival in the univariate analyses. In the multivariate



	5-year overall survival
High SMI	83.7%
Low SMI	44.0%

	5-year cancer-specific survival
High SMI	88.4%
Low SMI	70.0%



	5-year overall survival
MA Low	83.8%
MA High	47.6%

	5-year cancer-specific survival
MA Low	89.6%
MA High	66.7%

Figure 2. *Kaplan-Meier overall survival and cancer-specific survival curves in patients grouped according to (A) skeletal muscle index (SMI) and (B) skeletal muscle reduction rate (MR). A) Both overall and cancer-specific survival were significantly lower in the low SMI group than in the high SMI group (p<0.0001 and p=0.0115, respectively). B) Both were significantly lower in the high MR group than in the low MR group (p=0.0001 and p=0.0083, respectively).*

analysis, preoperative SMI (HR=0.927; 95%CI=0.877-0.979; *p*=0.0066) and MR (HR=0.954; 95%CI=0.917-0.992; *p*=0.0177) were independent prognostic factors. Overall and cancer-specific survival curves of patients stratified according to SMI and MR classifications are shown in Figure 2. Both overall and cancer-specific survival were significantly lower in the low SMI group than in the high SMI group (*p*<0.0001 and *p*=0.0115, respectively). Similarly, both were significantly lower in the high MR group than in the low MR group (*p*=0.0001 and 0.0083, respectively).

Characteristics of patients stratified according to SMI and MR classifications. Characteristics of patients stratified according to SMI and MR classifications are shown in Table III. Mean age was significantly higher and body mass index was significantly lower in the low SMI group than in the high SMI group (*p*=0.0004 and *p*<0.0001, respectively). Tumor diameter, depth of invasion, prevalence rates of lymph node metastasis and preoperative comorbidity, and recurrence rate were significantly higher in the low SMI group that in the high SMI group (*p*=0.0002, 0.0082, 0.0133, 0.0402, and 0.0016,

Table III. *Characteristics of patients grouped according to skeletal mass index and muscle reduction classifications.*

	High SMI N=115	Low SMI N=35	*p*-Value	Low MR N=87	High MR N=28	*p*-Value
Age†	65.5±1.0	73.2±1.9	0.0004	65.3±1.2	69.0±2.1	0.1371
Sex (male/female)	78 (67.9%)/37 (32.1%)	24 (68.6%)/11 (31.4%)	0.9340	62 (71.3%)/25 (28.7%)	15 (53.6%)/13 (46.4%)	0.1066
BMI†	22.9±3.2	19.9±2.1	<0.0001	22.4±0.3	21.9±0.6	0.4771
Operation method DG/TG/PG/other	74 (64.4%)/28 (24.4%) /8 (7.0%)/5 (4.4%)	17 (48.6%)/13 (37.1%) /3 (8.6%)/2 (5.7%)	0.4155	56 (64.3%)/21 (24.1%) /7 (8.0%)/3 (3.4%)	13 (46.4%)/14 (50.0%) /0/1 (3.6%)	0.0259
Upper location	27 (23.5%)	11 (31.3%)	0.3514	20 (23.0%)	7 (25.0%)	0.9043
Size of tumor‡ (mm)	40 (5-162)	61 (7-240)	0.0002	44 (4-240)	48 (20-170)	0.0191
T stage						
T1-T2/T3-T4	84 (73.0%)/31 (27.0%)	17 (48.6%)/18 (51.4%)	0.0082	60 (69.0%)/27 (31.0%)	15 (53.6%)/13 (46.4%)	0.1712
N stage						
N0/N1-N3	78 (67.8%)/37 (32.2%)	15 (44.1%)/19 (55.9%)	0.0133	51 (58.6%)/36 (41.4%)	15 (53.6%)/13 (46.4%)	0.6656
Lymphatic invasion	59 (51.3%)	23 (65.7%)	0.1305	52 (59.8%)	18 (64.3%)	0.8243
Venous invasion	45 (39.1%)	16 (45.7%)	0.4891	38 (43.7%)	13 (46.4%)	0.8296
Recurrence	12 (10.4%)	12 (34.3%)	0.0016	12 (13.8%)	7 (25.0%)	0.2395
Preoperative comorbidity	31 (27.0%)	16 (45.7%)	0.0402	25 (28.4%)	10 (35.7%)	0.4882
Circulatory	17 (14.8%)	9 (25.7%)	0.1488	15 (17.2%)	5 (17.9%)	0.9405
Dialysis	2 (1.7%)	1 (2.9%)	0.6913	2 (2.3%)	0	0.2883
Diabetes mellitus	9 (7.8%)	4 (11.4%)	0.5194	8 (9.2%)	2 (7.1%)	0.7323
Respiratory	9 (7.8%)	4 (11.4%)	0.5194	4 (4.6%)	5 (17.9%)	0.0358
Postoperative complications*	24 (20.8%)	12 (34.3%)	0.1132	17 (19.5%)	11 (39.3%)	0.0406
Bleeding	2 (1.7%)	1 (2.9%)	0.6913	1 (1.2%)	2 (7.1%)	0.1161
Anastomotic leakage	5 (4.4%)	3 (8.6%)	0.3552	5 (5.8%)	1 (3.6%)	0.6394
Pancreatic fistula	2 (1.7%)	0	0.3007	0	2 (7.1%)	0.0164
Abscess	4 (3.5%)	2 (5.7%)	0.5700	2 (2.3%)	1 (3.6%)	0.7226
Pneumonia	3 (2.6%)	2 (5.7%)	0.3987	3 (3.4%)	2 (7.1%)	0.5939
Other	9 (7.8%)	4 (11.4%)	0.5197	7 (8.1%)	3 (10.7%)	0.6693

†Mean±standard deviation. ‡Median (range). *Clavien-Dindo classification ≥2. SMI: Skeletal muscle index; MR: muscle reduction; BMI: body mass index; TG: total gastrectomy; DG: distal gastrectomy; PG: proximal gastrectomy; T1: tumor confined to the mucosa or submucosa; T2: tumor invading the muscularis propria; T3: tumor invading the subserosa; T4: tumor invasion beyond the serosa; N0: no regional lymph node metastasis; N1-N3: metastasis in regional lymph nodes.

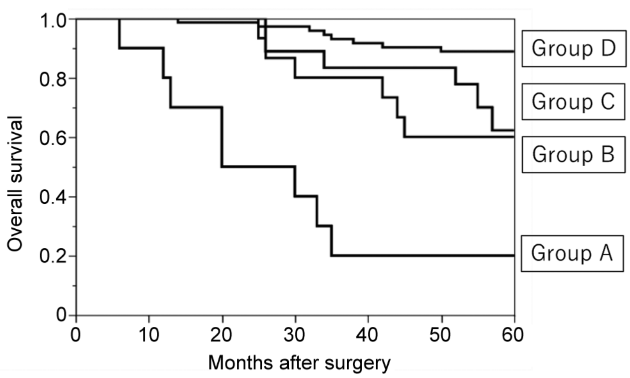
Table IV. *Univariate and multivariate analysis for overall survival according to group.*

			Univariate analysis			Multivariate analysis*		
Group	Pre SMI	Muscle reduction	HR (95%CI)	*p*-Value	Type 3 test	HR (95%CI)	*p*-Value	Type 3 test
A	Low	High	1		<0.0001	1		<0.0001
B	Low	Low	0.245 (0.084-0.713)	0.0099		0.082 (0.022-0.309)	0.0002	
C	High	High	0.189 (0.065-0.550)	0.0022		0.109 (0.030-0.390)	0.0007	
D	High	Low	0.064 (0.024-0.168)	<0.0001		0.047 (0.015-0.146)	<0.0001	

*Adjusted for age, tumor size, T stage, N stage, pathological stage, lymphatic invasion, venous invasion, preoperative comorbidity, postoperative complications, prognostic nutrition index, and neutrophil/lymphocyte ratio. Pre SMI: Preoperative skeletal muscle index; HR: hazard ratio; CI: confidence interval.

respectively). However, the TG rate, tumor diameter, prevalence of preoperative respiratory comorbidity, and postoperative complication rate were significantly higher in the high MR group than in the low MR group (p=0.0259, 0.0191, 0.0358, and 0.0406, respectively). In summary, oncological factors were significantly associated with preoperative SMI and surgery-related factors were significantly associated with MR.

Group analysis. Overall survival significantly differed among the four groups (p<0.0001) (Figure 3). The list of groups in



Group	Definition	5-year overall survival
D	High SMI/Low MR	88.8%
C	High SMI/High MR	62.0%
B	Low SMI/Low MR	60.0 %
A	Low SMI/High MR	20.0 %

Figure 3. *Kaplan-Meier overall survival curves in groups A (low SMI/high MR), B (low SMI/low MR), C (high SMI/high MR), and D (high SMI/low MR). Overall survival significantly differed among the four groups (p<0.0001). The list of groups in order of worsening overall survival is as follows: group D, group C, Group B, and group A.*

order of worsening overall survival is as follows: group D, group C, group B, and group A. The univariate and multivariate analyses of overall survival according to group are shown in Table IV. Patient group was an independent prognostic factor (p<0.0001). Compared with group A, survival was significantly better in groups B (HR=0.082; 95%CI=0.022-0.309; p=0.0002), C (HR=0.109; 95%CI=0.030-0.390; p=0.0007), and D (HR=0.047; 95%CI=0.015-0.146; p<0.0001).

Causes of MR. The decision tree algorithm is shown in Figure 4. As a result, the upper branches most associated with MR were an operation time of 430 min or more. The next branch was the involvement of surgical procedures, and TG affected MR.

Discussion

Aging is associated with progressive loss of muscle mass and physical function that may lead to progressive disability and loss of independence (17). Because the risks of cancer treatment increase with aging, accurate risk assessment and careful selection of appropriate treatment are necessary in older patients. This is of particular importance in Japan, where the population is aging rapidly.

Low skeletal muscle mass before surgery has been associated with increased incidence of postoperative complications and worse long-term outcomes in patients with gastric cancer (18-20). Sarcopenia has been shown to cause decreased patient compliance with postoperative chemotherapy (6), decreased muscular myokine secretion (2), and decreased immunocompetence (21). Skeletal muscle mass is frequently low in patients with gastric cancer because of tumor-induced digestive tract obstruction. Moreover, surgery and chemotherapy for gastric cancer may cause further MR. If MR contributes to worse long-term outcomes in these patients, preventing perioperative skeletal muscle loss might be beneficial.

In our study, low SMI had a significant relationship with patient and oncological factors, while high MR was significantly associated with surgical procedure and postoperative complications. In multivariate analysis, both postoperative MR and preoperative SMI were strong predictors of overall survival. Therefore, MR may be related to gastrectomy extent and method of reconstruction. Postoperative weight loss of 15% or more has been associated with poor compliance with postoperative chemotherapy and poor outcome (22). Another study reported that skeletal muscle loss of 5% or more at 6 months after surgery is associated with worse overall and recurrence-free survival (23). Kuwada *et al*. reported that postoperative muscle loss was an independent indicator of poor prognosis in gastric cancer patients who underwent gastrectomy (24). Therefore, perioperative management that focuses on postoperative skeletal muscle maintenance may be more important than postoperative weight loss.

As in previous studies, patients with low preoperative SMI, which presumably includes those with sarcopenia and cachexia, had worse survival outcomes in our study. In addition, patients with both low preoperative SMI and a high degree of MR had the worst survival. In patients with high preoperative SMI, overall survival significantly differed between those with low MR and high MR. Similar results were observed in patients with low preoperative SMI. These results suggest that treatment selection and perioperative management should focus on maintaining skeletal muscle.

Patient, oncological, and surgery-related factors involved in MR were analyzed using a decision tree algorithm. Surgical factors such as long operation time and total gastrectomy were more involved than oncological and patient factors. Surgeons should be aware that more invasive surgery is strongly associated with MR. Previous surgical clinical trials in patients with gastric cancer have shown that extensive measures such as para-aortic lymph node dissection and bursa resection are not necessary (25, 26); nor is splenectomy required for advanced gastric cancer that does not invade the greater curvature side (27). Another study of patients with upper gastric cancer showed that laparoscopic proximal gastrectomy with hand-sewn esophagogastrostomy results in less postoperative body weight loss and better quality of life than laparoscopic total gastrectomy (28), which is in line with our findings.

This study has several limitations. It was retrospective in design and was conducted in a single center. In addition, the sample size was relatively small and muscle mass was only

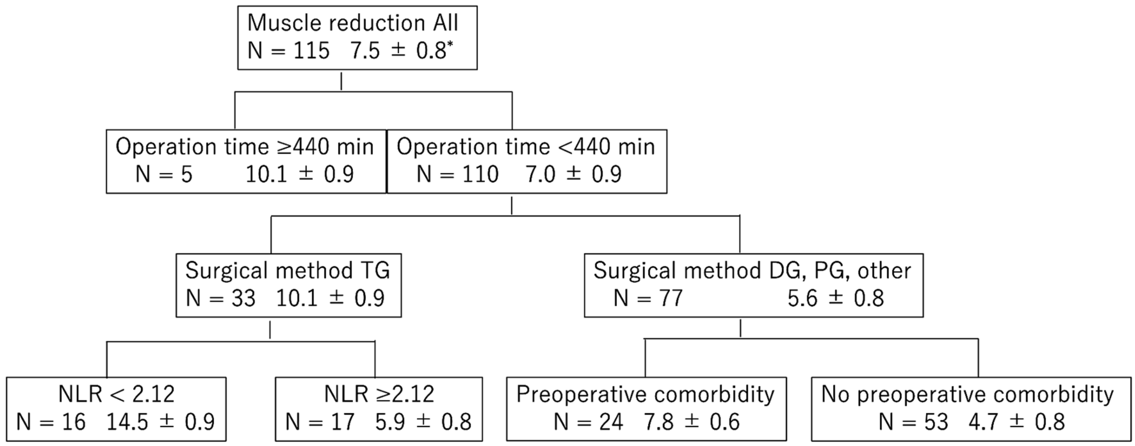


Figure 4. *Muscle reduction decision tree algorithm. The upper branches most associated with muscle reduction had an operation time of 430 min or more. The next branch was the involvement of surgical procedures, and total gastrectomy affected muscle reduction. *Values shown are mean muscle reduction%±standard deviation. TG: Total gastrectomy; DG: distal gastrectomy; PG: proximal gastrectomy; NLR: neutrophil/lymphocyte ratio.*

evaluated 1 year after surgery. Furthermore, muscle strength and function were not assessed.

In conclusion, preoperative SMI and reduction in skeletal muscle mass after gastric cancer surgery were significantly associated with overall survival. Long-term management of these patients should focus on maintenance of postoperative skeletal muscle mass.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Study design and data acquisition, analysis, and interpretation: Yuya Tanaka, Keishiro Aoyagi, Yuki Umetani, Yu Tanaka, Hiedaki Kaku, Taizan Minami, Taro Isobe. Manuscript writing and revision: Yuya Tanaka, Keishiro Aoyagi. Final approval of published version: Keishiro Aoyagi, Naotaka Murakami, Fumihiko Fujita, Yoshito Akagi.

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2 McGuire WL and Chamnes GC: Studies on the oestrogen receptor in breast cancer. In: Receptors for Reproductive Hormones. O' Malley BW, Chamnes GC (eds.). New York City, NY, USA, Plenum Publ Corp., pp 113-136, 1973.
3 Global Health Estimates 2015: Disease Burden by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva, Switzerland, World Health Organisation, 2016. Available at: **http://www.who.int/healthinfo/global_burden_disease/estimates/en/index2.html** [Last accessed on April 3, 2018] (The web address should link directly to the cited information and not to a generic webpage)

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