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Original Article

Diagnostic Performance of Four Screening Tools for Detecting Low Muscle Mass and Sarcopenia in Preoperative Older Japanese Patients with Colorectal Cancer According to the AWGS 2019 Criteria

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Abstract

Objectives: Sarcopenia and low muscle mass are distinct clinical conditions associated with adverse outcomes after colorectal cancer (CRC) surgery. Due to limited effective screening methods, we evaluated the diagnostic performance of four tools for these conditions in preoperative patients with CRC. **Methods**: This cross-sectional study included patients aged ≥65 years with stage I–III CRC scheduled for elective surgery. Sarcopenia and low appendicular skeletal muscle mass (ASM) were diagnosed according to the Asian Working Group for Sarcopenia 2019 criteria. Diagnostic performance of calf circumference (CC), SARC-F, SARC-CalF, and the Ishii score was evaluated. **Results**: Ninety-eight patients (48.0% females, mean age 77.4 ± 6.2 years) were included. Prevalences of low ASM and sarcopenia were 56.1% and 41.8%, respectively. For low ASM, CC demonstrated the highest accuracy (AUC 0.907, sensitivity 80.0%, specificity 86.0%), significantly outperforming SARC-F and SARC-CalF (AUCs 0.617 and 0.854; *p*<0.005), and comparable to the Ishii score (AUC 0.895). For sarcopenia, the Ishii score exhibited the highest accuracy (AUC 0.957, sensitivity 100%, specificity 75.4%), significantly surpassing CC, SARC-F, and SARC-CalF (AUCs 0.875, 0.704, and 0.865; *p*<0.001). **Conclusions**: The Ishii score demonstrated superior diagnostic performance for sarcopenia, underscoring the importance of muscle strength assessment for effective screening.

Keywords: Colorectal cancer, Sarcopenia, Low muscle mass, Calf circumference, Ishii score

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide, with an estimated 1.9 million new cases and 903,859 deaths reported in 2022[1]. Surgical resection remains the cornerstone of curative treatment for CRC. Despite advances in surgical techniques, including minimally invasive surgery, approximately 20% of patients experience postoperative complications[2]. These complications are typically associated with prolonged hospital stay, increased healthcare costs, and decreased long-term survival[3–5]. Sarcopenia, characterized by the loss of skeletal muscle mass and decline in muscle strength

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and/or physical performance[6], has emerged as a strong predictor of postoperative complications in patients with CRC[7].

The Asian Working Group for Sarcopenia (AWGS) 2019 consensus emphasizes the importance of muscle mass assessment for diagnosing sarcopenia. Likewise, the Global Leadership Initiative on Malnutrition (GLIM) criteria include reduced muscle mass as a phenotypic criterion, underscoring its clinical relevance[8]. Nevertheless, the accurate assessment of muscle mass typically requires expensive and inaccessible modalities such as computed tomography (CT), magnetic resonance imaging, bioelectrical impedance analysis (BIA), and dual-energy X-ray absorptiometry, thereby presenting a barrier to the widespread diagnosis of sarcopenia. This highlights the need for simple, accurate, and accessible screening tools to assess low muscle mass and sarcopenia.

To address this need, AWGS 2019 recommends screening for sarcopenia using calf circumference (CC), SARC-F (strength, assistance with walking, rising from a chair, climbing stairs, and falls)[9], or SARC-CalF (SARC-F combined with CC)[10]. Additionally, the Ishii score, which integrates age, handgrip strength (HGS), and CC into a predictive formula, has been proposed for sarcopenia screening[11]. A meta-analysis in older Chinese adults found that CC and the Ishii score demonstrated high sensitivity (both 81%) but moderate specificity (73% and 76%, respectively). Conversely, SARC-F and SARC-CalF showed high specificity (90% and 85%) but low sensitivity (34% and 59%), raising concerns regarding the underestimation of sarcopenia prevalence[12]. However, these data are primarily derived from community-dwelling or institutionalized older adults, and their applicability to patients with cancer, including those with CRC, remains unclear.

Additionally, in clinical research involving patients with CRC, sarcopenia is often assessed solely based on low skeletal muscle mass using CT imaging[7]. However, sarcopenia is a multifactorial syndrome distinct from low muscle mass[13]. In patients with CRC, sarcopenia may be appropriately diagnosed using a comprehensive approach, as recommended by consensus criteria such as AWGS 2019, which emphasize the assessment of muscle strength and physical performance in addition to muscle mass.

Therefore, the aim of this study was to determine the prevalence of low muscle mass and sarcopenia according to the AWGS 2019 criteria and to evaluate the diagnostic performance of CC, SARC-F, SARC-CalF, and the Ishii score for detecting these conditions in preoperative older Japanese patients with CRC.

Materials and Methods

Study design and population

This single-center, cross-sectional study evaluated consecutive patients aged ≥65 years with stage I–III CRC scheduled for elective laparoscopic or robot-assisted laparoscopic surgery at Kobe City Medical Center West Hospital between April 2023 and March 2025. The exclusion criteria were refusal to participate, contraindications to BIA, presence of bilateral pitting edema, diagnosis of dementia, and missing data.

Data collection

The following baseline clinicopathological data were collected from medical records: age, sex, body mass index (BMI), American Society of Anesthesiologists Physical Status (ASA-PS), Charlson Comorbidity Index (CCI), preoperative body weight loss (>5% within 6 months or >10% beyond 6 months), preoperative bowel obstruction, neoadjuvant therapy (chemotherapy, radiotherapy, or chemoradiotherapy), tumor location (colon or rectum), and cancer stage based on the TNM classification of malignant tumors.

On the day before surgery, anthropometric measurements, body composition, muscle strength, and physical performance were assessed, and the Japanese version of the SARC-F questionnaire[14] was administered via face-to-face interviews. Anthropometric data included height, weight, BMI, and CC. CC was measured at the widest point of each calf using nonelastic tape with the patient seated and the knees flexed at approximately 90°. Maximum values for each calf were recorded. Body composition was assessed using BIA (InBody S10; InBody Japan, Tokyo, Japan) in the standing position; for patients unable to maintain a standing position, measurements were performed in the supine position. The appendicular skeletal muscle mass index (ASMI) was calculated as appendicular skeletal muscle mass (ASM) divided by height squared (kg/ m^2). Muscle strength was assessed using the maximum HGS measured with a digital hand dynamometer (T.K.K.5401; Takei-Kiki-Kogyo Corporation, Niigata, Japan). Measurements were conducted in a standing position with arms straight at the sides; if patients were unable to maintain a standing position, the test was performed with the elbows flexed at 90°. Each hand was alternately tested twice, and the maximum value (kg) was recorded. Physical performance was evaluated using the 5-chair stand test (5CST). The patients were instructed to stand up from a chair five times with their arms crossed over their chest, and the total time was recorded. The inability to rise or be deemed unsuitable for safe testing was classified as low physical performance.

Diagnosis of sarcopenia and cutoff values of screening tools

Sarcopenia was diagnosed according to the AWGS 2019 criteria, which define low skeletal muscle mass (ASMI<7.0 kg/m^2 for males and <5.7 kg/m^2 for females), and require the presence of either low muscle strength

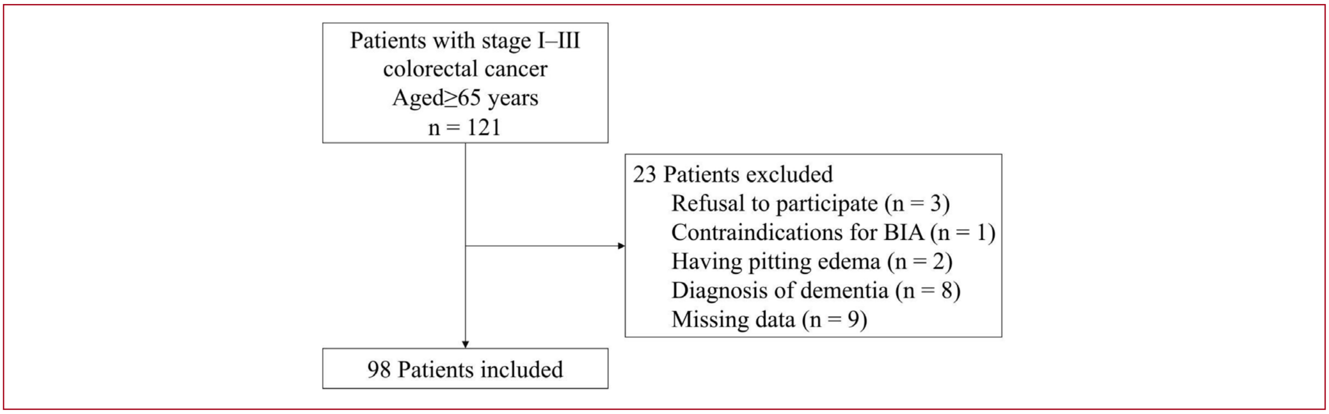

Figure 1. Flowchart of patient inclusion and exclusion criteria, showing the number of patients.

(HGS<28 kg for males and <18 kg for females) or low physical performance (5CST >12 seconds)[6]. For screening, we used CC, SARC-F, and SARC-CalF, applying the cutoff values recommended by AWGS 2019. The CC cutoff was <34 cm for males and <33 cm for females. SARC-F comprises five items scored 0–2, with a total score ranging 0–10; a score ≥4 indicates a positive screening. SARC-CalF adds 10 points to the SARC-F score if CC is below the cutoff, yielding a total score of 0–20; a score ≥11 indicates a positive screening. The Ishii score predicts sarcopenia using the following formulas [11]:

Males: $0.62 \times (\text{age} - 64) - 3.09 \times (\text{HGS} - 50) - 4.64 \times (\text{CC} - 42)$

Females: $0.80 \times (\text{age} - 64) - 5.09 \times (\text{HGS} - 34) - 3.28 \times (\text{CC} - 42)$.

A score of ≥105 for males and ≥120 for females was considered positive.

Statistical Analysis

Normality of data distribution was assessed using the Shapiro–Wilk test. Parametric data are presented as means and standard deviations, non-parametric data are expressed as medians and interquartile ranges, and categorical data are reported as counts and percentages. Continuous variables were compared between patients with or without low ASM and sarcopenia using the *t*-test (parametric) or the Mann–Whitney U test (non-parametric). Categorical variables were compared using Fisher's exact test. Using the AWGS 2019 criteria as the reference standard, sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were calculated for CC, SARC-F, SARC-CalF, and the Ishii score to assess their accuracy in identifying low ASM and sarcopenia. Receiver operating characteristic (ROC) curves were generated to evaluate diagnostic performance, and the area under the curve (AUC) with 95% confidence intervals (CI) was calculated. The AUCs were compared using the Delong method. The optimal cutoff values were determined based on the shortest distance to the upper-left corner of the ROC plot. Two-sided *p*-values were used, with $p \leq 0.05$ considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria), which is a modified version of R Commander designed to add statistical functions frequently used in biostatistics[15].

Results

Study population and baseline characteristics

A total of 121 patients aged ≥65 years with stage I–III CRC underwent elective colorectal surgery during the study period. Patients who refused to participate (n = 3), had contraindications to BIA (n = 1), exhibited bilateral pitting edema (n = 2), were diagnosed with dementia (n = 8), or had missing data (n = 9) were excluded. Finally, 98 patients (47 females and 51 males) were included in the analysis, with the mean age was 77.4 ± 6.2 years (Figure 1).

Table 1 presents the baseline characteristics of the study population stratified according to low ASM and sarcopenia status. The prevalences of low ASM and sarcopenia were 56.1% and 41.8%, respectively according to the AWGS 2019 criteria. Among the patients, those with low ASM and those with sarcopenia were older and had lower BMI, CC, HGS, and ASMI, as well as impaired physical performance. They also exhibited a higher prevalence of preoperative body weight loss, preoperative bowel obstruction, and elevated SARC-F, SARC-CalF, and Ishii score (all $p<0.005$).

Table 1. Baseline characteristics of the study population.

	Low ASM			Sarcopenia		
	Negative (n = 43)	Positive (n = 55)	p value	Negative (n = 57)	Positive (n = 41)	p value
Age (years) [a]	75.9 ± 6.0	78.7 ± 6.1	0.024	76.1 ± 5.8	79.3 ± 6.3	0.013
Sex, n (%)			0.314			0.414
Male	25 (58.1)	26 (47.3)		32 (56.1)	19 (46.3)	
Female	18 (41.9)	29 (52.7)		25 (43.9)	22 (53.7)	
BMI (kg/m^2) [a]	23.3 ± 2.6	20.3 ± 2.1	<0.001	22.8 ± 2.6	20.0 ± 2.2	<0.001
ASA-PS score, n (%)			0.724			0.566
1	4 (9.3)	8 (14.5)		5 (8.8)	7 (17.1)	
2	36 (83.7)	44 (80.0)		48 (84.2)	32 (78.0)	
3	3 (7.0)	3 (5.5)		4 (7.0)	2 (4.9)	
CCI (points) [b]	0 (0–1)	0 (0–1)	0.827	0 (0–1)	0 (0–1)	0.823
Preoperative body weight loss, n (%)	7 (16.3)	27 (49.1)	0.001	12 (21.1%)	22 (53.7%)	0.001
Preoperative bowel obstruction, n (%)	2 (4.7)	17 (30.9)	0.001	5 (8.8)	14 (34.1)	0.003
Neoadjuvant therapy, n (%)	4 (9.3)	14 (25.5)	0.064	7 (12.3)	11 (26.8)	0.111
Tumor location, n (%)			0.533			0.405
Colon	24 (55.8)	35 (63.6)		32 (56.1)	27 (65.9)	
Rectum	19 (44.2)	20 (36.4)		25 (43.9)	14 (34.1)	
TNM stage, n (%)			1.000			0.663
I–II	14 (32.6)	18 (32.7)		20 (35.1)	12 (29.3)	
III	29 (67.4)	37 (67.3)		37 (64.9)	29 (70.7)	
CC (cm) [a]	35.6 ± 2.5	31.2 ± 2.6	<0.001	34.8 ± 2.7	30.7 ± 2.7	<0.001
CC below cutoff, n (%)	6 (14.0%)	44 (80.0%)	<0.001	15 (26.3)	35 (85.4)	<0.001
SARC-F score (points) [b]	0 (0–1)	1 (0–2)	0.027	0 (0–1)	1 (0–3)	<0.001
SARC-F ≥4, n (%)	2 (4.7%)	10 (18.2%)	0.062	2 (3.5%)	10 (24.4%)	0.003
SARC-CalF score (points) [b]	0 (0–1)	10 (10–11)	<0.001	0 (0–10)	11 (10–13)	<0.001
SARC-CalF ≥11, n (%)	2 (4.7%)	23 (41.8%)	<0.001	3 (5.3%)	22 (53.7%)	<0.001
Ishii score (points) [a]	89.2 ± 27.0	133.4 ± 28.3	<0.001	93.0 ± 26.1	143.2 ± 23.9	<0.001
Ishii score above cutoff, n (%)	7 (16.3%)	48 (87.3%)	<0.001	14 (24.6%)	41 (100%)	<0.001
HGS (kg) [a]	28.9 ± 8.3	21.1 ± 7.2	<0.001	28.4 ± 8.1	19.0 ± 5.9	<0.001
HGS below cutoff, n (%)	5 (11.6%)	35 (63.6%)	<0.001	5 (8.8%)	35 (85.4%)	<0.001
5CST ≥12 sec, n (%)	6 (14.0%)	22 (40.0%)	0.006	6 (10.5%)	22 (53.7%)	<0.001
ASMI (kg/m^2) [a]	7.0 ± 0.9	5.6 ± 0.9	<0.001	6.7 ± 1.0	5.4 ± 0.9	<0.001

ASM, appendicular skeletal muscle mass; BMI, body mass index; ASA-PS, American Society of Anesthesiologists Physical Status; CCI, Charlson Comorbidity Index; CC, calf circumference; SARC-F, strength, assistance with walking, rising from a chair, climbing stairs, and falls; SARC-CalF, SARC-F combined with CC; HGS, handgrip strength; 5CST, 5-chair stand test; ASMI, appendicular skeletal muscle mass index. Data are presented as mean ± standard deviation[a], median (interquartile range) [b], or number (%), as appropriate. Comparisons between negative and positive groups for low ASM and sarcopenia were conducted using the t-test or Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables.

Table 2. Diagnostic performance of screening tools for low ASM and sarcopenia.

Screening tools	Sensitivity (%)	Specificity (%)	PLR	NLR	AUC (95% CI)
Low ASM					
CC	80.0	86.0	5.733	0.232	0.907 [bc] (0.850–0.965)
SARC-F	18.2	95.3	3.909	0.858	0.617 [acd] (0.519–0.715)
SARC-CalF	41.8	95.3	8.991	2.242	0.854 [ab] (0.777–0.931)
Ishii score	87.3	83.7	5.361	0.152	0.895 [b] (0.829–0.960)
Sarcopenia					
CC	85.4	73.7	3.244	0.199	0.875 [bd] (0.808–0.941)
SARC-F	24.4	96.5	6.951	0.784	0.704 [acd] (0.606–0.801)
SARC-CalF	53.7	94.7	10.195	0.489	0.865 [bd] (0.794–0.936)
Ishii score	100	75.4	4.071	0	0.957 [abc] (0.920–0.993)

ASM, appendicular skeletal muscle mass; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUC, area under the curve; CI, confidence interval; CC, calf circumference; SARC-F, strength, assistance with walking, rising from a chair, climbing stairs, and falls; SARC-CalF, SARC-F combined with CC. [a] p<0.05 vs. CC; [b] p<0.05 vs. SARC-F; [c] p<0.05 vs. SARC-CalF; [d] p<0.05 vs. Ishii score.

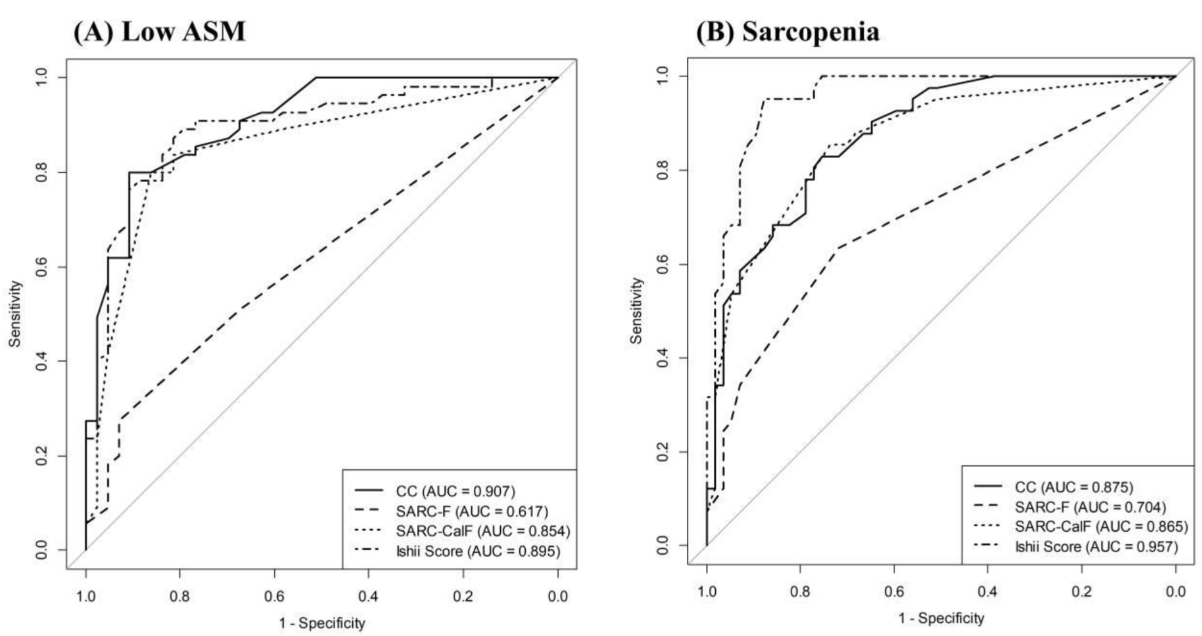

Figure 2. Receiver operating characteristic curves of four screening tools for detecting (A) low ASM and (B) Sarcopenia, according to the AWGS 2019 criteria. ASM, appendicular skeletal muscle mass; CC, calf circumference; SARC-F, strength, assistance with walking, rising from a chair, climbing stairs, and falls; SARC-CalF, SARC-F combined with CC; AUC, area under the curve.

Diagnostic performance of four screening tools for detecting low muscle mass and sarcopenia

Table 2 summarizes the diagnostic performance of the four screening tools—CC, SARC-F, SARC-CalF, and the Ishii score—using low ASM and sarcopenia as reference standards, in accordance with the AWGS 2019 criteria. For detecting low ASM, CC demonstrated the highest diagnostic performance, with an AUC of 0.907 (95% CI 0.850–0.965), significantly outperforming SARC-F (AUC 0.617, 95% CI 0.519–0.715), and SARC-CalF (AUC 0.854, 95% CI 0.777–0.931) ($p<0.05$), while showing no significant difference compared with the Ishii score (AUC 0.895, 95% CI 0.829–0.960). Sensitivity and specificity were 80.0% and 86.0% for CC, 18.2% and 95.3% for SARC-F, 41.8% and 95.3% for SARC-CalF, and 87.3% and 83.7% for the Ishii score, respectively. For detecting sarcopenia, the Ishii score exhibited the highest diagnostic performance, with an AUC of 0.957 (95% CI 0.920–0.993), significantly outperforming CC (AUC 0.875, 95% CI 0.808–0.941), SARC-F (AUC 0.704, 95% CI 0.606–0.801), and SARC-CalF (AUC 0.865, 95% CI 0.794–0.936) ($p<0.05$). Sensitivity and specificity were 100% and 75.4% for the Ishii score, 85.4% and 73.7% for CC, 24.4% and 96.5% for SARC-F, and 53.7% and 94.7% for SARC-CalF, respectively. Figure 2 illustrates the ROC curves corresponding to the data in Table 2, stratified by diagnostic reference (low ASM and sarcopenia). For low ASM, CC, which demonstrated the highest diagnostic accuracy, had optimal cutoff values of 33.8 cm for males and 33.2 cm for females. For sarcopenia, the Ishii score, which exhibited the highest diagnostic performance, had optimal cutoff values of 121 points for males and 120 points for females.

Discussion

The present study revealed three key findings in preoperative patients with stage I–III CRC aged ≥65 years scheduled for elective surgery: (1) the prevalences of low muscle mass and sarcopenia, defined by the AWGS 2019 criteria, were 56.1% and 41.8%, respectively; (2) CC had the highest accuracy for predicting low ASM; and (3) the Ishii score exhibited the highest diagnostic performance for sarcopenia.

A previous meta-analysis reported a sarcopenia prevalence of 37% in patients with CRC; however, most included studies assessed only skeletal muscle mass, with few incorporating evaluations of muscle strength or physical performance.7 One recent study reported that the prevalence of sarcopenia in Chinese preoperative patients with CRC, defined using the AWGS 2019 criteria, including muscle strength and physical performance, was 21.5%[16]. Conversely, the prevalence of sarcopenia based on the AWGS 2019 criteria in community-dwelling Japanese individuals was 11.5% in males and 16.7% in females[17]. In our study, the higher prevalence of sarcopenia may be attributed to the greater proportion of patients experiencing unintentional body weight loss, suggesting that sarcopenia in these patients may represent a secondary, cancer-related sarcopenia. Cancer-related sarcopenia is typically more severe and less responsive to nutritional and exercise interventions than age-related sarcopenia[18]. However, distinguishing between the two forms is often difficult in clinical practice. Therefore, regardless of etiology, patients with sarcopenia require similar nutritional and physical intervention.

Although the clinical utility of CC for detecting low ASM has been validated in community-dwelling populations[19], our findings further substantiate its effectiveness in patients with CRC. In Japanese community-dwelling individuals, CC exhibited high diagnostic accuracy for low ASM assessed using BIA (AUC = 0.93 in males and 0.89 in females)[19]. Consistently, our study demonstrated similarly high diagnostic accuracy of CC for low ASM measured by BIA in preoperative patients with CRC (AUC 0.907, sensitivity 80.0%, specificity 86.0%). Furthermore, a previous study reported that SARC-F exhibits poor diagnostic performance for detecting low skeletal muscle mass using CT imaging (sensitivity 35%, specificity 76%)[20], and our results similarly confirmed its limited diagnostic value for detecting low ASM assessed by BIA (sensitivity 18.2%, specificity 95.3%). By contrast, SARC-CalF and the Ishii score, both incorporating CC, outperformed SARC-F but remained inferior to CC alone. Collectively, these results support the clinical utility of CC as a simple and effective screening measure for low ASM in patients with CRC. Furthermore, the optimal cutoff values identified—33.8 cm for males and 33.2 cm for females—were closely aligned with those recommended by the AWGS 2019 criteria[6]. However, these cutoff values were derived from a relatively small sample and should be interpreted as reference points rather than as definitive thresholds. Additionally, given that the GLIM criteria for diagnosing malnutrition require the assessment of muscle mass, demonstrating the utility of CC for detecting low muscle mass specifically in patients with CRC adds clinical relevance to our findings.

While CC exhibited the highest accuracy for detecting low ASM, the Ishii score emerged as the most effective screening tool for sarcopenia in our study. A meta-analysis of older Chinese adults found that CC and the Ishii score exhibited comparable diagnostic accuracy (AUC = 0.88 both), outperforming SARC-F and SARC-CalF (AUC = 0.78 both) according to the AWGS 2019 criteria[12]. In contrast, although SARC-F and SARC-CalF also showed limited diagnostic utility in our cohort, the Ishii score clearly outperformed CC. This discrepancy is likely attributable to differences in study populations, as previous studies predominantly included community-dwelling individuals, whereas our study focused on patients with cancer. A recent study of preoperative Chinese patients with CRC reported that SARC-CalF and the Ishii score outperformed SARC-F (AUCs: 0.90, 0.88, and 0.80, respectively), but did not assess the diagnostic performance of CC[16]. Our findings build upon this evidence by demonstrating the superiority of the Ishii score over CC, representing, to the best of our knowledge, the first study to demonstrate this advantage in patients with cancer.

The observed superiority of the Ishii score in our study may be attributed to a subset of patients with cancer who preserve muscle strength or physical performance despite reduced muscle mass. A previous study in patients with digestive cancers undergoing chemotherapy revealed a moderate correlation between HGS and SMI (r = 0.53), but poor concordance between low muscle strength and low SMI based on consensual criteria (κ = 0.14)[21]. This suggests that loss of muscle mass and decline in strength may not occur simultaneously. This characteristic may make the Ishii score particularly valuable in patients with cancer susceptible to unintentional weight loss, as observed in approximately half of our cohort. These findings underscore the utility of the Ishii score, which incorporates both muscle mass and strength, as a more appropriate tool for sarcopenia screening than muscle mass-only indicators, such as CC. Furthermore, given the high cost and limited availability of devices for measuring muscle mass, which limit their widespread clinical application, our findings suggest that combining CC and HGS may offer a simple, cost-effective, and accurate approach for sarcopenia screening in these patients. Considering the ongoing international initiatives, such as the Global Leadership Initiative on Sarcopenia, aimed at standardizing diagnostic criteria[22], further validation of these screening tools according to emerging global standards is warranted.

This study has several limitations. First, it was conducted at a single center with a relatively small sample size. A multicenter prospective study should be conducted to validate the diagnostic accuracy of sarcopenia screening tools in preoperative older patients with CRC. Second, we used BIA rather than "gold standard" method, such as CT, to estimate muscle mass. While BIA has inherent limitations, it demonstrates a strong correlation with CT-based measurements, is practical and non-invasive, and endorsed as an alternative method by the AWGS 2019 consensus[6]. Finally, a prospective cohort study should be undertaken to evaluate the utility of muscle mass and strength measurements, or composite sarcopenia assessments, in predicting adverse postoperative outcomes, including postoperative complications, length of hospital stay, and 30-day readmission.

In conclusion, the Ishii score demonstrated superior diagnostic performance for sarcopenia in preoperative older patients with CRC, underscoring the importance of assessing muscle strength in addition to muscle mass for effective screening. It may serve as a practical tool for early sarcopenia detection prior to surgical admission and inform preoperative intervention strategies, potentially improving surgical outcomes in this population.

Ethics approval

The study was approved by the Ethics Review Board of Kobe City Medical Center West Hospital (Approval No. 22-037) and conducted in accordance with the principles of the Declaration of Helsinki and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects.

Consent to participate

Written informed consent was obtained from all the participants.

Authors' contributions

Yasuhiro Shimamura: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. Naomi Akazawa: Writing – review & editing. Sanae Nakajima: Writing – review & editing. Yukiko Kobayashi: Writing – review & editing. Wataru Aoi: Writing – review & editing. Masashi Kuwahata: Conceptualization, Methodology, Supervision, Writing - review & editing. All authors read and approved the final version of the manuscript.

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