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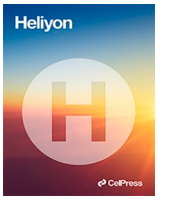

Research article

Predictive impact of sarcopenia in advanced non-small cell lung cancer patients treated with immune checkpoint inhibitors: A retrospective study

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ABSTRACT

Background: Sarcopenia, characterised by an ongoing loss of skeletal muscle mass and reduced strength and function, is frequently observed in patients with non-small cell lung cancer (NSCLC). However, the relationship between sarcopenia and the prognosis of NSCLC treated with immune checkpoint inhibitors (ICIs) remains unclear. This aimed to assess whether sarcopenia is an independent prognostic factor for survival in patients with advanced NSCLC receiving ICIs.

Methods: For this retrospective cohort study, we analysed the medical records of patients attending our hospital aged 18–75 years who were newly diagnosed with stage IIIB to stage IV NSCLC, and who had received ICIs as first- or second-line therapy between May 2019 and April 2022. The skeletal muscle index (SMI) was calculated from computed tomography (CT) images and relevant clinical characteristics within 4 weeks of initiating treatment and used to diagnose sarcopenia status. The Kaplan-Meier method and log-rank test were used to calculate and compare patients' progression-free survival (PFS). Cox proportional hazard regression was used to examine the associations between sarcopenia and survival outcomes. The chi-square test was used to compare treatment response outcomes, such as the objective response rate (ORR), disease control rate (DCR), and immunotherapy-related adverse events (irAEs), between individuals with and without sarcopenia. Additionally, the Student's t-test was utilised to compare SMI values between patients by their objective response (OR) and disease control (DC). Finally, the Mann-Whitney *U* test was used to compare nutritional and inflammatory indicators between the sarcopenia groups.

Results: The study enrolled 70 patients, of whom 34 (48.6%) were diagnosed with sarcopenia. The median PFS of patients with and without sarcopenia was 7.5 *vs.* 13.4 months, respectively (p =

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0.006). The proportional hazards regression analysis showed sarcopenia to be an independent prognostic factor for shorter PFS (hazard ratio (HR): 0.504, 95% CI: 0.265–0.962, $p = 0.038$). Using chi square tests, we found significant differences in the ORR (20.59% *vs.* 58.33%, $p = 0.001$) and occurrence of any irAEs (44.1% *vs.* 22.2%, $p = 0.028$) between the sarcopenia and the non-sarcopenia groups, respectively. The Student's t-test showed a significant difference in SMI between the ORR group and the non-ORR group (49.99 ± 7.00 *vs.* 42.98 ± 2.18 cm^2/m^2, $p = 0.0015$). While the sarcopenia group were with significantly a lower $CD4^+/CD8^+$ ratios and a higher C-reactive protein (CRP) level ($p = 0.026$, $p = 0.011$, respectively). **Conclusions:** This study found that sarcopenia is a significant predictor of a poor prognosis for patients with advanced NSCLC receiving ICIs. Multiple inflammatory and immune functions related to prognosis also differ by sarcopenia status.

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide [1]. Approximately 85% of lung cancer cases are non-small cell lung cancer (NSCLC), which is characterised by its ability to invade surrounding tissues and distant organs [2]. Immunotherapy has revolutionised cancer treatment and has been successfully used in the treatment of NSCLC [3,4]. In particular, the application of immune checkpoint inhibitors (ICIs) such as anti-PD-1/PD-L1 antibodies has been shown to improve overall survival, with higher expression of PD-L1 biomarkers predicting the greatest benefit [5]. However, many patients do not respond to ICI treatments. Thus, there is a need to develop predictive models that can identify patients most likely to benefit from ICI treatments.

Sarcopenia, the loss of muscle mass and strength, is a common condition in elderly people and patients with cancer [6]. Distinct from aging-related sarcopenia, cancer-related sarcopenia is often related to cancer cachexia, elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), and with diminished immune cell activity. Thus, a range of negative outcomes occur in these patients, including increased treatment-related toxicities, a lower response to treatment, and decreased survival rates [7]. Cancer-related sarcopenia not only affects patients' quality of life but is also strongly linked to poorer immunotherapy outcomes and shortens their survival time [8]. We are unaware of previous studies examining these outcomes by sarcopenia and inflammatory status.

Elevated levels of inflammatory factors promote the development of sarcopenia by disrupting protein metabolism [9]. Overexpression of IL-6 and decreased levels of IL-7 lead to the depletion of T-cell function, thereby affecting immune system functioning and negatively influencing ICI outcomes [10]. Therefore, sarcopenia may indirectly impair immune function in cancer patients by aggravating the chronic inflammatory milieu, leading to suboptimal outcomes of ICI treatments.

Table 1
Characteristics of patients with advanced NSCLC in the sarcopenia and non-sarcopenia groups receiving ICIs treatments.

Characteristics	Total	Sarcopenia (n = 34)	Non-Sarcopenia (n = 36)	*p* value
Age, median (IQR)	67 (61.75, 72)	68 (62, 73)	65.5 (60, 69.25)	0.212
Gender, n (%)				0.071
Male	60 (85.7%)	26 (76.5%)	34 (94.4%)	
Female	10 (14.3%)	8 (23.5%)	2 (5.6%)	
Smoke History, n (%)				0.459
YES	32 (45.7%)	14 (41.2%)	18 (50.0%)	
NO	38 (54.3%)	20 (58.8%)	18 (50.0%)	
BMI (kg/cm2), n (%)				0.259
<18.5 kg/cm^2	12 (17.1%)	4 (11.8%)	8 (22.2%)	
18.5–24.9 kg/cm^2	46 (65.7%)	22 (64.7%)	24 (66.7%)	
≥25 kg/cm^2	12 (17.1%)	8 (23.5%)	4 (11.1%)	
TNM Stage, n (%)				0.730
IIIB	4 (5.7%)	2 (5.9%)	2 (5.6%)	
IIIC	10 (14.3%)	6 (17.6%)	4 (11.1%)	
IV	56 (80%)	26 (76.5%)	30 (83.3%)	
Histopathology, n (%)				0.770
Adenocarcinoma, n (%)	42 (60%)	21 (61.8%)	21 (58.3%)	
Squamous cell carcinoma, n (%)	28 (40%)	13 (38.2%)	15 (41.7%)	
Treatment line, n (%)				0.030
First-line immunotherapy , n (%)	55 (78.6%)	23 (67.6%)	32 (88.9%)	
Second-line immunotherapy , n (%)	15 (21.4%)	11 (32.4%)	4 (11.1%)	
SMI, median (IQR)	44.6 (39.225, 50.425)	39.1 (35.525, 41.05)	50.05 (46.675, 56.95)	<0.001
Treatment strategy, n (%)				0.185
PD-1/PD-L1 only	1 (1.4%)	1 (2.9%)	0 (0%)	
Combined with chemotherapy/Radiotherapy	59 (84.3%)	26 (76.5%)	33 (91.7%)	
Combined with anti-VEGF	10 (14.3%)	7 (20.6%)	3 (8.3%)	

ICIs: immune checkpoint inhibitors; IQR; interquartile range; BMI, body mass index; TNM, tumor-node-metastasis; SMI, skeletal muscle index; PD-1, programmed death receptor-1; PD-L1, programmed cell death 1 ligand 1; VEGF, vascular endothelial growth factor.

Several studies have demonstrated that cancer-related sarcopenia is a critical factor in predicting the efficacy of ICI treatments for NSCLC; however, few considered a possible involvement of inflammatory factors [11,12]. In addition, the links between sarcopenia, inflammatory status and immune function need to be further investigated. Additionally, recent studies have enrolled patients receiving second-line or more treatment-line ICIs [13,14]. Finally, it is not known whether these relationships differ for first-line compared to second-line treatments.

Here, we report a retrospective study of patients with advanced NSCLC receiving ICIs as first- or second-line treatment, investigating the relationship of sarcopenia to poor progression free survival (PFS) and clinical inflammatory and immune status.

2. Results

2.1. Patient characteristics

Seventy patients were included in this study. Baseline patient characteristics are shown in Table 1. Patients were categorized according to their SMI score into sarcopenia (34 patients) and non-sarcopenia (36 patients) groups. There were no statistically significant differences in age, sex, smoking history, tumor node metastasis (TNM) stage, pathological type, or treatment strategy. Patients with sarcopenia were significantly more likely to be receiving second-line treatment. Thus, we conducted a stratified Kaplan-Meier analysis and Cox-regression analysis in patients receiving ICIs by treatment line (Fig. S1, Table S1).

2.2. Primary outcome: progression free survival (PFS)

2.2.1. Kaplan-Meier and log-rank tests

Kaplan-Meier and log-rank tests were used to generate PFS survival curves. The median PFS was 10.2 months (range: 1.2–40.1 months, 95% confidence interval (CI): 7.2–13.5; Fig. 1A), and significantly shorter for patients with sarcopenia than for those without (7.5 *vs.* 13.4 months, respectively, $p = 0.006$; Fig. 1B). PFS remained significantly shorter for patients with sarcopenia whether they had received first-line (7.5 months *vs.* 12.7 months, $p = 0.044$; Fig. S1) or second-line ICI therapy (6.97 months *vs.* 11.8 months, $p = 0.038$; Fig. S1).

2.2.2. Cox regression analysis

Similar to the Kaplan-Meier and log-rank test results, the univariate Cox regression analysis showed that the 34 patients with sarcopenia had lower PFS (hazard ratio (HR): 0.444, 95% CI: 0.247–0.798, $p < 0.01$; Table 2) than those without sarcopenia. This result was also seen in the 55 patients receiving ICIs as first-line therapy by univariate analysis (HR: 0.506, 95% CI: 0.259–0.988, $p < 0.05$; Table S1).

The univariate analysis also found that histopathology type, Karnofsky Performance Status (KPS) score for functional status, and the NRS 2002 nutritional risk screening score were significant predictors of PFS (all $p < 0.05$). In the multivariable model adjusted for KPS, sarcopenia remained independently associated with poor PFS (HR: 0.504, 95% CI: 0.265–0.962, $p < 0.05$; Table 2).

2.2.3. Objective response rate and disease control rate

The overall objective response rate (ORR) was 40.0%, with a significant difference between the sarcopenia and non-sarcopenia

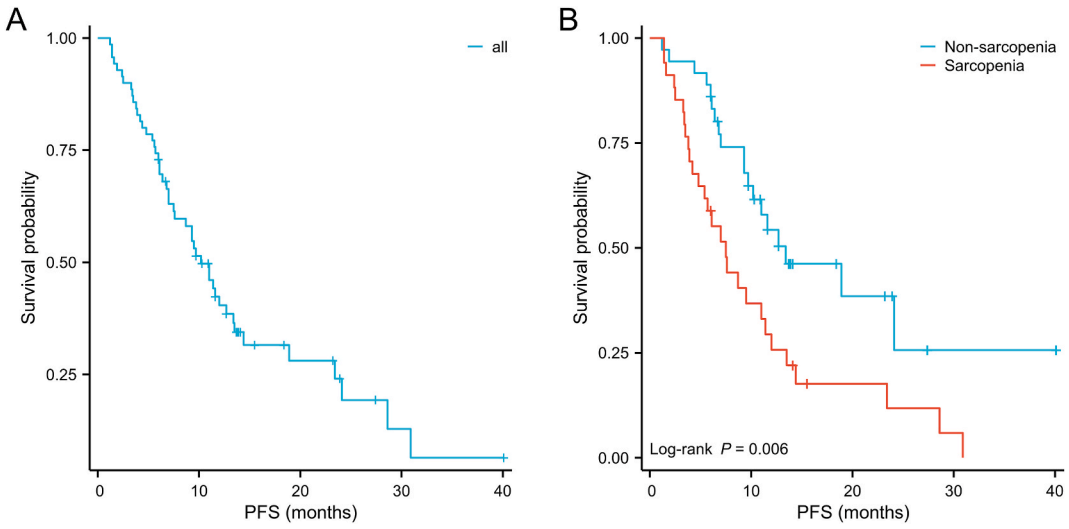

Fig. 1. Kaplan-Meier survival curves for the sarcopenia and non-sarcopenia groups. (A) Kaplan-Meier survival curve of PFS in all 70 patients; (B) Kaplan-Meier survival curve of PFS in the sarcopenia and non-sarcopenia groups (7.5 *vs.* 13.4 months, $p < 0.05$). PFS, progression free survival.

Table 2
Cox regression analysis of the risk of sarcopenia and clinicopathological factors on PFS in patients with advanced NSCLC receiving ICI therapy.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Age	70	0.990 (0.956–1.025)	0.562		
Smoke History	70		0.958		
YES	32	Reference			
NO	38	0.985 (0.550–1.762)	0.958		
BMI (kg/cm2)	70		0.744		
<18.5 kg/cm^2	12	Reference			
18.5–24.9 kg/cm^2	46	1.088 (0.497–2.384)	0.833		
≥25 kg/cm^2	12	1.419 (0.544–3.703)	0.474		
Gender	70		0.265		
Male	60	Reference			
Female	10	1.586 (0.732–3.438)	0.243		
Histopathology	70		0.027*		
Adenocarcinoma	42	Reference		Reference	
Squamous cell carcinoma	28	1.953 (1.082–3.526)	0.026*	1.720 (0.903–3.273)	0.099
NRS 2002	70	1.372 (1.095–1.718)	0.006**	1.008 (0.756–1.344)	0.955
KPS	70		<0.001***		
80	38	Reference		Reference	
70	19	12.876 (5.561–29.813)	<0.001***	10.651 (4.256–26.654)	<0.001***
90	13	1.627 (0.754–3.512)	0.215	1.630 (0.711–3.733)	0.248
Treatment line	70		0.800		
First-line immunotherapy	55	Reference			
Second-line immunotherapy	15	0.912 (0.447–1.865)	0.802		
SMI Stage	70		0.006**		
Sarcopenia	34	Reference		Reference	
Non-sarcopenia	36	0.444 (0.247–0.798)	0.007**	0.504 (0.265–0.962)	0.038*

BMI, body mass index; NRS 2002, Nutritional Risk Screening 2002 score; KPS, Karnofsky Performance Score; SMI, skeletal muscle index. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

groups (20.59% *vs.* 58.33%, respectively, $p = 0.001$). In contrast, the overall disease control rate (DCR) was 90.0% (63/70), with no statistically significant difference by sarcopenia status (85.29% *vs.* 94.44%, $p = 0.22$). Specifically, two patients with sarcopenia and five patients without sarcopenia experienced progressive disease (Table 3). The mean SMI of the ORR group was significantly higher than that of the non-ORR group (49.99 ± 7.00 *vs.* 42.98 ± 2.18 cm^2/m^2, respectively, $p = 0.0015$; Fig. 2A). No significant difference was seen in SMI by DCR status (46.16 ± 3.70 *vs.* 42.37 ± 3.79 cm^2/m^2, $p = 0.3078$; Fig. 2B).

2.2.4. Immunotherapy-related adverse events

A total of 23 patients (23/70, 32.86%) experienced irAEs during immunotherapy (Table 3). There was a significant difference in the incidence of irAEs between the two groups, with 15 patients in the sarcopenia group and 8 patients in the non-sarcopenia group, respectively ($p = 0.028$, Table 3). The highest incidence of irAEs in the sarcopenia group was for skin toxicity (4/34, 11.7%, Table S2), while immune thyroiditis was highest in the non-sarcopenia group (6/36, 16.7%, Table S2). However, the occurrence of severe irAEs (grade 4) in the sarcopenia group was not significantly different from the occurrence in the non-sarcopenia group (5.9% *vs.* 0%, respectively, $p > 0.05$, Table S3). The full results of the Cox analysis of irAEs were presented in Table S4.

2.3. Secondary outcomes: nutritional and inflammatory indices

Levels of sixteen inflammatory and nutritional indicators (including routine blood tests, renal and liver function tests, and immune cell analysis) were analysed using the Mann-Whitney *U* test, revealing several significant differences by sarcopenia status (Table 4). Specifically, patients with sarcopenia had significantly lower triglyceride levels, waist circumferences, KPS, and albumin/globulin ratios but higher NRS 2002 nutritional scores, globulin levels, and C-reactive protein (CRP) levels. Furthermore, flow cytometry analysis of immune cells revealed significantly lower $CD4^+/CD8^+$ ratios in patients with sarcopenia than in those without sarcopenia

Table 3
Response to treatment of patients with advanced NSCLC receiving ICI therapy, by sarcopenia status.

Variables	CR (n)	PR (n)	SD (n)	PD (n)	ORR (%)	DCR (%)	irAEs (n,any)
Sarcopenia	0	7	22	5	20.59%	85.29%	15 (44.1%)
Non-Sarcopenia	0	21	13	2	58.33%	94.44%	8 (22.2%)
X^2 Value					10.30	1.62	4.802
p Value					0.001**	0.202	0.028*

CR, complete remission; PR, partial remission; SD, stable disease; PD, disease progression; ORR, overall response rate; DCR, disease control rate; irAEs, immunotherapy-related adverse events. *$p < 0.05$, **$p < 0.01$.

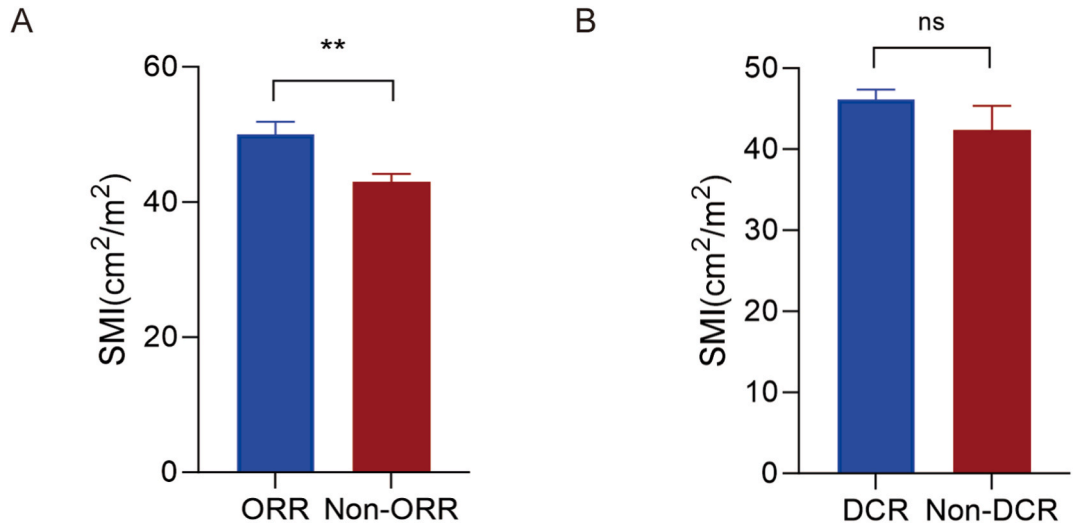

Fig. 2. Independent *t*-test of SMI by objective response rate (ORR) and disease control rate (DCR) status. (A) SMI by ORR status ($p = 0.001$). (B) SMI by DCR status ($p = 0.22$). $^{**}p < 0.01$.

Table 4
Mann-Whitney *U* test of inflammatory and nutritional indicators of patients with advanced NSCLC receiving ICI therapy, by sarcopenia status.

Variables (median/(IQR))	All Patients (n = 70)	Sarcopenia (n = 34)	Non-Sarcopenia (n = 36)	*p* value
Scr, μmol/L	68.00 (56.00,75.40)	65.60 (50.95,75.05)	68.75 (60.85,76.05)	0.194
TG, mmol/L	1.18 (0.85,1.56)	0.93 (0.74,1.48)	1.27 (1.06,1.66)	0.011*
Cholesterol, mmol/L	4.85 (3.88,5.52)	4.88 (4.08,5.67)	4.77 (3.85,5.41)	0.44
Prealbumin,mg/L	229.10 (181.90,285.50)	209.10 (178.75,267.40)	240.50 (214.20,296.25)	0.153
Albumin, g/L	40.80 (38.70,44.70)	41.30 (36.35,43.80)	40.50 (39.13,44.90)	0.668
Globulin, g/L	30.60 (26.70,33.90)	32.80 (28.40,39.45)	28.95 (26.20,31.58)	0.013*
PLR	153.85 (112.35,234.12)	160.00 (114.01,250.56)	149.54 (108.24,205.31)	0.13
NLR	2.91 (2.06,4.25)	2.85 (2.02,4.20)	3.03 (2.04,4.35)	0.88
CRP, mg/L	8.76 (2.12,23.50)	16.16 (5.98,40.89)	6.25 (1.82,14.47)	0.011*
LDH, U/L	187.00 (161.00,253.00)	183.00 (164.00,252.00)	187.00 (156.25,255.00)	0.76
A/G	1.37 (1.18,1.55)	1.24 (1.09,1.49)	1.47 (1.29,1.57)	0.017*
$CD4^+/CD8^+$	1.52 (1.21,2.43)	1.24 (1.00,1.60)	1.62 (1.31,2.86)	0.026*
Treg, %	5.80 (4.78,7.35)	6.25 (4.16,8.27)	5.62 (4.95,6.31)	0.289
Waistline, cm	84.80 (77.50,91.00)	81.20 (73.3,88.85)	87.65 (81.98,72.70)	0.008**
NRS 2002	3 (2.00,4.00)	3 (2.50,4.50)	2 (1.75,3.00)	0.001**
KPS	80 (70,80)	80 (70,80)	80 (80,90)	0.004**

Scr, serum creatinine; TG, triglyceride; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; LDH, lactate dehydrogenase; A/G, Albumin/globulin ratio; $CD4^+$ T cells, cluster of differentiation 4^+ T cells; $CD8^+$ T cells, cluster of differentiation 8^+ T cells; NRS 2002, Nutritional Risk Screening 2002 score; KPS, Karnofsky Performance Score. $^*p < 0.05$, $^{**}p < 0.01$.

($p < 0.05$).

3. Discussion

Sarcopenia, characterised by excessive muscle wasting and loss of muscle strength due to disrupted muscle homeostasis, is a common adverse outcome of both cancer and its therapeutic interventions [15]. The mechanisms underlying both primary and secondary sarcopenia are generally attributed to the gradual loss of motor neurons, which correlates with a decrease in the numbers and size of muscle fibres [16]. Sarcopenia exacerbates inflammation through a vicious, cyclic increase in the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 [17]. By combining predictive modelling biomarkers with inflammatory factors such as IL-6 and TNF-α levels, researchers have been able to identify patients with a high-risk of relapse and treat them with PD-1/PD-L1 inhibitors [10,18].

A previous study found that the co-occurrence of inflammation and sarcopenia was associated with a high mortality risk in patients with cancer [19]. Inflammation is an immune response that can lead to increased cancer progression and metastasis, resulting in immune escape [20]. The intricate mechanisms underlying the impact of skeletal muscle mass on the efficacy of ICIs have highlighted the involvement of chronic inflammation as a pivotal contributor [21]. Moreover, inflammatory indices such as PLR, NLR, A/G ratio, and CRP levels have also been reported to predict overall survival in patients treated with ICIs [22–24]. Lee et al. reported that a $CD4^+/CD8^+$ ratios of 1.93 or higher was a favorable predicting factor for lung cancer patients receiving ICIs [25]. Wang et al. have

reported that the underlying mechanisms between higher $CD4^+/CD8^+$ ratios and ICI efficacy in patients with NSCLC involved sphingolipid metabolism and ceramide synthases [26]. Several studies have reported that sphingolipid metabolism belonging to lipid metabolism, especially ceramide accumulation, was involved in multiple, complex physiological processes such as sarcopenia, aging, and tumor immunity [9,27,28]. Although several studies have discussed sarcopenia as a predictor of poor survival outcomes in patients with NSCLC receiving ICI therapy [11–13], few have clarified the associations among sarcopenia, inflammatory status, and immune function in these populations [11]. Our study aimed to investigate whether sarcopenia could be used to predict the prognosis of patients with advanced NSCLC treated with ICIs, and to untangle aspects of their relationship with immune function and inflammation status.

In our study, patient characteristics other than SMI stage did not differ significantly by sarcopenia status (Table 1). We found that PFS and ORR, were significantly lower in patients with sarcopenia, which is consistent with other studies (Fig. 1, Tables S2 and 3) [12, 24]. Similarly, SMI, but not DCR, was significantly different between ORR groups [29]. The overall incidence of irAEs was significantly higher in patients with sarcopenia, but there was no difference in the occurrence of grade 4 irAEs (Table 3, Table S3) [30]. Several studies have reported that sarcopenia is not a significant predictor of irAEs [31–33]. According to our study, sarcopenia may not directly affect the incidence of irAEs, however impaired immune function in the sarcopenia group will result in these circumstances (Tables S2, S3, S4).

Patients with sarcopenia had significantly lower triglyceride levels, NRS 2002 nutritional scores, globulin , CRP levels, and significantly higher $CD4^+/CD8^+$ ratios, these markers are thought to represent dysfunctional immune activation associated with chronic inflammation (Table 4). Although skeletal muscle mass loss in cancer patients has long been considered an irreversible process, recent data indicate that muscle wasting is reversible in some patients. For example, skeletal muscle depletion is associated with reduced plasma (n-3) fatty acid levels in patients with NSCLC [34]; several studies have found improved levels of sarcopenia, inflammatory status and survival for patients receiving oral supplements containing n-3 and n-6 fatty acids [35–38].

Differences in our findings with those of other studies may reflect variations in study design, inclusion and exclusion criteria, sample sizes, cut-off points, and methods for measuring muscle area or defining sarcopenia. A strength of this study is our access to a

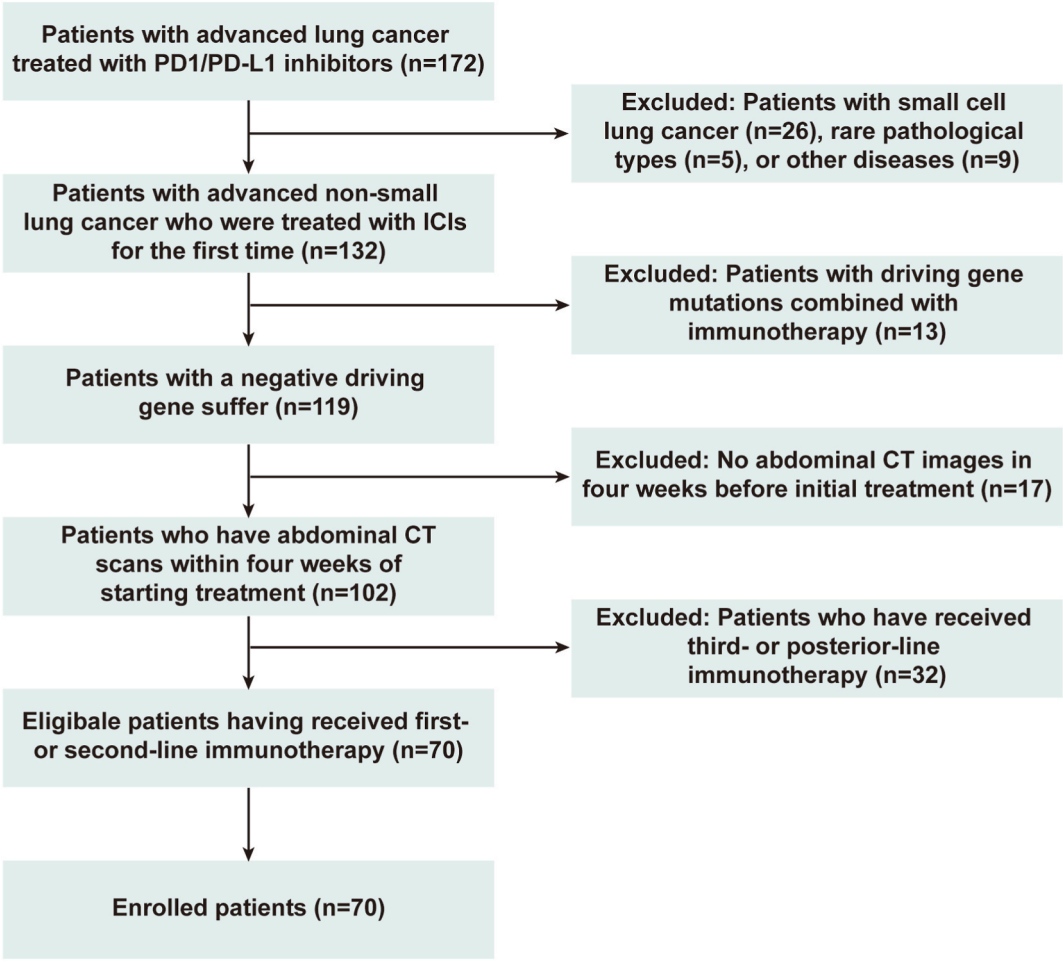

Fig. 3. Patient selection flow-chart. ICIs, immune checkpoint inhibitors; CT, computed tomography.

homogeneous population of NSCLC patients with CT scan data from 30 consecutive slices of the L3 area. This provided high-quality data for minimising errors in SMI differences in different slices.

This study had several limitations. First, a lack of consensus on optimal cut-off points for sarcopenia poses a challenge for cross-study comparisons. Second, current definitions of sarcopenia commonly used in the literature are based on North American populations, which may not be globally applicable. Further studies with larger sample sizes are needed to reach consensus on sarcopenia cut-off points, the relationship between sarcopenia and overall survival in different populations, and the impact of nutritional interventions on cancer-related sarcopenia.

4. Conclusions

In conclusion, we found that sarcopenia and high inflammatory levels were associated with poor outcomes in patients with NSCLC treated with PD-1/PD-L1 inhibitors. Further research is needed to fully understand the mechanisms of sarcopenia on the development of NSCLC and optimal treatment strategies.

5. Materials and methods

5.1. Study population

The study enrolled 70 patients with advanced NSCLC (stage IIIB–IV) who received ICIs as first- or second-line therapy at the Department of Oncology, Suzhou Municipal Hospital, Affiliated Suzhou Hospital of Nanjing Medical University, Nanjing, China, between May 1, 2019 and April 30, 2022 (Fig. 3). The inclusion criteria were as follows: (1) age between 18 and 75 years, regardless of sex; (2) a diagnosis with advanced NSCLC not appropriate for resection or advanced NSCLC, with specific pathological types including adenocarcinoma and squamous cell carcinoma; (3) negative driver genes; (4) the first receipt of immunotherapy as a first- or second-line treatment was in our hospital; and (5) an abdominal CT examination in our hospital within one month of subsequent PD1/PD-L1 inhibitor treatment. The exclusion criteria were as follows: (1) lack of specific pathological support; (2) rare pathological cancers, such as sarcoma and large-cell neuroendocrine carcinoma; (3) any current serious or uncontrollable disease; (4) severe infections and rheumatoid or other chronic diseases that may have affected their routine blood and biochemical test results during the previous month; and (5) diagnosed degeneration of L3 skeletal muscle.

5.2. Data collection and follow-up

The following clinical data were extracted from electronic medical records: age, sex, smoking history, KPS, NRS 2002, height, weight, routine blood, renal and liver function tests, immune cell analysis (including frequency of $CD4^+$ T, $CD8^+$ T, Treg cells, and $CD4^+/CD8^+$ ratios), abdominal CT images, histopathology, TNM tumor classification, treatment regimens, abdominal CT images and interpretations, date of initiation of immunotherapy, treatment response, date of progression defined by CT, and date of last follow-up. The last follow-up date was December 30, 2023.

The primary study endpoint was progression-free survival (PFS), defined as the time between the initiation of immunotherapy and the date of disease progression or death. Secondary endpoints included the objective response rate (ORR), disease control rate (DCR), and occurrence of immunotherapy-related adverse events (irAEs). We used the immune-related response evaluation criteria for solid tumors (iRECIST) criteria to define ORR as the sum of complete response (CR) and partial response (PR), and DCR as the sum of CR, PR, and stable disease (SD). The irAEs were graded and classified according to the criteria established by the Chinese Society of Clinical Oncology. Dermatological (rash and pruritus), gastrointestinal (colitis and diarrhoea), and endocrine (thyroid dysfunction) toxicities were obtained from the medical records.

5.3. Sarcopenia definition and body composition analysis

We imported abdominal computed tomography (CT) images featuring a layer thickness of 1 mm into 3D slicer software (version 5.2.2, NIH, USA). We meticulously delineated the musculature in each layer, from the superior margin to the inferior edge of the third lumbar spine, to compute volumetric measurements of the skeletal muscle at the L3 level. Subsequently, we determined the average skeletal muscle area (SMA) at L3 by dividing the acquired volume by the total number of layers [39]. Senior radiologists reviewed all image-drawing processes.

To mitigate variations in height, weight, and other physiologic factors, we calculated the skeletal muscle mass index (SMI) to portray the comprehensive muscle profile of patients [40,41]. The formula was defined as SMI = L3 SMA/height^2, the prevailing gold standard for quantifying skeletal muscle area [42]. Sarcopenia was defined as low SMI, with separate criteria by sex. For women, sarcopenia was defined as SMI <41 cm^2/m^2, regardless of BMI. For men, sarcopenia was defined as SMI <43 cm^2/m^2 for BMI <25 kg/m^2, and SMI <53 cm^2/m^2 for BMI ≥ 25 kg/m^2 [43,44].

5.4. Flow cytometry

Peripheral blood samples were collected prior to the administration of anti-PD-1 immunotherapy. The staining antibodies were anti-CD4 (PE-cy7-labelled CD4), anti-CD8 (APC-cy7-labelled CD8), anti-CD3 (FITC-labelled CD3), and anti-CD45 (PerCP-cy5.5-

labelled CD45). Isotype-matched negative controls were included for each antibody (BD Biosciences, San Jose, CA, USA).

Next, 100 μl of well-mixed, anticoagulated whole blood were gently vortexed with 20 μl of antibodies and incubated for 15 min in the dark at room temperature following the procedure for the BD Multitest™ CD3/CD8/CD45/CD4 kit (No. 662967, BD Biosciences). Subsequently, 450 μl of 1 × BD Multitest IMK Kit lysing solution was added and incubated for 15 min in the dark at room temperature. For the Treg test, we labelled one tube with the identification number and added 10 μL of anti-CD4 antibody, 10 μL of anti-CD25 antibody, and 10 μL of anti-CD127 antibody. Then, we pipetted 50 μL of well-mixed, anticoagulated whole blood into the tube, capped the tubes, and gently vortexed to ensure proper mixing. The mixture was then incubated for 25 min in the dark at room temperature. 2 ml of 1X BD Multitest IMK Kit Lysing Solution was added to each tube for an 11-min incubation period. The tubes were then centrifuged at 1500 rpm for 5 min, and 1–2 ml PBS added to wash the cells. After centrifuging again at 1500 rpm for 5 min, we finally added 200–300 μL of PBS to each tube. The samples were analysed on the flow cytometer, with the $CD4^{+}/CD25^{+}/CD127$ low cell population identified as Treg cells. The stained cells were analysed on a FACS Canto II flow cytometry system using FACS Diva software (BD Biosciences).

5.5. Statistical analyses

All data analysed using SPSS software (version 25.0, IBM, USA). We utilised the Kaplan-Meier method and log-rank test to delineate survival curves and assess differences in progression-free survival (PFS) between the sarcopenia and non-sarcopenia groups. We conducted univariate Cox regression analysis to identify clinical and pathological factors influencing PFS, and multivariate Cox regression to ascertain their independent prognostic significance. Associations between qualitative variables, including the overall response rate (ORR), disease control rate (DCR), and incidence of immune-related adverse events (irAEs), were evaluated by the chi-square test. Furthermore, we employed an independent *t*-test to compare variations in SMI values between the ORR and non-ORR groups, as well as between the DCR and non-DCR groups. Finally, we used the Mann-Whitney *U* test to compare the nutritional and inflammatory indicators between the sarcopenia and non-sarcopenia groups. Statistical significance was defined as $p < 0.05$.

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Ethics approval and consent to participate

This study was approved by the ethics committee of the Suzhou Municipal Hospital (No.K-2022-144-K01) and complied with the standards recognized by the Declaration of Helsinki. Written informed consent was waived as no new or invasive procedures were conducted for the study purposes.

Availability of data and materials

Data included in article/supp. material/referenced in article. The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Ying Feng: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Fen Guo:** Formal analysis, Data curation. **Yan Wang:** Project administration, Methodology. **Yingru Zhang:** Supervision, Software. **Xiaofeng Chen:** Visualization, Validation. **Liyu Wang:** Methodology. **Fan Zhu:** Investigation. **Jianming Shi:** Resources. **Luyao Zhang:** Conceptualization, Data Curation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27282.

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