Research Article

Cancer Epidemiology, Biomarkers & Prevention

Postdiagnosis Loss of Skeletal Muscle, but Not Adipose Tissue, Is Associated with Shorter Survival of Patients with Advanced Pancreatic Cancer

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Abstract

Background: Pancreatic cancer is associated with development of cachexia, a wasting syndrome thought to limit survival. Few studies have longitudinally quantified peripheral tissues or identified biomarkers predictive of future tissue wasting.

Methods: Adipose and muscle tissue were measured by computed tomography (CT) at diagnosis and 50 to 120 days later in 164 patients with advanced pancreatic cancer. Tissue changes and survival were evaluated by Cox proportional hazards regression. Baseline levels of circulating markers were examined in relation to future tissue wasting.

Results: Compared with patients in the bottom quartile of muscle change per 30 days (average gain of 0.8 ± 2.0 cm^2), those in the top quartile (average loss of 12.9 ± 4.9 cm^2) had a hazard ratio (HR) for death of 2.01 [95% confidence interval (CI), 1.12–3.62]. Patients in the top quartile of muscle attenuation change (average decrease of 4.9 ± 2.4 Hounsfield units) had an HR of 2.19 (95% CI, 1.18–4.04) compared with those in the bottom quartile (average increase of 2.4 ± 1.6 Hounsfield units). Changes in adipose tissue were not associated with survival. Higher plasma branched chain amino acids (BCAA; $P = 0.004$) and lower monocyte chemoattractant protein-1 (MCP-1; $P = 0.005$) at diagnosis were associated with greater future muscle loss.

Conclusions: In patients with advanced pancreatic cancer, muscle loss and decrease in muscle density in 2 to 4 months after diagnosis were associated with reduced survival. BCAAs and MCP-1 levels at diagnosis were associated with subsequent muscle loss.

Impact: BCAAs and MCP-1 levels at diagnosis could identify a high-risk group for future tissue wasting.

Introduction

Patients with advanced pancreatic cancer have particularly poor survival, with overall 5-year survival less than 10% (1). Several factors are thought to limit patient survival, including a tissue wasting syndrome commonly referred to as cachexia (2). An international expert consensus defined cachexia as >5% weight loss over past 6 months, >2% weight loss in patients with body mass index (BMI) < 20 kg/m^2, or appendicular skeletal mass consistent with sarcopenia (2). Nevertheless, studies have examined pancreatic cancer–associated cachexia and patient outcomes with differing results, likely due to differing definitions of cachexia, measurement approaches, and study designs (3–5). In most studies, body composition was measured only at diagnosis. These static pretreatment measurements may not capture the dynamic nature of tissue wasting and its relationship with patient survival.

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Cachexia is thought to occur in up to 80% of patients with advanced pancreatic cancer during the course of their disease (6). While several biomarkers for detecting cachexia in newly diagnosed patients have been described previously (7), there are currently no validated biomarkers that predict the severity of future wasting in the period following diagnosis. Among the most studied candidates are inflammatory cytokines interleukin-6 (IL6), tumor necrosis factor-α (TNFα; ref. 7), and monocyte chemoattractant protein-1 (MCP-1; ref. 8). We have previously demonstrated that circulating branched chain amino acids (BCAA; isoleucine, leucine, and valine) are liberated from tissues in mouse models and patients with early pancreatic cancer (9). However, the ability of these circulating markers to predict future tissue wasting is not known.

We measured tissue compartments using CT imaging, a precise and reproducible method for tissue quantification in patients with cancer (10), before treatment and at restaging in patients with advanced pancreatic cancer. Using these imaging studies, we examined whether changes in tissue compartments predicted patient survival, and whether circulating markers measured at the time of cancer diagnosis could identify patients at higher risk of future tissue wasting.

Materials and Methods

Study population

This study included patients from Dana-Farber/Brigham and Women's Cancer Center (DF/BWCC, Boston, MA; $N = 117$) and Mayo Clinic (Rochester, MN; $N = 47$) with the following requirements: (i) diagnosed with advanced pancreatic ductal adenocarcinoma between 2000 and 2015, (ii) available CT scan prior to receiving any cancer-directed treatment, including surgery, chemotherapy, or radiation, (iii) available follow-up CT scan 50–120 days after the baseline scan, which is the common time interval of the first restaging scan to evaluate treatment efficacy, and (iv) stored pretreatment plasma sample obtained within 30 days before to 60 days after cancer diagnosis. From a population of patients previously evaluated in a cross-sectional study of tissue compartments and patient outcomes (11), 164 patients met the four requirements (Fig. 1). The study was approved by Institutional Review Boards of Dana-Farber/Harvard Cancer Center and Mayo Clinic. All patients provided informed consent.

CT analysis of body composition

CT scans were acquired as part of regular clinical care using imaging hardware and acquisition protocols from multiple institutions. Reconstructed slice thicknesses were 5 mm in 92% of patients, 3 mm in 5%, and other values 1–5 mm in 2%. Skeletal muscle, visceral adipose tissue, and subcutaneous adipose tissue areas were measured on axial CT images at the level of the L3 vertebral body, as described previously (11). Skeletal muscle index (SMI) was calculated as the ratio of muscle area (cm^2) to squared height (m^2). We also measured muscle attenuation, a marker of muscle density, which can capture the intramuscular accumulation of lipid droplets (12). Mean muscle attenuation was calculated as the average CT attenuation in Hounsfield units (HU) across all pixels in the labeled muscle region. Pretreatment and restaging scans were concordant for intravenous contrast administration in 97% of patients, with 97% of scans performed with intravenous contrast. Because administration of intravenous contrast may affect muscle attenuation measurements on CT imaging (13), the 5 patients with discordant intravenous contrast use were excluded from the analyses of muscle attenuation with patient survival and biomarker levels. Scans from DF/BWCC were analyzed by manual segmentation using Slice-O-Matic Software (v4.3; TomoVision; ref. 11). Images were analyzed by trained image analysts blinded to study question, study design, and image order (baseline vs. follow-up scan). Aggregate intra-analyst coefficients of variation (CV) were 0.53% for muscle (individual reader range: 0.48%–1.14%), 0.44% for subcutaneous adipose (0.19%–0.55%), and 0.66% (0.41%–0.97%) for visceral adipose tissue. Final data verification was performed by a board-certified radiologist. Scans from Mayo Clinic were analyzed using software developed at the Mayo Clinic with manual review by radiologists at that site. A version of this software was described by Weston and colleagues (14). To calculate the variation between analysis methods used at the two study sites, we analyzed scans from 20 patients using both methods, and obtained similar results, with CV of 2.4% for muscle, 3.8% for visceral adipose tissue, and 1.7% for subcutaneous adipose tissue.

Plasma marker measurements

Plasma isoleucine, leucine, and valine were measured by LC/MS as described previously (11). The mean CVs were 7.6% for isoleucine, 8.0% for leucine, and 7.3% for valine. Total plasma BCAAs were derived by summing the concentrations of individual BCAAs. We previously measured plasma IL6, MCP-1, and soluble TNF receptor type II (sTNF-RII) in a subset of 92 patients (15). sTNF-RII is an established surrogate for TNFα due to its lower diurnal variation and higher stability in frozen samples (16). CVs were calculated using blinded duplicate samples, and were 3.6% for IL6, 10.5% for MCP-1, and 6.1% for sTNF-RII.

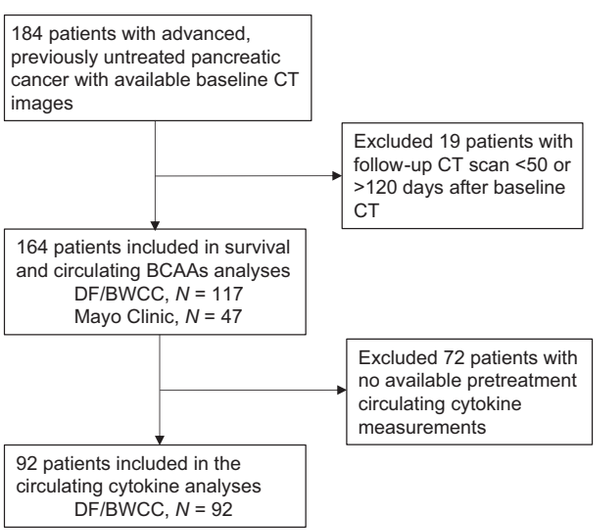

Figure 1.

Selection of patients with pancreatic cancer included in the study of postdiagnosis body composition change. Patients were selected on the basis of the availability of pretreatment CT images, CT images obtained 50 to 120 days after diagnosis, and pretreatment plasma samples ($N = 164$). A subset of those patients ($N = 92$) had cytokine measurements in pretreatment plasma samples.

Covariate data

Patient demographic, clinical, and treatment data were obtained from medical records and patient questionnaires, including age, sex, height, weight at the time of diagnosis, race/ethnicity, smoking status, history of diabetes, year of diagnosis, cancer stage, cancer treatment type and duration, and date of death or last follow-up visit.

Statistical analysis

To evaluate the association between change in body composition and patient survival, we used multivariate Cox proportional hazards regression models and calculated hazard ratios (HR) and 95% confidence intervals (CI). Overall survival was defined as time from diagnosis to death from any cause or end of follow-up, whichever came first. In an initial multivariate model, we adjusted for age at diagnosis (years), study site (DF/BWCC and Mayo Clinic), race (white and non-white), baseline body composition measurement (continuous), sex, year of diagnosis (2000–2010 and 2011–2015), and disease stage (locally advanced and metastatic). In the second multivariate model, we additionally adjusted for BMI (continuous), smoking history (never, past, current, and unknown), history of diabetes (no diabetes, diabetes duration $\leq$4 years, diabetes duration >4 years, and unknown), and type of treatment (FOLFIRINOX/FOLFOX/FOLFIRI, gemcitabine/gemcitabine combination, chemoradiation, and no treatment/unknown). We calculated median survival times for patents in each quartile of body composition change using direct adjusted survival estimation (17). Change in body composition was expressed as the difference per 30 days in tissue measurements between the follow-up and baseline CT scans. Because of differences in body composition change by sex, we calculated sex-specific quartiles of change for each measurement. The bottom quartile (i.e., patients with the least change) was taken as the reference group, and a *P* value for trend was evaluated by entering the median value of sex-specific quartiles in Cox proportional hazards models. Similarly, the association between baseline body composition and patient survival was examined using sex-specific quartiles of baseline measurements. Muscle wasting was also categorized by the presence of sarcopenia using established cut-off points of SMI (BMI $\leq$ 24.9 kg/m^2: <43 cm^2/m^2 for men and <41 cm^2/m^2 for women; BMI $\geq$ 25 kg/m^2: <53 cm^2/m^2 for men and <41 cm^2/m^2 for women; ref. 18). Heterogeneity of the association across the two study sites was assessed using Cochran Q-statistic (19). We performed stratified analyses by sex, cancer stage, and treatment type, and evaluated the statistical interaction using the Wald test of the cross-product term of change in body composition and stratification variables.

Differences in body composition change across quartiles of plasma markers were evaluated using the Kruskal–Wallis test. The association between BCAAs and MCP-1 with change in muscle area was further modeled using multivariate linear regression. All *P* values were two-sided. All statistical analyses were performed using SAS 9.4 (SAS Institute).

Results

Patient characteristics overall and by study site are shown in Table 1. All patients had advanced disease at diagnosis, with 106 (65%) having metastatic disease and 58 (35%) having locally advanced disease. Median overall survival times were 14.8 months for patients with locally advanced disease and 10.2 months for metastatic disease. Median time between pathologic diagnosis and baseline scans was 1 day [interquartile range (IQR): 26 days], and the median time between baseline and follow-up scans was 80 days (IQR: 28 days). By the end of follow-up, 140 (85%) patients had died.

Between the baseline and follow-up scans, patients lost an average of 9.9% of muscle, 14.7% of subcutaneous adipose tissue, and 7.5% of visceral adipose tissue (Table 2). Muscle attenuation declined on average 3.2 HU between the two scans, reflecting decreasing muscle density. Changes were similar in patients from the two study sites (Supplementary Table S1). Muscle and visceral adipose loss per 30 days was greater among men (Table 2; Supplementary Table S2). At baseline, 86 (52%) of patients were sarcopenic, and 113 (69%) were sarcopenic at follow-up scan. Correlation coefficients for clinical characteristics and body composition measurements are shown in Supplementary Table S3.

Compared with patients in the bottom quartile of muscle change per 30 days (i.e., those that lost the least muscle), patients in the top quartile experienced a 2-fold increase in the hazards for mortality (HR, 2.01; 95% CI, 1.12–3.62; $P_{trend} = 0.01$; Table 3). Similarly, patients in the top quartile of muscle density change per 30 days (i.e., those with the greatest reduction in muscle density) had a 2.2-fold increased mortality (HR, 2.19; 95% CI, 1.18–4.04; $P_{trend} = 0.02$) compared with those in the bottom quartile. No association was identified between patient survival and change in subcutaneous ($P_{trend} = 0.52$) or visceral ($P_{trend} = 0.20$) adipose tissue. The associations were similar across the two study sites (all $P_{heterogeneity} > 0.15$). Mean duration of cancer treatment was similar across quartiles of muscle area ($P = 0.16$), or muscle density ($P = 0.77$) change (Supplementary Table S4).

In stratified analyses by sex, disease stage, and treatment type, no statistically significant interactions were identified for change in muscle or adipose areas (all $P_{interaction} \geq 0.16$). In contrast, the association between change in muscle density and survival was more pronounced among women (per sex-specific standard deviation HR, 2.27; 95% CI, 1.35–3.84) than men (HR, 1.16; 95% CI, 0.80–1.69; $P_{interaction} = 0.05$). However, interaction tests were limited by modest sample sizes within strata.

We analyzed four circulating biomarkers from baseline plasma samples to identify patients at risk of developing tissue wasting after diagnosis. Patients in the top quartiles of BCAAs had a greater loss of muscle per 30 days ($P = 0.004$; Table 4), but a similar change in other tissue measurements. Loss of muscle or adipose tissues was similar across quartiles of IL6 and sTNF-RII (all $P \geq 0.11$), while patients in the highest quartile of MCP-1 experienced significantly less muscle loss ($P = 0.005$; Table 4). In multivariate adjusted models, the association of higher BCAA levels with greater loss of muscle was attenuated, while the association of higher MCP-1 levels with lower loss of muscle was similar (Supplementary Table S5).

Discussion

In this study of patients with advanced pancreatic cancer treated at two academic cancer centers, loss of muscle area and decrease in muscle density as assessed by CT imaging in the 2–4 months after diagnosis were associated with a significant reduction in patient survival. Specifically, patients in the top quartile of muscle loss or

Table 1. Baseline characteristics of patients with pancreatic cancer

Patient characteristics[a]	DF/BWCC ($N = 117$)	Mayo Clinic ($N = 47$)	Overall ($N = 164$)
Age at diagnosis, years	63.6 (9.5)	62.9 (10.3)	63.4 (9.7)
Female sex	52 (44)	17 (36)	69 (42)
Race/ethnicity			
White	106 (91)	47 (100)	153 (93)
Black	7 (6)	0 (0)	7 (4)
Other	3 (3)	0 (0)	3 (2)
Unknown	1 (1)	0 (0)	1 (1)
BMI, kg/m^2	26.5 (5.4)	29.1 (5.7)	27.2 (5.6)
Diabetes history			
No diabetes	78 (67)	36 (77)	114 (70)
Diabetes $\leq$4 years	19 (16)	7 (15)	26 (16)
Diabetes >4 years	9 (8)	2 (4)	11 (7)
Unknown	11 (9)	2 (4)	13 (8)
Smoking history			
Never	54 (46)	16 (34)	70 (43)
Past	53 (45)	20 (43)	73 (45)
Current	10 (9)	2 (4)	12 (7)
Unknown	0 (0)	9 (19)	9 (5)
Year of diagnosis			
2000–2010	32 (27)	25 (53)	57 (35)
2011–2015	85 (73)	22 (47)	107 (65)
Cancer stage			
Locally advanced	30 (26)	28 (60)	58 (35)
Metastatic	87 (74)	19 (40)	106 (65)
Median survival time, months			
All patients	11.0	13.9	11.4
By stage			
Locally advanced	13.3	16.0	14.8
Metastatic	10.2	10.8	10.2
Initial treatment program			
FOLFIRINOX/FOLFOX/FOLFIRI[b]	56 (48)	15 (32)	71 (43)
Gemcitabine or gemcitabine combination[c]	53 (45)	17 (36)	70 (43)
Chemoradiation (RT with 5-FU or capecitabine)	7 (6)	9 (19)	16 (10)
No treatment/unknown	1 (1)	6 (13)	7 (4)
Median time (IQR) between pathologic diagnosis and baseline CT scan (days)	2 (28)	1 (14)	1 (26)
Median time (IQR) between baseline and follow-up CT scan (days)	80 (28)	84 (27)	80 (28)
Median time (IQR) between baseline CT scan and blood draw (days)	12 (21)	1 (8)	8 (19)

Abbreviations: 5-FU, 5-fluorouracil; RT, radiotherapy.

[a]Continuous variables are reported as mean (SD), and categorical variables are reported as number (percentage), unless noted otherwise.

[b]FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; $N = 61$), FOLFOX (5-fluorouracil, leucovorin, and oxaliplatin; $N = 9$), or FOLFIRI (5-fluorouracil, leucovorin, and irinotecan; $N = 1$).

[c]Gemcitabine ($N = 41$) or gemcitabine combinations ($N = 29$), including gemcitabine plus: bevacizumab/erlotinib ($N = 2$), capecitabine ($N = 3$), cisplatin ($N = 3$), erlotinib ($N = 2$), nab-paclitaxel/momelotinib ($N = 2$), nab-paclitaxel ($N = 4$), panitumumab/erlotinib ($N = 1$), temsirolimus ($N = 1$), AGS-1C4D4 ($N = 1$), AMG-479 ($N = 2$), IPI-926 ($N = 6$), or TH-302 ($N = 2$).

decrease in muscle density had an adjusted median overall survival 4–5 months shorter than those in the bottom quartile of these measurements. In contrast, changes in adipose tissues were not associated with patient survival even though large losses were also identified for these tissues, suggesting that muscle and adipose tissue wasting may mark biologically distinct processes, a finding supported by data in preclinical models (11). Circulating levels of BCAAs and MCP-1 at diagnosis were associated with future muscle loss.

Previous studies of postdiagnosis body composition change reported no significant association between patient survival and muscle loss (5, 20), and either no association (5) or shorter survival in patients with greater visceral adipose tissue loss (20). While both studies included patients with advanced disease and identified similar rates of change in body composition, patient outcomes were evaluated using unadjusted Kaplan–Meier survival curves. Given the association of tissue changes with patient characteristics such as age, sex, and BMI, multivariate modeling is necessary to evaluate independent associations between body composition change and patient survival. Dalal and colleagues observed shorter survival in patients with locally advanced disease receiving chemoradiation with higher loss of visceral adipose tissue (21), and we cannot rule out that body composition change might be differentially associated with patient survival depending on stage and type of treatment.

Several studies have examined muscle area measured at diagnosis in patients with pancreatic cancer with advanced disease and observed either no difference (4, 5, 20, 21) or worse survival in patients with sarcopenia (3) or lower muscle density (4). Our recent study of baseline body composition among 682 patients with previously untreated pancreatic cancer included the patients analyzed here, and baseline body composition measurements were not associated with patient survival (11). These data suggest that the rate of change in body composition measurements in these patients may better reflect the adverse biology of tissue wasting, rather than the static measurements taken at the time of diagnosis.

Table 2. Body composition changes between baseline and follow-up CT scans in patients with advanced pancreatic cancer

	N	Baseline measurement[a]	Change between baseline and follow-up CT scan[a]	% change between baseline and follow-up CT scan[a]	Change per 30 days[a]	% change per 30 days[a]
Overall						
Skeletal muscle area (cm^2)	164	136.5 (36.3)	−14.6 (16.4)	−9.9 (11.0)	−5.4 (5.9)	−3.7 (3.9)
Skeletal muscle attenuation (HU)	159	36.8 (9.2)	−3.2 (7.6)	N/A[b]	−1.1 (3.0)	N/A[b]
Subcutaneous adipose tissue area (cm^2)	164	190.2 (103.0)	−27.5 (35.4)	−14.7 (19.7)	−9.8 (13.2)	−5.3 (8.0)
Visceral adipose tissue area (cm^2)	164	162.1 (104.6)	−24.5 (39.4)	−7.5 (58.3)	−9.2 (14.8)	−2.5 (23.1)
Women						
Skeletal muscle area (cm^2)	69	104.1 (19.1)	−8.5 (14.4)	−7.8 (12.9)	−3.3 (4.9)	−2.9 (4.5)
Skeletal muscle attenuation (HU)	66	35.4 (9.8)	−3.1 (6.7)	N/A[b]	−1.2 (2.9)	N/A[b]
Subcutaneous adipose tissue area (cm^2)	69	211.0 (118.4)	−28.9 (43.6)	−12.7 (22.3)	−9.8 (16.3)	−4.2 (9.3)
Visceral adipose tissue area (cm^2)	69	101.6 (76.1)	−8.3 (23.9)	5.6 (85.0)	−3.0 (9.1)	3.0 (33.8)
Men						
Skeletal muscle area (cm^2)	95	160.6 (25.7)	−19.0 (16.5)	−11.4 (9.2)	−7.0 (6.0)	−4.2 (3.3)
Skeletal muscle attenuation (HU)	93	37.8 (8.7)	−3.3 (8.2)	N/A[b]	−1.1 (3.1)	N/A[b]
Subcutaneous adipose tissue area (cm^2)	95	175.1 (87.9)	−26.5 (28.3)	−16.2 (17.6)	−9.8 (10.5)	−6.1 (7.0)
Visceral adipose tissue area (cm^2)	95	206.0 (100.7)	−36.2 (44.1)	−17.0 (21.1)	−13.7 (16.5)	−6.6 (8.0)

[a]Mean (SD).
[b]The HU scale has a zero value set in the middle of its range, so percentage calculations relative to the arbitrary zero are not meaningful.

Table 3. HRs for mortality by quartiles of body composition change per 30 days in patients with advanced pancreatic cancer

	Quartile of body composition change per 30 days				
	1 Less loss of tissue area	2	3	4 More loss of tissue area	P_{trend}[c]
Skeletal muscle area					
Mean (SD) change per 30 days (cm^2)	0.8 (2.0)	−3.0 (1.7)	−6.8 (2.7)	−12.9 (4.9)	
Person-months	552.2	578.5	511.4	399.8	
Deaths/cases	35/41	36/42	36/41	33/40	
Median survival, months	13.9	12.8	10.2	9.8	
Model I[a]	Reference	1.29 (0.78–2.12)	1.53 (0.92–2.56)	1.84 (1.06–3.20)	0.03
Model II[b]	Reference	1.25 (0.73–2.14)	1.83 (1.07–3.15)	2.01 (1.12–3.62)	0.01
Visceral adipose tissue area					
Mean (SD) change per 30 days (cm^2)	6.4 (7.8)	−4.4 (5.1)	−12.6 (6.2)	−26.5 (13.6)	
Person-months	558.6	579.2	444.0	401.8	
Deaths/cases	32/40	35/40	38/40	31/39	
Median survival, months	11.3	13.3	11.0	10.2	
Model I[a]	Reference	0.79 (0.47–1.33)	1.20 (0.71–2.01)	1.29 (0.74–2.27)	0.13
Model II[b]	Reference	0.76 (0.44–1.28)	1.04 (0.59–1.83)	1.31 (0.72–2.39)	0.20
Subcutaneous adipose tissue area					
Mean (SD) change per 30 days (cm^2)	5.6 (7.2)	−5.4 (3.1)	−13.9 (3.0)	−26.1 (9.5)	
Person-months	569.4	487.1	434.6	492.6	
Deaths/cases	33/40	32/40	36/40	35/39	
Median survival, months	11.3	12.9	10.2	11.3	
Model I[a]	Reference	0.78 (0.46–1.32)	1.26 (0.75–2.12)	0.97 (0.57–1.65)	0.45
Model II[b]	Reference	0.85 (0.49–1.49)	1.27 (0.72–2.23)	1.00 (0.57–1.75)	0.52
	1 Less decrease in muscle attenuation	**2**	**3**	**4 More decrease in muscle attenuation**	P_{trend}[c]
Skeletal muscle attenuation					
Mean (SD) change per 30 days (HU)	2.4 (1.6)	−0.3 (0.5)	−1.7 (0.5)	−4.9 (2.4)	
Person-months	528.8	524.0	506.0	419.4	
Deaths/cases	34/39	35/41	34/40	33/39	
Median survival, months	13.9	12.9	10.8	8.8	
Model I[a]	Reference	1.30 (0.79–2.13)	1.72 (1.02–2.90)	2.51 (1.42–4.43)	0.002
Model II[b]	Reference	1.27 (0.75–2.14)	1.69 (0.97–2.94)	2.19 (1.18–4.04)	0.02

Abbreviations: FOLFIRINOX, 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan.
[a]Adjusted for age (continuous), baseline body composition measurement (continuous), sex, years of diagnosis (2000–2010 and 2011–2015), race (white and non-white), stage (locally advanced and metastatic), and study site (DF/BWCC and Mayo Clinic).
[b]In addition, adjusted for BMI (continuous), diabetes (no diabetes, diabetes duration ≤4 years, and diabetes duration >4 years), smoking (never, past, and current), and treatment type (FOLFIRINOX/FOLFOX/FOLFIRI, gemcitabine/gemcitabine doublet, chemoradiation, and no treatment/unknown).
[c]Calculated using sex-specific quartile median as a continuous variable.

Table 4. Baseline circulating markers and subsequent body composition changes between baseline and follow-up CT scans in patients with advanced pancreatic cancer

	Circulating total BCAAs				
	Q1	**Q2**	**Q3**	**Q4**	***P* value[a]**
N	40	41	42	41	
Range (μmol/L)	83.8–275.1	275.7–322.1	323.3–388.2	390.1–673.8	
Skeletal muscle area (cm^2)	−2.6 (5.4)	−6.2 (5.5)	−6.4 (6.5)	−6.2 (5.2)	0.004
Skeletal muscle attenuation (HU)	−0.3 (2.6)	−2.1 (3.2)	−1.4 (3.2)	−0.7 (2.8)	0.06
Visceral adipose tissue area (cm^2)	−6.0 (9.9)	−9.2 (17.3)	−10.6 (14.2)	−10.8 (16.6)	0.24
Subcutaneous adipose tissue area (cm^2)	−13.1 (13.6)	−7.8 (13.7)	−8.5 (13.7)	−10.0 (11.4)	0.23
	Circulating IL6				
	Q1	**Q2**	**Q3**	**Q4**	***P* value[a]**
N	23	23	23	22	
Range (pg/mL)	0.2–1.5	1.5–2.5	2.6–3.3	3.6–33.2	
Skeletal muscle area (cm^2)	−5.8 (6.0)	−6.9 (6.1)	−5.2 (4.5)	−6.2 (7.2)	0.91
Skeletal muscle attenuation (HU)	−1.3 (3.5)	−1.2 (4.0)	−1.7 (2.6)	−1.5 (4.2)	0.89
Visceral adipose tissue area (cm^2)	−7.4 (11.0)	−11.2 (16.7)	−9.2 (18.2)	−11.5 (18.1)	0.75
Subcutaneous adipose tissue area (cm^2)	−10.5 (11.8)	−9.1 (15.0)	−14.0 (15.9)	−8.5 (15.0)	0.57
	Circulating sTNF-RII				
	Q1	**Q2**	**Q3**	**Q4**	***P* value[a]**
N	23	23	24	22	
Range (pg/mL)	1,397.2–2,316.8	2,318.2–3,228.9	3,244.0–4,899.7	4,910.9–10,000.0	
Skeletal muscle area (cm^2)	−7.2 (4.7)	−7.7 (6.5)	−5.8 (5.5)	−4.0 (6.9)	0.11
Skeletal muscle attenuation (HU)	−0.6 (2.5)	−2.6 (4.0)	−1.6 (3.3)	−1.6 (4.0)	0.32
Visceral adipose tissue area (cm^2)	−8.6 (14.3)	−12.3 (17.3)	−6.0 (15.3)	−11.5 (13.9)	0.59
Subcutaneous adipose tissue area (cm^2)	−10.4 (10.6)	−10.5 (13.9)	−9.1 (12.7)	−11.5 (17.5)	0.58
	Circulating MCP-1				
	Q1	**Q2**	**Q3**	**Q4**	***P* value[a]**
N	21	24	23	23	
Range (pg/mL)	4.3–41.5	41.6–77.7	91.2–176.8	181.7–448.3	
Skeletal muscle area (cm^2)	−9.7 (5.7)	−6.3 (4.9)	−5.0 (6.3)	−3.4 (5.3)	0.005
Skeletal muscle attenuation (HU)	−1.8 (3.7)	−1.0 (3.3)	−1.2 (3.3)	−1.8 (4.0)	0.94
Visceral adipose tissue area (cm^2)	−12.9 (13.4)	−7.0 (11.2)	−7.7 (21.1)	−12.3 (16.9)	0.39
Subcutaneous adipose tissue area (cm^2)	−11.0 (9.0)	−12.4 (14.0)	−10.0 (15.6)	−9.0 (17.9)	0.72

[a]*P* value is calculated using Kruskal–Wallis test.

Loss of muscle mass and muscle density may reflect a more aggressive disease biology, whereby muscle is reactive to a rapidly growing malignancy without contributing to either the specific growth of the tumor or compromise of the host (22). Alternatively, muscle loss might have a causal effect leading to reduced patient survival. Reversal of cancer-associated muscle loss in animal models has been shown to increase survival in some cancer types (23, 24). In patients, muscle wasting can result in generalized weakness and poor tolerance of cancer-directed treatments, which may contribute to worsened survival (25). Furthermore, nutritional starvation could lead to muscle catabolism and less effective antitumoral immune response (26), which may also have an adverse impact on patient survival.

No biomarkers are currently used in the clinic to identify patients with cancer at increased risk of muscle wasting. The majority of studies examining potential cachexia biomarkers have used a cross-sectional design, comparing levels of biomarkers between patients with and without cachexia at the time of diagnosis (7). While cross-sectional studies can identify biomarkers differentiating patients with or without cachexia, longitudinal studies are needed to identify biomarkers associated with future tissue depletion. Using a retrospective, longitudinal cohort study design, we have shown that higher levels of baseline BCAAs are associated with accelerated loss of muscle in the 2–4 months after diagnosis and thus may reflect higher ongoing rates of muscle degradation. These data are consistent with a previous study in mouse models of pancreatic cancer showing that circulating levels of BCAAs are increased early in the development of pancreatic cancer as a consequence of muscle wasting (9).

We observed no association between plasma IL6 and sTNF-RII levels at diagnosis and subsequent tissue wasting. In some, but not all prior cross-sectional studies, IL6 and TNFα levels were higher in patients with pancreatic cancer with cachexia compared with patients without cachexia defined as weight loss (7, 27). Our results suggest that IL6 and sTNF-RII at diagnosis do not predict subsequent tissue loss as quantified on CT images. Interestingly, we observed less future muscle loss in patients with higher circulating levels of MCP-1, which is not consistent with a prior cross-sectional study in which higher circulating MCP-1 levels were seen in patients with cachexia defined by weight loss (8). In animal models of acute (28) and chronic (29) muscle injury, MCP-1 was shown to facilitate muscle repair and regeneration. Thus, one might speculate that patients with higher MCP-1 levels may have greater ability to repair muscle damage induced by pancreatic cancer.

An important strength of this study was the precise tissue quantification at two time points during disease using CT imaging. Thus, the dynamics of body composition change over time could be evaluated in relation to both patient outcomes and circulating biomarkers. Importantly, baseline CT imaging and blood collection were performed prior to any cancer-directed treatment, reducing confounding by treatment status. Furthermore, we collected data for multiple potential confounding covariates, allowing for determination of the independent association of body composition changes and patient survival.

Our study has several limitations. Muscle attenuation is widely used as a proxy for intramuscular fat accumulation and area-preserving atrophy (12), but attenuation values can also be decreased by fluid accumulation from anasarca. Further work is required to differentiate the aspects of altered muscle attenuation most related to poor patient survival. Body composition change was followed for 50–120 days after diagnosis, rather than for the patient's entire treatment course. However, a previous study that evaluated body composition changes for up to four consecutive CT scans (419 days after diagnosis) found little difference in rate of change in muscle or adipose tissue across study intervals (20). Furthermore, the initial several months after diagnosis are critical for identification of cachexia, as this period provides a window of opportunity for interventions to reduce tissue wasting and improve patient outcomes. In addition, patients must have undergone repeat imaging at least 50 days after the baseline scan, such that patients with extremely rapid progression of their cancer may not have been included in our study population. Information on use of nutritional supplements such as L-carnitine or omega-3 polyunsaturated fatty acids (30) was not available in our study population, so their impact on skeletal muscle or adipose tissue changes could not be assessed. Finally, most patients were white; therefore, these observations need to be validated in additional, more diverse populations.

In conclusion, in this large study of patients with advanced pancreatic cancer and serial quantification of body composition using CT imaging, postdiagnosis loss of muscle, but not of adipose tissue was associated with reduced patient survival. Furthermore, baseline plasma levels of BCAAs and MCP-1 were associated with future muscle loss, potentially marking a patient group at high risk for future complications due to cancer cachexia.

Disclosure of Potential Conflicts of Interest

R.F. Dunne is consultant/advisory board member for Exelixis Inc. J.A. Meyerhardt is an advisory board member for Cota and Ignyta, and is a member of the grant review panel through NCCN for Taiho Pharmaceutical. K. Ng reports receiving commercial research grants from Gilead and Celgene. M.G. Vander Heiden is a scientific advisory board member for Agios Pharmaceuticals, Aeglea Biotherapeutics, and Auron Therapeutics and is a founder of Auron Therapeutics. B.M. Wolpin reports receiving commercial research grants from Celgene and Eli Lilly and has provided one-time consulting for Celgene, GRAIL, G1 Therapeutics, and BioLineRx. No potential conflicts of interest were disclosed by the other authors.

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