Universidade do Estado do Rio de Janeiro

Centro de Educação e Humanidades

Instituto de Nutrição

Larissa Calixto Lima

Avaliação da massa muscular e da mioesteatose em pacientes com câncer incurável

Rio de Janeiro

2020

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Tese apresentada, como requisito parcial para obtenção do título de doutor, ao Programa de Pós-Graduação em Alimentação, Nutrição e Saúde, da Universidade do Estado do Rio de Janeiro. Área de concentração: Alimentação, Nutrição e Saúde.

Orientadora: Profª. Drª. Carla Maria Avesani

Coorientadora: Profª. Drª. Flávia Fioruci Bezerra

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DEDICATÓRIA

Ao meu Arthur, meu amor maior.

Ao meu querido Fabio.

Aos meus pais, Borges e Gislaine.

Aos meus irmãos, Luciana, Márcio, Francisco e Rafael.

Vocês são os pilares da minha vida!

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RESUMO

CALIXTO-LIMA, L. Avaliação da massa muscular e da mioesteatose em pacientes com câncer incurável. 2020. 110 f. Tese (Doutorado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2020.

Ao longo da última década, o número de estudos sobre sarcopenia em indivíduos com neoplasias malignas aumentaram consideravelmente. Nos pacientes com câncer a sarcopenia é definida como depleção muscular grave, miopenia ou sarcopenia secundária. A tomografia computadorizada (TC) é considerada método padrão-ouro para a avaliação da massa muscular, uma vez que, além de fazer a quantificação da massa muscular com precisão, permite a identificação da infiltração de tecido adiposo muscular, denominada mioesteatose. Os objetivos deste estudo foram: (1) desenvolver e validar uma equação de predição de massa muscular esquelética (MME) baseada em variáveis antropométricas e na força de preensão manual (FPM); (2) investigar os fatores associados à radiodensidade do músculo esquelético (RME) avaliada por TC; e (3) investigar a associação entre diferentes fenótipos de músculo esquelético com resposta inflamatória sistêmica, capacidade funcional e sobrevida global. Três artigos serão apresentados, sendo que cada um responderá a um dos três objetivos propostos. Para todos os artigos, os critérios de inclusão adotados compreenderam idade maior que 20 anos, apresentar *Karnofsky Performance Status* (KPS) superior a 30% no momento da avaliação e possuir imagens de TC das regiões pélvica e abdominal no intervalo de até 30 dias da data de inclusão na pesquisa. O *artigo 1* desenvolveu e validou uma equação de predição da MME usando como referência a MME avaliada por TC em 272 pacientes (68% do sexo feminino; mediana de idade de 60 anos) com câncer incurável. A equação de estimativa desenvolvida neste estudo incluiu as variáveis sexo, raça/cor da pele autorrelatada, peso corporal, área muscular do braço corrigida (AMAc) e FPM, apresentando R^2 ajustado de 0,60 e raiz do erro quadrático médio de 5,82, cuja fórmula segue descrita: massa muscular (cm^2) = 57,37 + peso (kg) x 0,38 + AMAc (cm) x 1,17 + FPM (kg) x 0,65 + 7,75 (se raça/cor da pele negra) – 22,96 (se sexo feminino). O *artigo 2*, de delineamento transversal, analisou os fatores associados à RME por meio de modelos de regressões lineares em uma amostra de 393 pacientes (mediana de idade de 61 anos, 69,7% do sexo feminino) com câncer incurável. Os modelos multivariados demostraram associação significativa e direta entre a RME e as variáveis albumina sérica, FPM, MME e câncer gastrintestinal e associação significativa e indireta entre RME e idade, raça/cor da pele branca, adiposidade corporal e diagnóstico de câncer ginecológico ou tecido ósseo e conectivo. O *artigo 3* inclui 326 pacientes com câncer incurável (mediana de idade de 60 anos, 67,5% do sexo feminino) e demonstrou que fenótipos de baixa MME e mioesteatose, particularmente quando combinados, se associaram a pior desempenho de função física e maior resposta inflamatória sistêmica avaliada por meio de diferentes indicadores inflamatórios. Além disso, a presença de mioesteatose aumentou o risco de morte em um período de seguimento de 90 dias. Em conclusão, os três artigos apresentados na presente tese possibilitaram propor uma equação como alternativa simples e de baixo custo para a avaliação da MME de pacientes com câncer avançado. Ademais, mostraram os fatores associados à RME e aos diferentes fenótipos musculares.

Palavras-chave: Câncer Avançado. Cuidados Paliativos. Massa Muscular. Sarcopenia. Mioesteatose. Tomografia Computadorizada. Antropometria.

ABSTRACT

CALIXTO-LIMA, L. Evaluation of muscle mass and myosteatosis in patients with incurable cancer. 2020. 110 f. Tese (Doutorado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2020.

Over the last decade, studies on sarcopenia in individuals with cancer have increased considerably. In the oncologic setting, sarcopenia has been used as a synonym for severe muscle wasting, myopenia, or secondary sarcopenia. Computed tomography (CT) is considered a gold standard method due to its high accuracy and reproducibility for muscle mass assessment and good accuracy to infer muscle fat infiltration (i.e., myosteatosis). The aim of this study was to: (1) develop and validate an equation using anthropometric and handgrip strength (HGS) measurements to estimate skeletal muscle mass (SM) in incurable patients with cancer; (2) examine the factors associated with skeletal muscle radiodensity (SMD) evaluated by CT; and (3) investigate whether distinctive skeletal muscle phenotypes are associated with systemic inflammatory response, functional impairment, and survival in patients with incurable cancer. Three articles will be presented, each answering one of these objectives. For all articles, the inclusion criteria were patients aged 20 years or older, Karnofsky Performance Status (KPS) ≥30% and those who had abdominal or pelvic CT scans up to 30 days before the inclusion in the study. *Article 1* developed and validated an equation for estimating SM in 272 patients (68.0% female; median age of 60 years) with incurable cancer. The predicted equation developed in this study included sex, race/skin color, body weight, corrected arm muscle area (CAMA) and HGS, with an adjusted R^2 of 0.60 and the root mean square error of 5.82 The best predictive equation was muscle mass (cm^2) = 57.37 + weight (kg) x 0.38 + CAMA (cm) x 1.17 + HGS (kg) x 0.65 + 7.75 (black race/skin color) – 22.96 (female). *Article 2* was a cross-sectional study that analyzed the factors associated with SMD using linear regression models in 393 patients (median age 61 years, 69.7% female) with incurable cancer. The results showed that age, white race/skin color, adiposity markers and gynecological and BCT tumors were inversely associated with SMD, while HGS, SM index (SMI) and gastrointestinal tumor was directly associated with SMD. *Article 3* comprised 326 patients with incurable cancer (median age 60 years, 67.5% female) and demonstrated that low SM index (SMI) and myostatosis phenotypes, particularly when combined, were associated with worse physical functional impairment and altered inflammatory response evaluated by different inflammation-based prognostic scores. Moreover, the presence of myosteatosis increased 90-day mortality risk. In conclusion, an equation was developed and validated using simple and low-cost measurements to be used as an alternative for assessing SM in patients with incurable cancer. Additionally, this work highlighted factors associated with SMD and with different muscle phenotypes.

Keywords: Advanced Cancer. Palliative Care. Muscle Mass. Sarcopenia. Myosteatosis. Computed Tomography. Anthropometry.

RESUMEN

CALIXTO-LIMA, L. Evaluación de la masa muscular y miosteatosis en pacientes con cáncer incurable. 2020. 110 f. Tese (Doutorado em Alimentação, Nutrição e Saúde) – Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2020.

Durante la última década, los estudios sobre sarcopenia en personas con neoplasias malignas han aumentado considerablemente. En pacientes con cáncer, la sarcopenia se define por una depleción muscular grave, miopenia o sarcopenia secundaria. La tomografía computarizada (TC) se considera un método padrón oro para evaluar la masa muscular, ya que además de cuantificar con precisión la masa muscular, permite identificar la infiltración de tejido adiposo intramuscular, conocida como miosteatosis. El objetivo de este estudio fue: (1) Desarrollar y validar una ecuación de predicción de masa del músculo esquelético (MME) basada en variables antropométricas y fuerza de prensión manual (FPM); (2) Investigar los factores asociados con la radiodensidad del músculo esquelético (RME) evaluados por TC; (3) Investigar la asociación entre los diferentes fenotipos de músculo esquelético con respuesta inflamatoria sistémica, capacidad funcional y supervivencia global. Se presentarán tres artículos, cada artículo respondiendo a uno de los tres objetivos de la tesis. Para todos los artículos, los criterios de inclusión adoptados incluyeron ser mayor de 20 años, presentar el stado de desempeño Karnofsky (KPS) superior al 30% al momento de la evaluación y tener una TC de las regiones abdominal o pélvica hasta 30 días antes de la evaluación inicial. El artículo 1 desarrolló y validó una ecuación de predicción de la MME utilizando MME según la evaluación de la TC en 272 pacientes (68,0% mujeres; mediana de edad 60 años) con cáncer incurable. La ecuación de estimación desarrollada en este estudio incluyó las variables sexo, raza / color de piel (según por la propia persona), peso corporal, área muscular del brazo corregido (AMAc) y FPM, mostrando un R2 ajustado de 0.60 y la raíz del error cuadrático medio de 5.82, cuya fórmula se describe a continuación: masa muscular (cm2) = 57,37 + peso (kg) x 0,38 + AMAC (cm) x 1,17 + FPM (kg) x 0,65 + 7,75 (si es de raza / color de la piel negra) - 22,96 (si es mujer). El artículo 2 transversal analizó los factores asociados con la RME utilizando modelos de regresión lineal en una muestra de 393 pacientes (mediana de edad 61 años, de estes 69,7% mujeres) con cáncer incurable. Los modelos multivariados han mostrado asociación significativa y directa entre RME y las variables albúmina sérica, FPM, MME y cáncer del tracto gastrointestinal y asociación significativa y indirecta entre RME y edad, la raza / el color de la piel, adiposidad corporal y el diagnóstico de cáncer ginecológico o de tejido óseo y conectivo. El *artículo 3* incluye a 326 pacientes con cáncer avanzado (mediana de edad 60 años, de estes 67,5% mujeres) y demostró que los fenotipos de MME baja y miosteatosis, especialmente cuando se combinan, se asociaron con una peor función física y una mayor respuesta inflamatoria sistémica, evaluado utilizando diferentes indicadores inflamatorios. Además, la presencia de miosteatosis aumentó el riesgo de muerte en un período de seguimiento de 90 días. En conclusión, los tres artículos presentados en la presente tesis permitieron proponer una ecuación como una alternativa simple y de bajo costo para la evaluación de MME en pacientes con cáncer avanzado. Además, se mostraron los factores asociados con RME y los diferentes fenotipos musculares,

Palabras llave: Cáncer Avanzado. Cuidados Paliativos. Masa Muscular. Sarcopenia. Miosteatosis. Tomografía Computarizada. Antropometría.

LISTA DE ILUSTRAÇÕES

Revisão da literatura

Artigo 1

Artigo 2

Artigo 3

LISTA DE TABELAS

Artigo 1

Artigo 2

Artigo 3

LISTA DE ABREVIATURAS E SIGLAS

Português

AMBc	Área muscular do braço corrigida
ASG-PPP	Avaliação Subjetiva Global Produzida Pelo Paciente
CB	Circunferência do braço
CMB	Circunferência muscular do braço
CP	Circunferência da panturrilha
CQ	Circunferência do qaudril
DCT	Dobra cutânea tricipital
DXA	Absorciometria por dupla emissão de raios X
FPM	Força de preensão manual
GLOBOCAN	Agência Internacional de Pesquisa sobre o câncer
HU	Unidade de Hounsfield
IMC	Índice de massa corporal
INCA	Instituto Nacional de Câncer José Alencar Gomes da Silva
KPS	*Karnofsky Performance Status*
L3	Terceira vértebra lombar
NHANHES	National Health and Nutrition Examination Survey
PCR	Proteína C-reativa
RM	Ressonância magnética
RNL	Relação neutrófilo-linfócito
RPL	Relação plaqueta-linfócito
TA	Tecido adiposo
TAIM	Tecido adiposo intramuscular
TASC	Tecido adiposo subcutâneo
TAV	Tecido adiposo visceral
TC	Tomografia computadorizada
TCLE	Termo de Consentimento Livre e Esclarecido

Inglês

%WL	Percentage of weight loss
AMA	Arm muscle area
BMI	Body mass index
CAMA	Corrected arm muscle area
CC	Calf circumference

CI	Confident interval
CT	Computed tomography
CRP	C-reactive protein
ECOG PS	Eastern cooperative oncology group performance status
HGS	Handgrip strength
HR	Hazard ratios
HU	Hounsfield unit
ICC	Intraclass correlation coefficient
IMAT	Intermuscular adipose tissue
IQR	Interquartile range
KPS	Karnofsky performance status
L3	Third lumbar vertebra
mGPS	Modified Glasgow Prognostic Score
NLR	Neutrophil-lymphocyte ratio
PCU	Palliative care unit
PG SGA SF	Patient-generated subjective global assessment short form
PLR	Platelet-lymphocyte ratio
PNI	Prognostic nutrition index
RMSE	Root mean squared error
SD	Standard deviation
SIR	Systemic inflammatory response
SM	Skeletal muscle mass
SMI	Skeletal muscle index
SMD	Skeletal muscle radiodensity
SAT	Subcutaneous adipose tissue
SATI	Subcutaneous adipose tissue index
VIF	Variance inflation factors
TAT	Total adipose tissue
TATI	Total adipose tissue index
TSF	Triceps skinfold thickness
VAT	Visceral adipose tissue
VATI	Visceral adipose tissue index

SUMÁRIO

APRESENTAÇÃO

Há alguns anos, durante um curso de qualificação para preceptores e docentes do Instituto Nacional de Câncer (INCA), local onde atuo como nutricionista, conheci Chimamanda Ngozi, uma feminista e escritora nigeriana que usa seu exemplo de vida para destacar "o perigo da história única". Chimamanda acredita que "insistir somente numa única face de uma narrativa significa negligenciar e tornar superficiais experiências complexas e tão ricas em detalhes. Isso inevitavelmente cria estereótipos, que se tornam um problema a partir do momento em que deixam incompleta a nossa compreensão sobre algo ou alguém". Quando me foi perguntado se já havia experienciado o perigo da história única, imediatamente pensei no estereótipo dos cuidados paliativos. Por incontáveis vezes, ao comentar que trabalhava com paciente com câncer avançado, ouvi: "Ahh, nessa fase da doença o paciente come o que ele quer, não é mesmo!?"

O termo cuidado paliativo é frequentemente associado ao fim da vida, à concepção de que "não há mais nada pra fazer". Respeitar a autonomia e os desejos dos pacientes, o que muitas vezes inclui fornecer alimentos de sua preferência, é de fato um dos pilares dos cuidados paliativos. Porém, para muito além disso, os cuidados paliativos são embasados em ciência, que fornecem subsídio para o controle da dor e dos demais sintomas que o paciente possa apresentar. Além disso, é por meio da ciência da nutrição que buscamos estratégias para reduzir a perda ponderal e de massa muscular, prevenir a caquexia e preservar ou quem sabe recuperar a autonomia dos pacientes, sempre objetivando a melhora da qualidade de vida. O primeiro passo para tal é conhecer melhor o perfil desses pacientes, seu estado nutricional, sua composição corporal, seu estado inflamatório. Foi em busca destas respostas que, no início de 2016, eu e duas outras nutricionistas, hoje estimadas amigas, criamos o NutriPali, Grupo de pesquisa em nutrição e cuidados paliativos. Assim, os resultados da presente tese são fruto de um esforço coletivo desse grupo de pesquisa em conjunto com as queridas orientadoras Carla Maria Avesani e Flavia Fioruci Bezerra.

Os dados amostrais desta tese fazem parte do projeto denominado "Diagnóstico nutricional diferencial e qualidade de vida de pacientes com câncer avançado em cuidados paliativos" em desenvolvimento na unidade de cuidados paliativos (Hospital do Câncer IV) do Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). A partir destes dados, três artigos independentes foram produzidos para compor esta tese, modelo este aceito na Deliberação número 45/2019 do programa de Pós-graduação em Alimentação, Nutrição e Saúde da Universidade do Estado do Rio de Janeiro (UERJ). Os três artigos seguem descritos

nesta tese formatados de acordo com as normas dos periódicos aos quais serão submetidos. O primeiro intitulado *Development and validation of a predictive equation using anthropometry and handgrip strength to estimate muscle mass in patients with incurable câncer"* será submetido ao periódico *Nutrition* (Fator de impacto 3,630; 2019-20) e teve por objetivo desenvolver e validar uma equação de predição da massa muscular utilizando medidas de baixo custo e não são invasivas, passíveis de aplicação na prática clínica. O segundo artigo "*Factors associated with skeletal muscle radiodensity in patients with incurable cancer*" explorou os fatores associados a radiodensidade do músculo esquelético, particularmente fatores relacionados ao câncer, e será submetido ao periódico Journal of Parenteral and Enteral Nutrition. (Fator de impacto: 2,853 2019-20:). Por fim, o terceiro artigo denominado "*Altered skeletal muscle phenotypes are associated with functional impairment, worse systemic inflammatory response, and reduced survival in patients with incurable cancer*" teve por objetivo investigar o comportamento de diferentes fenótipos de músculo esquelético derivados de uma combinação de baixa/alta massa muscular e ausência/presença de mioesteatose. Este manuscrito foi submetido ao periódico *Clinical Nutrition* (Fator de impacto 2019-20: 6,766).

Espera-se que os resultados desta tese possam contribuir não só para o desenvolvimento de equação de predição de massa muscular esquelética (MME) baseada em variáveis antropométricas e na força de preensão manual (FPM), mas também para a disseminação da pesquisa em cuidados paliativos de pacientes com câncer incurável. Paliar, do latim *pallium*, termo que nomeia o manto que os cavaleiros usavam para os proteger das tempestades pelos caminhos que percorriam. Proteger, cuidar... Que possamos, por meio de intervenções nutricionais, levar mais VIDA aos dias dos pacientes com câncer incurável, pois SEMPRE há algo que possa ser feito. "O sofrimento só é intolerável quando ninguém cuida" (Cicely Saunders).

INTRODUÇÃO

Câncer é o nome dado a um conjunto de doenças que têm em comum o crescimento desordenado de células anômalas que tendem a invadir tecidos e órgãos vizinhos.[1] Nos países em desenvolvimento, a maioria dos indivíduos apresenta a doença em estágio avançado no momento do diagnóstico.[2,3] Sob tal foco, ganha importância o cuidado paliativo, abordagem destinada ao cuidado global, ativo e multidisciplinar de indivíduos cuja doença não apresenta mais resposta aos tratamentos modificadores disponíveis.[4,5]

A presença de células malignas resulta no desenvolvimento de alterações metabólicas que expõem o indivíduo ao risco nutricional. Particularmente no paciente com câncer, as alterações ocasionadas pela interação tumor-hospedeiro resultam na depleção preferencial dos depósitos proteicos,[6,7,8,9] de forma que a característica fenotípica clinicamente mais relevante da desnutrição associada ao câncer é a perda de massa muscular esquelética (MME), definida nesse contexto como sarcopenia.[8,10-13]

A massa muscular pode ser avaliada por diversos métodos, sendo os mais utilizados em pacientes com câncer a tomografia computadorizada (TC), a ressonância magnética (RM), a absorciometria por dupla emissão de raios X (DXA), a impedância bioelétrica e a antropometria.[14,15] Para tal fim, a técnica de TC é reconhecida por ser de alta acurácia e reprodutibilidade, sendo, por isso, considerada padrão-ouro.[15-17] O emprego da TC no corte da terceira vértebra lombar (L3) tem se tornado uma medida de conveniência para a avaliação de composição corporal do paciente com tumores nas regiões pélvica e abdominal por ser rotineiramente utilizada para estadiamento tumoral e monitoramento da resposta ao tratamento.[15,16]

Apesar de métodos como a TC e a RM serem considerados precisos e de alta acurácia, os mesmos são considerados exames de conveniência para avaliação da composição corporal, pois expõem o paciente à radiação – especialmente a TC. Dessa forma, o uso desses métodos para a avaliação de massa muscular limita-se para fins de pesquisa.[18,19] As medidas antropométricas são consideradas de baixo custo, não invasivas e de fácil aplicabilidade para uso clínico hospitalar e ambulatorial.[19,20] De particular interesse, inúmeras equações de regressões que utilizam medidas antropométricas têm sido propostas na tentativa de estimar os valores de massa muscular.[21-24]

Além da quantificação da MME, a avaliação da composição corporal por TC permite a identificação da mioesteatose por meio da infiltração de tecido adiposo (TA) muscular.[25] Apesar de os mecanismos envolvidos no desenvolvimento da mioesteatose em pacientes com

câncer ainda não serem totalmente elucidados, acredita-se que a redução de massa muscular e a mioesteatose representam fenótipos clínicos distintos e que as duas condições concomitantes podem conferir efeito adicional para o pior prognóstico clínico.[26-28]

Sendo assim, estudos que avaliem a massa muscular de pacientes com câncer avançado por meio da TC justificam-se pela alta precisão e qualidade do método e por permitir a avaliação da mioesteatose por meio da avaliação da radiodensidade do músculo esquelético (RME). Essas características da TC a tornam um método de referência para desenvolvimento e validação de uma equação de predição da massa muscular, bem como para avaliar os fatores associados à RME em indivíduos com câncer avançado em cuidados paliativos.

1 REVISÃO DA LITERATURA

1.1 Câncer e cuidados paliativos

Câncer é um termo genérico utilizado para designar um conjunto de doenças que se caracterizam pela falha dos mecanismos de regulação do crescimento celular, resultando em proliferação anormal do tecido que tende à autonomia e à perpetuação, com invasão de estruturas adjacentes e efeitos agressivos sobre o hospedeiro.[1,29] O câncer representa a segunda maior causa de morte por doença.[2,29] Segundo dados da Agência Internacional de Pesquisa sobre o Câncer (IARC), as estimativas de 2018 foram de 18,1 milhões novos casos de câncer no mundo e 9,6 milhões de óbitos por esta enfermidade.[2] Para o ano de 2030, são estimados 21,6 milhões de casos novos e 13 milhões de mortes pela doença.[1] No Brasil, conforme estimativas do INCA, para cada ano do triênio 2020-2020 são esperados aproximadamente 625.000 novos casos no país. Os tumores malignos mais incidentes, excluindo-se os de pele não melanoma, são os de próstata, cólon e reto, pulmão, estômago e cavidade oral nos homens; e os de mama, cólon e reto, colo de útero, pulmão e glândulas da tireoide nas mulheres. Os responsáveis pelas maiores taxas de mortalidade são, respectivamente, os cânceres de pulmão, estômago, próstata, cólon e reto e mama.[30]

Nos países em desenvolvimento, é comum o diagnóstico tardio do câncer, momento no qual a doença se encontra já em estágio avançado.[2,3] Nesse sentido, ganha importância o cuidado paliativo, abordagem que enfatiza o cuidado global, ativo e multidisciplinar voltado aos pacientes cuja doença não apresenta mais resposta aos tratamentos modificadores disponíveis. Para tanto, prioriza-se o controle da dor e o alívio dos demais sintomas que o paciente possa apresentar, além de atuar também nas dimensões psicossociais e espirituais. Nessa fase, o objetivo principal é o de reduzir o sofrimento do paciente e melhorar a qualidade de vida do indivíduo e de sua família.[4,5,31] Estima-se que anualmente mais de 20 milhões de pessoas em todo o mundo necessitem desse tipo de assistência ao final da vida, sendo em sua maioria indivíduos acima de 60 anos de idade. O câncer representa a segunda maior causa da demanda pelos cuidados paliativos.[3,5]

A presença de células malignas resulta no desenvolvimento de alterações metabólicas que expõem o indivíduo ao risco nutricional.[6,7,32] A prevalência de desnutrição em pacientes com câncer varia conforme o tipo histológico do tumor, sua localização, o estágio da doença e o método de avaliação adotado para o diagnóstico nutricional.[32-34] O comprometimento do estado nutricional está diretamente associado à diminuição da MME, à deterioração da função

muscular e à redução da capacidade funcional, o que pode levar à piora da qualidade de vida.[35,36] Vale destacar que as alterações na composição corporal observadas em pacientes com câncer diferem das encontradas naqueles sem a enfermidade. Estas decorrem, principalmente, das várias alterações metabólicas ocasionadas pela interação tumor-hospedeiro, caracterizadas pela depleção preferencial dos depósitos proteicos.[6-9,11] Assim, a característica fenotípica clinicamente mais relevante da desnutrição associada ao câncer é a perda de MME, definida neste contexto como sarcopenia.[8,10-13]

1.2 **Sarcopenia e câncer**

Conceitualmente, a sarcopenia é uma síndrome geriátrica formalmente reconhecida como um distúrbio muscular caracterizado pela baixa força muscular associada às baixas quantidade ou qualidade da massa muscular.[37] Em pacientes com câncer, no entanto, o termo sarcopenia vem sendo utilizado como um sinônimo de depleção muscular grave.[8,12,13,38] Isso porque, particularmente no cenário oncológico, a massa muscular, além de consistir em um importante componente para identificação da caquexia,[8,39] é o principal fator associado a complicações do tratamento anticâncer, redução da qualidade de vida e mortalidade.[12,13,38,40] Ademais, em diversos tipos de câncer a reduzida massa muscular tem sido associada à diminuição da capacidade funcional, ao aumento do risco de morbidade pós-operatória e ao maior tempo de hospitalização.[40-42] Shachar et al. (2016),[43] em uma metanálise com 7.853 pacientes com tumores sólidos, descreveram a prevalência de sarcopenia avaliada por TC no nível de L3 variando de 11% a 69%, chegando a 74% em indivíduos com doença avançada.

O impacto da reduzida massa muscular foi avaliado em várias doenças malignas, incluindo câncer de ovário, pâncreas, mama, gastrintestinal, colorretal e de fígado. Pamoukdjian et al. (2018),[40] em uma meta-análise composta por 35 estudos que incluiu 6.894 pacientes com câncer, independentemente da localização tumoral, demostraram que a baixa massa muscular no período pré-tratamento se associou de forma significativa a complicações pós-operatórias, toxicidade induzida por quimioterápicos e menor sobrevida.

Entre os fatores envolvidos na gênese da depleção muscular em pacientes com câncer, os de maior relevância clínica são o estado pró-inflamatório crônico caracterizado por níveis elevados de citocinas, como a interleucina-6 e o fator de necrose tumoral alfa. Estas citocinas, além de induzirem perda de peso e diminuição do apetite, estimulam respostas metabólicas de fase aguda, caracterizadas pelo aumento de proteínas positivas de fase aguda como a proteína C-reativa (PCR) e pela redução de proteínas negativas de fase aguda, como a albumina e a

pré-albumina, causando aumento do catabolismo proteico.[6,9,44,45] Outros fatores relacionados com a doença *per se* ou as múltiplas opções de tratamento antineoplásico, como obstruções, má absorção de nutrientes, intervenções cirúrgicas ou toxicidade dos fármacos e presença de sintomas de impacto nutricional, como hiporexia, disfagia, odinofagia, mucosite, náuseas, vômitos, xerostomia, diarreia e alterações quimiossensoriais, entre outros, também contribuem para a redução da massa muscular nesse grupo de pacientes.[13,41,44,46] Considerando o exposto, a avaliação da massa muscular torna-se clinicamente relevante.

1.2.1 Métodos de avaliação da massa muscular

Os métodos de avaliação da massa muscular podem ser agrupados em dois níveis de análise: indireto e duplamente indireto. O primeiro inclui métodos validados a partir do método direto (pesagem dos diferentes constituintes do peso corporal por meio da dissecação de cadáveres), não havendo avaliação dos componentes corporais separadamente, mas a partir de princípios químicos e físicos que possibilitam sua quantificação; ou ainda a partir de outros métodos indiretos já validados. A análise do potássio corporal total, a excreção de creatinina urinária, a ultrassonografia, a RM, a DXA e a TC são exemplos de métodos indiretos.[47,48] Na prática clínica, o uso da maioria dessas técnicas torna-se inviável devido a seu alto custo e à necessidade de profissional treinado, além da exposição à radiação, como no caso da TC.[19] Desse modo, a utilização de técnicas duplamente indiretas, como a impedância bioelétrica e a antropometria, faz-se útil e aplicável na rotina clínica em hospitais e ambulatórios. Para que um método de avaliação da massa muscular tenha relevância clínica, ele deve reunir características como ser de fácil aplicabilidade e reprodutível, com boa acurácia, precisão e especificidade e que seja sensível às modificações corporais.

A avaliação da composição corporal por TC baseia-se na discriminação dos diferentes tecidos (músculo esquelético, tecidos adiposos subcutâneo, visceral e intramuscular) de acordo com os valores de radiodensidade de cada tecido estimados em unidades de Hounsfield (HU).[16-18,48] Além disso, permitem a avaliação da radiodensidade muscular, que fornece informações adicionais sobre o grau de infiltração de TA intramuscular.[25] A imagem do corte da L3 é um ponto de referência validado para tal fim. Nessa região, a massa muscular é avaliada nos músculos psoas, sacroileolombar, quadrado lombar, transverso abdominal, oblíquos interno e externo e reto abdominal.[15,16] A avaliação da composição corporal na região abdominal foi validada por Shen et al. (2004),[49] que demostraram em um estudo com adultos saudáveis que a região em L3 apresentou a melhor associação ao volume muscular

corporal total avaliado por RM. Em pacientes com câncer, Mourtzakis et al. (2008)[16] demostraram que a massa muscular da região da L3 apresentou forte associação à massa corporal magra avaliada por DXA.

O emprego da TC no corte de L3 tem se tornado uma medida de conveniência para a avaliação do paciente com tumores nas regiões pélvica e abdominal por ser rotineiramente utilizado para estadiamento tumoral e monitoramento da resposta ao tratamento. Esta técnica é reconhecida por ser de alta acurácia e reprodutibilidade, sendo, por isso, considerada padrão-ouro.[15,16,18,50] Apesar disso, apenas pacientes com determinados tipos tumorais são, geralmente, submetidos à TC com imagens disponíveis na região da L3 que possibilite a avaliação da composição corporal. Em virtude das peculiaridades relacionadas ao uso desse método, ele se limita para fins de uso em pesquisa científica.[15,19]

As medidas antropométricas são consideradas de baixo custo, pouco invasivas e de fácil aplicabilidade. Ademais, fornecem resultados rápidos, mas requerem treinamento adequado e cuidadoso de um examinador.[19] Para a avaliação da massa muscular por antropometria, algumas medidas foram descritas, como a circunferência da panturrilha (CP) e a área muscular do braço (CMB).[19,50] Vale mencionar, no entanto, que o uso de medidas antropométricas, especialmente nos pacientes com câncer avançado, pode apresentar algumas desvantagens, devido à influência de fatores não nutricionais que precisam ser considerados no momento da avaliação. Como por exemplo, as frequentes variações do estado de hidratação, decorrentes de edema, linfedema, ascite ou, ainda, alterações corporais provocadas por metástases e crescimento tumoral extenso.

De particular interesse, inúmeras equações provenientes de modelos de regressão que utilizam medidas antropométricas tem sido propostas na tentativa de predizer os valores de massa muscular.[21-24] A exemplo, Santos et al. (2019),[22] em um estudo utilizando dados do *National Health and Nutrition Examination Survey* (NHANES), composto por indivíduos norte-americanos saudáveis, demostraram boa correlação entre quatro modelos de equações compostas por medidas antropométricas e a massa muscular apendicular avaliada por DXA. Entre as equações, a que apresentou melhor resultado era constituída pelas variáveis CP, sexo, raça/cor da pele e idade. No estudo de Baumgartner et al. (1998),[23] em uma subamostra de 199 idosos saudáveis residentes no Novo México, os autores propuseram uma equação de predição da massa muscular apendicular utilizando as variáveis peso, altura, circunferência de quadril e força de preensão manual (FPM), tendo como medida de referência a massa muscular apendicular analisada por DXA. Al-Gindan et al. (2014),[24] tendo como medida critério a RM, propuseram equações de predição da massa muscular para indivíduos norte-

americanos sem problemas de saúde conhecidos utilizando as variáveis peso corporal e circunferência do quadril para mulheres e adicionando a tais medidas a circunferência da cintura para homens. Não há na literatura estudos que tenham desenvolvido e validado equações preditivas de massa muscular em pacientes com câncer.

As equações preditivas podem ser generalizadas ou específicas. Equações generalizadas podem ser utilizadas em indivíduos de ambos os sexos e diferentes faixas etárias; no entanto, a estimativa da massa muscular gerada por essas equações pode apresentar baixa acurácia devido às diferenças entre a amostra de origem da equação e as diferentes populações que a utilizam.[51] De acordo com Lohman (1981),[52] para o uso de equações generalizadas em populações específicas é necessário que elas sejam previamente validadas a fim de testar sua acurácia. Já as equações específicas são recomendadas a um grupo de indivíduos, como de acordo com a faixa etária, o nível de atividade física ou a presença de uma enfermidade específica. Para sua utilização, convém a amostra ter características similares às da população utilizada em sua validação.[51] O emprego de equações antropométricas pode ser, portanto, ferramenta de fácil aplicabilidade para a identificação da baixa massa muscular como parte dos critérios diagnósticos da sarcopenia, desde que seja validado para a população na qual será usada.

1.3 **Mioesteatose**

Em pacientes com câncer, além da baixa massa muscular, a infiltração de TA no músculo esquelético, denominada mioesteatose, também está associada a desfechos desfavoráveis.[28,42,53-57] Duas modalidades de deposição de TA podem ser identificadas no músculo esquelético, com papéis metabólicos distintos: (1) infiltração de TA intramuscular ou intramiocelular, representada por gotículas esféricas microscópicas no interior dos miócitos; e (2) infiltração de TA intermuscular, representada por filamentos de adipócitos extracelulares.[25,58,59] A primeira está prontamente disponível para ser utilizada como substrato energético durante o exercício, enquanto o TA intermuscular apresenta *turnover* mais lento e serve como local de armazenamento de energia de longo prazo.[25,60]

Os mecanismos envolvidos no desenvolvimento da mioesteatose em pacientes com câncer ainda estão pouco elucidados. A resposta inflamatória sistêmica associada a doença é um provável fator determinante da síntese e da eliminação de triglicerídeos no músculo esquelético.[61,62] Independentemente, acredita-se que a redução de massa muscular e a mioesteatose representam fenótipos clínicos distintos, mas com mecanismos sobrepostos, e

que alterações na composição muscular podem preceder a perda de massa muscular.[26-28] Ainda, as duas condições concomitantes – baixa massa muscular e mioesteatose – podem conferir efeito adicional no prognóstico clínico do paciente com câncer.[26]

A mioesteatose vem sendo reconhecida como um importante fator subjacente à qualidade do músculo, assim como um preditor da função muscular, independentemente da área muscular.[63,64] A explicação para esse achado possivelmente dá-se pelo fato de o excesso de TA no músculo diminuir a área contrátil dele, o que reduz o desempenho muscular.[25,58,65] Ademais, a mioesteatose também tem sido identificada como fator de risco independente para mortalidade.[53]

O método padrão-ouro para o diagnóstico da mioesteatose é a biópsia muscular. No entanto, por ser um exame invasivo, seu uso não é viável, mesmo no âmbito da pesquisa clínica.[25,60] Técnicas não invasivas baseadas em imagens de alta resolução, como TC e RM, apresentam especificidade para inferir indiretamente o conteúdo de TA muscular.[25,59] A TC tem sido uma ferramenta de pesquisa frequentemente utilizada para esse fim em pacientes com câncer. Contudo, uma limitação desta técnica é que ela não consegue diferenciar TA intramiocelular de TA intermuscular, este último presente em quantidade muito pequena no músculo.[25,60]

A avaliação da composição corporal pela técnica de TC baseia-se na medição da atenuação de raios X dos diferentes tecidos, que se refere ao valor médio de radiodensidade destes.[18,50,60] Os valores de radiodensidade são determinados pela velocidade com que a radiação passa através dos tecidos e são medidos e avaliados em HU, em que a água e o ar têm um valor de 0 HU e 1.000 HU, respectivamente.[59,60] Se por um lado a radiação passa mais lentamente através do tecido magro do que da água, dando ao tecido muscular um valor médio de radiodensidade em torno de +50HU, a radiação passa mais rapidamente pelo tecido gorduroso, de forma que a radiodensidade média do TA está em torno de –110HU.[18,59,60] Faixas de radiodensidade do tecido muscular (–29 a +150HU) e TA subcutâneo, intramuscular (–190 a –30HU) e visceral (–150 a –50HU) já são conhecidas.[16] Assim, a avaliação da mioesteatose por meio da TC parte da premissa de que o valor de radiodensidade do músculo reduz com o aumento da quantidade de TA intramuscular.[25] Diante do exposto, estudos que avaliem a composição corporal de pacientes com câncer por meio da TC justificam-se pelas elevada precisão e qualidade do método e por possibilitar a avaliação da radiodensidade muscular/mioesteatose.

2 JUSTIFICATIVA

Tendo em vista que a depleção muscular e a mioesteatose são condições frequentes em pacientes com câncer avançado e conhecendo-se ainda a influência destas anormalidades musculares sobre o prognóstico, fica clara a importância de se estudar medidas de composição corporal, especialmente o compartimento de massa muscular nesse grupo de indivíduos. Os métodos de referência disponíveis para a avaliação da massa muscular não estão sempre disponíveis, inviabilizando seu uso no ambiente hospitalar e/ou ambulatorial, bem como em estudos populacionais. Assim, o desenvolvimento de equações de predição utilizando medidas simples e de fácil obtenção constitui uma alternativa viável para a quantificação da massa muscular. Além disso, conhecer as variáveis associadas à mioesteatose, importante item subjacente à qualidade do músculo, contribuirá para melhorar a avaliação e a interpretação da qualidade do músculo esquelético. Essas análises facilitarão o planejamento de intervenções com o objetivo de diminuir anormalidades musculares e de promover melhor qualidade de vida em pacientes com câncer avançado em cuidados paliativos.

3 OBJETIVOS E HIPÓTESES

3.1 Artigo 1

Objetivo: desenvolver e validar uma equação de predição de massa muscular baseada em variáveis antropométricas e na força de preensão manual, utilizando a TC como método de referência.

Hipótese: variáveis antropométricas e a força de preensão manual podem compor uma equação de predição da massa muscular para utilização na prática clínica de hospitais e ambulatórios.

3.2 Artigo 2

Objetivo: investigar os fatores associados à radiodensidade do músculo esquelético avaliada por TC.

Hipótese

a) A idade, o sexo, a etnia e a composição corporal associam-se de forma significativa à radiodensidade do músculo esquelético.
b) Fatores intrínsecos ao câncer, como o sítio tumoral primário e o estágio do tumor, influenciam a radiodensidade do músculo esquelético.

3.3 Artigo 3

Objetivo: investigar a associação entre diferentes fenótipos do músculo esquelético derivados de uma combinação de baixa/alta massa muscular e ausência/presença de mioesteatose com resposta inflamatória sistêmica, capacidade funcional e sobrevida global.

Hipótese: a baixa massa muscular e a mioesteatose representam fenótipos clínicos distintos que, quando combinados, levam a pior prognóstico clínico.

4 MÉTODOS

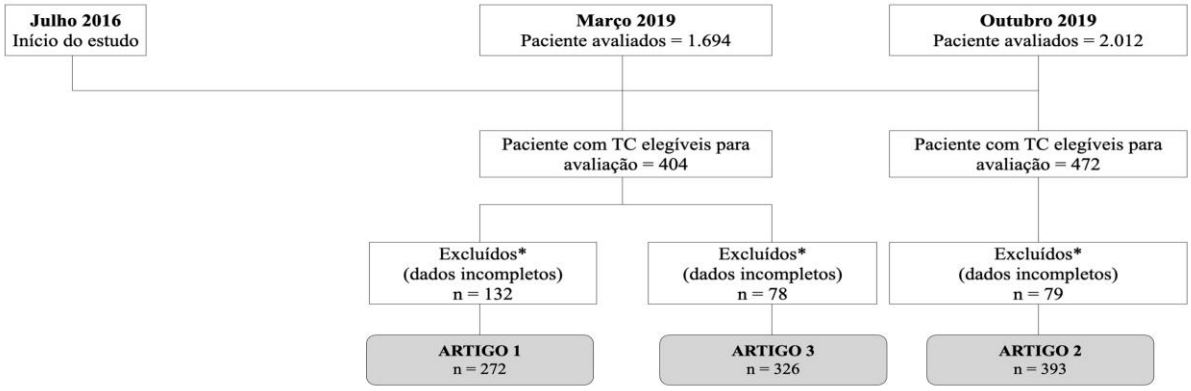
4.1 Delineamento e local do estudo

Trata-se de um estudo de coorte, com pacientes com câncer incurável atendidos na unidade de cuidados paliativos (HC IV) do INCA, na cidade do Rio de Janeiro (RJ).

4.2 Participantes da pesquisa

Foram incluídos adultos de ambos os sexos com diagnóstico histopatológico confirmado de neoplasia maligna incurável, independentemente da localização. Os pacientes foram avaliados na primeira consulta ambulatorial ou em até 48 horas após a primeira internação hospitalar, no período de 1º de julho de 2016 a 31 de outubro de 2019 e acompanhados para fins de análise de sobrevivência até 90 dias após a inclusão. O grupo de entrevistadores foi composto por nutricionistas e estudantes de Nutrição vinculados à instituição de ensino superior previamente treinados. Os seguintes critérios de inclusão foram adotados: idade maior que 20 anos e aqueles que apresentaram *Karnofsky Performance Status* (KPS) superior a 30% no momento da avaliação. Ademais, apenas os pacientes que possuíam imagens de TC das regiões pélvica e abdominal realizadas como rotina médica no intervalo de até 30 dias da data de inclusão na pesquisa foram selecionados. Foram excluídos do estudo indivíduos com doença de Alzheimer ou qualquer outra comorbidade que impossibilitasse o indivíduo de responder às informações necessárias para a pesquisa, que apresentavam amputação de algum membro, ou que possuíam dados incompletos para análise. O fluxograma do estudo encontra-se descrito na **Figura 1**.

Figura 1. Fluxograma de inclusão dos pacientes no estudo



Nota: TC= tomografia computadorizada
*O número de pacientes excluídos por dados incompletos nos Artigos 1, 2 e 3 variou a depender do objetivo central e das medidas necessárias para atender aos respectivos objetivos. A explicação detalhada segue descrita nos artigos.

A inclusão de cada indivíduo no projeto foi feita mediante autorização formal pela assinatura do termo de consentimento livre e esclarecido (TCLE) (Apêndice A**)**, feita após esclarecimentos sobre os objetivos e procedimentos do projeto, de acordo com a Resolução nº 466/2012 do Conselho Nacional de Saúde. O presente projeto faz parte de um estudo maior intitulado "Diagnóstico nutricional diferencial e qualidade de vida de pacientes com câncer avançado em cuidados paliativos", aprovado pelo Comitê de Ética em Pesquisa do INCA, conforme protocolo número 001488/2016 (Anexo A).

4.3 **Variáveis de análise**

4.3.1 Antropometria

As seguintes medidas antropométricas foram aferidas pelos pesquisadores:

a) *Peso corporal:* foi obtido de acordo com a metodologia proposta por Gordon et al. (1988).[66] Orientou-se o paciente a posicionar-se em pé, no centro da balança, sem sapatos e com roupas leves. Solicitou-se que ele distribuísse o peso corporal igualmente sobre os pés. A balança utilizada foi do tipo portátil digital (Wiso®, modelo 905, Brasil), com capacidade máxima de 180 kg e precisão de 100 g. Pacientes acamados, avaliados na internação hospitalar, foram pesados deitados em cama tipo balança (Stryker®, modelo Go Bed II, EUA).

b) *Estatura:* para quantificar a estatura (m), utilizou-se fita métrica fixada na parede. A medida foi realizada com o paciente em pé, descalço ou com meias finas, e com o mínimo de roupa possível para que a posição do corpo pudesse ser vista. Ele foi orientado a ficar em posição ortostática olhando para um ponto fixo na altura dos olhos (plano horizontal de Frankfurt), distribuir o peso igualmente entre os pés e manter os braços livremente soltos ao longo do tronco, com as palmas voltadas para as coxas. Cada indivíduo manteve os calcanhares unidos e tocando a parede. Solicitou-se que realizasse uma inspiração profunda e que se mantivesse em posição completamente ereta. O cursor do aparelho foi colocado sobre o ponto mais alto da cabeça com pressão suficiente para comprimir o cabelo.[66]

c) *Índice de massa corporal (IMC):* foi determinado pela divisão do peso pelo quadrado da estatura, o que resultou em um valor expresso em kg/m^2.

d) *Circunferência do braço (CB):* foi medida no braço dominante utilizando-se uma fita métrica inextensível (Sanny®, modelo TR-4010, Brasil) no ponto médio entre o

acrômio e o olecrano. Para a obtenção desse ponto, o indivíduo permaneceu em pé, com o braço flexionado em direção ao tórax, formando um ângulo de 90°. Com a fita, mediu-se a distância entre os dois pontos citados anteriormente, sendo que o ponto equidistante foi marcado. Para a obtenção da CB, o paciente manteve o braço relaxado e a fita contornou o ponto marcado de forma ajustada, porém evitando a compressão da pele. A leitura foi realizada no centímetro mais próximo. [67]

e) *Dobra cutânea tricipital (DCT):* foi obtida utilizando o adipômetro do tipo *Lange*® *Skinfold Caliper* (*Cambridge Scientific Industries Inc*.), que possui escala de 0 a 60 mm e resolução de 1,0 mm e mantém pressão constante de 10 g/mm^2. A medida foi determinada paralelamente ao eixo longitudinal do braço dominante, na face posterior, sendo seu ponto exato de reparo a distância entre o acrômio e o olécrano, seguindo a mesma técnica descrita para medida da CB. Para a medição, o tecido gorduroso foi levemente desprendido do tecido muscular e pinçado com o calibrador formando um ângulo reto exatamente no local marcado. O braço manteve-se relaxado e solto ao lado do corpo durante a medição.[68]

f) *Circunferência muscular do braço (CMB):* foi obtida por meio da equação proposta por Gurney & Jelliffe (1973),[69] que emprega as medidas de CB e DCT.

$$\text{CMB (cm)} = \{\text{PB (cm)} - [\pi \times \text{DCT (cm)}]\}$$

g) *Área muscular do braço corrigida (AMBc):* foi obtida por meio da equação proposta por Heymsfield (1982),[70] que emprega a CB e a DCT.

$$\text{Homens: AMBc (cm}^2\text{)}: \frac{\{\text{PB (cm)} - [\pi \times \text{DCT (cm)}]\}^2 - 10\ \text{cm}^2}{4\pi}$$

$$\text{Mulheres: AMBc (cm}^2\text{)}: \frac{\{\text{PB (cm)} - [\pi \times \text{DCT (cm)}]\}^2 - 6{,}5\ \text{cm}^2}{4\pi}$$

h) *Circunferência da panturrilha (CP):* foi realizada adotando-se a medida da maior circunferência (medida máxima no plano perpendicular à linha longitudinal da panturrilha), com o paciente sentado, os joelhos e tornozelos flexionados a um ângulo de 90° e os pés afastados 20 cm um do outro. Utilizando uma fita inextensível (Sanny®, modelo TR-4010, Brasil), a medida foi registrada no 0,1 cm mais próximo.[71]

i) *Circunferência do quadril (CQ):* foi realizada na região de maior proeminência entre a cintura e a coxa.[67]

4.3.2 Composição corporal

A análise da composição corporal foi realizada por meio da leitura da TC utilizando-se a imagem do corte L3. Para tanto, foram selecionadas as imagens no nível da L3 que exibiam mais claramente ambos os processos transversos vertebrais. A imagem selecionada tinha de ser de qualidade suficiente para analisar o músculo esquelético, o que significa: (1) sem artefatos; (2) nenhum corte do músculo; e (3) clara diferenciação entre músculo e tecido circundante.

A determinação das MME (músculos psoas, sacroileolombar, quadrado lombar, transverso abdominal, oblíquo interno e externo e reto abdominal) e adiposa (subcutânea, visceral e intramuscular) foi realizada utilizando-se o *software* Slice-O-Matic®, versão 5.0, (Tomovision, Montreal, Canadá), de acordo com os valores de radiodensidade de cada tecido estimada em HU, a saber: de –29 a +150 para MME, de –190 a –30 para TA subcutâneo (TASC) e intramuscular (TAIM) e de –150 a –50 para TA visceral (TAV).[16] As áreas transversais dos três tipos de TA foram calculadas automaticamente pela soma dos pixels. O TA total (TAT) refere-se à soma destes compartimentos. A radiodensidade do músculo esquelético também foi avaliada a partir da TC e derivada da média do valor HU da área muscular total.

Um pesquisador treinado realizou a leitura de todas as imagens e um segundo experiente avaliador revisou todas as leituras. Aproximadamente 10% das imagens também foram analisadas pelo revisor, e a variância interobservador para as medidas de MME, TASC, TAIM e TAV foram de 0,5%, 0,2%, 1,3% e 0,1%, respectivamente.

4.3.3 Força de preensão manual

A FPM foi avaliada a partir da medida da contração isomérica dos músculos da mão, com a utilização do dinamômetro hidráulico Jamar®, que possui escala de 0 a 100 kg e resolução de 2,0 kg. Para sua obtenção, os participantes foram orientados a permanecer sentados, com pés apoiados no chão, pernas levemente afastadas, joelhos flexionados a noventa graus e com os braços estendidos ao longo do corpo. O membro superior avaliado permaneceu com cotovelo em flexão de 90º, antebraço em posição neutra entre supinação e pronação.[72] Olhando para um ponto fixo, indicou-se que o paciente pressionasse o dinamômetro ao sinal do avaliador. A preensão manual foi realizada em ambos os braços e

repetida por três vezes, alternadamente, com intervalos de cerca de 1 minuto, sendo considerada a maior medida das seis contrações para representar a FPM.

4.3.4 Avaliação subjetiva global produzida pelo paciente

Foi utilizada a versão reduzida em português da Avaliação Subjetiva Global Produzida pelo Paciente (ASG-PPP), disponível em pt-global.org. A ASG-PPP é um questionário estruturado dividido em duas partes. A versão resumida do questionário utiliza somente a primeira parte, respondida pelo paciente com ou sem auxílio do cuidador responsável, e contém perguntas sobre alteração do peso corporal, história alimentar, presença de sintomas de impacto nutricional e avaliação da capacidade funcional. Ao final da avaliação, foi gerado um escore numérico total baseado no somatório de cada um dos itens do questionário (quanto mais alta é a pontuação, maior o risco nutricional).[73]

4.3.5 *Karnofsky Performance Status*

O KPS é utilizado para avaliar a capacidade funcional do paciente. Trata-se de uma escala percentual que classifica o indivíduo diante da capacidade de realizar trabalho ativo e autocuidado e necessidade de cuidados médicos regulares, devido à maior evidência de doença. O KPS possui 11 categorias, e cada uma é pontuada em 10%. Uma pontuação mais baixa indica pior função (100%: função completa – 0%: morte).[74] Os pacientes foram classificados com os valores de 30 a 100% pelo pesquisador no momento da avaliação do indivíduo.

4.3.6 Exames laboratoriais

As seguintes dosagens foram avaliadas: hemograma completo, albumina sérica e PCR. A coleta de sangue foi realizada, conforme rotina da unidade, por um técnico de enfermagem durante a consulta ambulatorial e, para os pacientes internados, pelo técnico de laboratório, em no máximo 48 horas após a internação hospitalar. Os resultados obtidos a partir de alguns parâmetros laboratoriais foram utilizados para avaliar a resposta inflamatória sistêmica. O escore prognóstico de Glasgow modificado (EPGm) foi determinado associando-se as concentrações séricas de PCR e de albumina. Tal classificação varia de 0 a 2, sendo albumina: ≥3,5 mg/dL e PCR <10 mg/L = 0, PCR >10 mg/dL = 1 e PCR >10 mg/dL e albumina <3,5

mg/dL = 2.[75] A relação neutrófilo-linfócito (RNL) e a relação plaqueta-linfócito (RPL) foram calculadas como as razões entre o número absoluto de neutrófilos e o de linfócitos; e entre o número de plaquetas e o de linfócitos, respectivamente.

4.3.7 Sobrevida global

A data do óbito por qualquer causa foi coletada no prontuário dos pacientes. A sobrevida foi definida como o tempo em dias contados a partir da data da inclusão do paciente no estudo até a data do óbito ou censura em 90 dias.

4.4 **Análises estatísticas**

Estão descritas nos artigos derivados da presente tese, uma vez que os testes estatísticos diferiram a depender do estudo e de seu respetivo objetivo. Para todas as análises foram considerados intervalo de confiança de 95% e $p<0,05$.

4.5 **Qualidade dos dados**

Elaboraram-se manuais com instruções para a aplicação de cada instrumento de coleta de dados empregado na pesquisa. Os pesquisadores, a saber, nutricionistas e estudantes de Nutrição, participaram de um treinamento realizado no INCA sob a supervisão dos coordenadores da pesquisa, com duração total de 24h. Tal atividade constou de uma etapa teórica com o estudo de manuais e publicações científicas pertinentes aos questionários utilizados. Em seguida, na etapa prática, os pesquisadores foram padronizados para a aplicação dos questionários e técnicas de acordo com a proposta de Habicht et al. (1974),[76] sendo avaliados segundo a exatidão e a precisão. A entrada de novos entrevistadores foi condicionada à realização de novos treinamentos.

Durante a coleta de dados, após as entrevistas, cada um dos formulários foi revisado imediatamente por quem o aplicou e posteriormente por um segundo revisor, visando a minimizar possíveis erros de preenchimento. Realizou-se um estudo piloto (n = 81) com o objetivo de testar os instrumentos elaborados para a coleta de dados. Foram propostas e executadas modificações nos questionários; e as técnicas de aferição e a logística, igualmente aperfeiçoadas.

5 RESULTADOS E DISCUSSÃO

Os itens Resultados e Discussão serão apresentados separadamente nos artigos propostos para publicação. Os artigos estão escritos em língua inglesa e apresentados conforme as normas dos respectivos periódicos aos quais foram ou serão submetidos, a depender do artigo em questão.

Artigo 1

Título: Development and validation of a predictive equation using anthropometry and handgrip strength to estimate muscle mass in patients with incurable cancer

Será submetido no periódico *Nutrition* (Fator de impacto 2019-20: 3,630)

Artigo 2

Título: Factors associated with skeletal muscle radiodensity in patients with incurable cancer

Será submetido ao periódico *Journal of Parenteral and Enetral Nutrition* (Fator de impacto 2019-20: 2,853)

Artigo 3

Título: Altered skeletal muscle phenotypes are associated with functional impairment, worse systemic inflammatory response and reduced survival in patients with incurable cancer

Submetido ao periódico *Clinical Nutrition* (Anexo B) (Fator de impacto 2019-20: 6,766)

5.1 **Artigo 1**: **Development and validation of a predictive equation using anthropometry and handgrip strength to estimate muscle mass in patients with incurable cancer**

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Highlights / Key points of this study:

- The predict equation using anthropometric and handgrip strength measures is a feasible and low-cost alternative for estimating muscle mass in patients with incurable cancer.
- The predicted equation showed a good agreement and was validated to estimate muscle mass.
- The estimated muscle mass showed good reproducibility in relation to CT-measured muscle mass

Abstract

Background & aims: Skeletal muscle mass (SM) of patients with cancer remains difficult to quantify in clinical practice due to the availability of methods that offer accurate measures and at the same time can be easily performed. The aim of this study was to develop and validate an equation using anthropometric and handgrip strength (HGS) measurements to estimate SM in patients with incurable cancer. **Methods:** This population-based cohort study was performed in the palliative care unit of the National Cancer Institute (Brazil). The sample comprised 272 patients with incurable cancer (68.0% female; median age of 60 years). The equation for estimating SM was developed by backward linear regression analysis using the SM obtained by CT as the dependent variable and the anthropometric measurements, HGS, demographic and cancer-related factors as independent variables. The equations were validated using bootstrapping methods, while the concordance and overall, 95% limits of agreement between the predicted equation and CT-measured SM were assessed using intraclass correlation coefficient (ICC) and Bland-Altman's plot analysis. **Results:** The model to estimate SM providing the highest R^2 included the variables sex, self-reported race/skin color, body weight, corrected arm muscle area (CAMA) and HGS, with an adjusted R^2 accuracy of 0.60 and the root mean square error of 5.82. The selected predictive equation was: **Muscle mass (cm^2) = 57.37 + weight (kg) x 0.38 + CAMA (cm) x 1.17 + HGS (kg) x 0.65 + 7.75 (black race/skin color) – 22.96 (female).** The concordance between the predicted and CT-measured SM was indicative of good reproducibility for the overall sample (ICC=0.85; 95% CI: 0.81-0.88) as well as in the groups stratified by sex, age, race/skin color, primary tumor site, and health care setting. **Conclusion:** The estimative equation developed in this study to predict SM in patients with incurable cancer was cross-validated and provided a reproducible measurement to infer the SM in this patient group. More studies testing its reproducibility in other similar groups are warranted.

Keywords: incurable cancer; muscle mass; computed tomography; anthropometry; handgrip strength

5.1.1 Introduction

Sarcopenia is recognized as an aging muscle disorder involving low muscle strength and low muscle quantity or quality [1,2]. However, in patients with cancer in particular, sarcopenia has been used as a synonym for severe muscle wasting [2,3]. In view of the fact that incidence of most cancer types increases with age [4], the etiology of muscle loss in oncologic patients can result from both age and, with greater strength, metabolic changes induced by the tumor's malignancy and its treatment [5].

Low skeletal muscle mass (SM) is one of the components used to diagnose cancer cachexia [6], and it is an important factor associated with cancer treatment complications, impaired quality of life, and increased mortality rates [5,7,8]. In addition, in several types of cancer, low SM has been associated with reduced functional capacity, increased risk of post-operatory morbidity, and longer hospital length stay [5,9].

The use of computed tomography (CT) at the third lumbar vertebra (L3) is known for its high accuracy and reproducibility for muscle mass assessment in oncologic setting and is considered a gold standard method for this purpose [5,10,11]. However, CT is a convenient measurement for evaluating body composition in patients with cancer, as it exposes individuals to radiation, so that its use is restricted to individuals undergoing the test for diagnosis and clinical monitoring [5,12].

Anthropometric measurements have been used as low-cost, non-invasive method of assessing SM in hospital and outpatient settings [12]. Many regression equations that use anthropometric measurements have been proposed in an attempt to predict SM values in healthy individuals [13,14,15]. However, to our knowledge, there are no studies that attempted to develop and validate an equation to estimate SM in patients with cancer. If validated, the main advantage of such equation would be to allow an easy assessment of SM to identifying sarcopenia and as an objective and robust marker for cachexia diagnosis. Therefore, the aim of this study is to develop and validate an equation for estimating SM in patients with incurable cancer based on easily assessable methods, such as anthropometric and handgrip strength (HGS) measurements, using CT at L3 as the reference method for the SM measurement.

5.1.2 Methods

5.1.2.1 Study population and data collection

The research ethics committee of the Brazilian National Cancer Institute José Alencar Gomes da Silva (INCA) in Rio de Janeiro approved the study (protocol number 1.407.458, of 2016), and all the participants signed the informed consent form.

This is a cross-sectional study that evaluated patients admitted to the palliative care unit at INCA, in Brazil, from July 1st 2016 to March 31st 2019. The cohort inclusion criteria were both sex, age equal or higher that 20 years, Karnofsky Performance Status (KPS) ≥30%, ability to provide the necessary information and histopathological confirmation of advanced-stage malignant cancer irrespective of the tumor site. Incurable cancer was defined as locally recurrent or metastatic cancer proven by histological, cytological, or radiological evidence, and not receiving any antineoplastic treatment with curative intent. The patients were assessed by trained researchers at their first outpatient consultation or within 48 hours of their first hospitalization in the unit.

Information on age, sex, and self-reported race/skin color were collected in interviews. The following data were collected from the medical records: extent of the disease (locally recurrent or distant metastasis) and site of the primary tumor, categorized into six groups according to the prevalence encountered in the sample: gastrointestinal tract, gynecological, bone and connective tissue, lung, breast, and "others" (less prevalent tumor sites).

Only patients for whom CT images in the third L3 region were available and with the anthropometric and HGS measurements needed to construct the models for the equations were considered for the development and validation of the equation for estimating SM. Baseline data were collected from 1,694 patients, of which 446 conduced CT scans up to 30 days before the date of the initial assessment. Forty-two scans were excluded due to not available body composition analyses at the level of L3, in addition to 132 patients due to missing data regarding anthropometric measurements, leaving 272 patients for the present analyses. Since only 16.0% of the patients fulfilled all these criteria, this sub-sample was weighted to assure coherence with the total number of patients assessed in the study.

5.1.2.2 Computed tomography as reference method for skeletal muscle mass assessment

CT images at the third lumbar vertebra (L3) was used as reference method for the assessment of SM. For this purpose, CT scans taken routinely for clinical reasons up to 30 days before the patients' inclusion in the study were used. One trained researcher analyzed the images and all readings were subsequently reviewed by an experienced evaluator. Approximately 10% of those images were also analyzed by the reviewer and the inter-observer concordance was below 1.0% (inter-observer intraclass correlation coefficient, ICC= 0.99; 95% CI: 0.99-0.99). The images that showed most clearly both transverse processes were selected to be analyzed. These images had to be of quality high enough for the assessment of skeletal muscle, which meant to show: (1) no artifacts, (2) no muscle out of field of view, and (3) clear differentiation between muscle and surrounding tissue. Muscle area (psoas, erector spinae, quadratus lumborum, transversus abdominus, external and internal obliques, and rectus abdominus) was determined using sliceOmatic, version 5.0 (Tomovision™, Canada), according to the attenuation values of the tissue, estimated using the

scale of Hounsfield units (from -29 to +150). SM obtained by CT was used as the reference measurement for development and validation of the predictive equation.

5.1.2.3 Muscle strength

Muscle strength was assessed by handgrip strength (HGS) using a Jamar® hydraulic hand dynamometer, with a range of 0 – 100 kg and 2.0 kg resolution. Each participant was instructed to sit in a chair with their feet on the floor, their knees flexed at 90° and their legs slightly apart. They were asked to look at a fixed point and to squeeze the dynamometer when indicated by the evaluator. Three trials were performed per hand, alternating the hands, with one-minute rest intervals. The highest of the six measurements was used to represent the HGS.

5.1.2.4 Anthropometric measurements

All the anthropometric assessments were performed by trained nutritionists. Body weight was measured using a portable digital scale (Wiso®) with 180 kg capacity. For patients unable to stand, the Stryker® GoBed II in-bed weight system was used. Height was measured using a tape measure fixed to the wall. When this could not be used, height was taken from the medical records or as self-reported by the patient. Body-mass index (BMI) was determined by dividing weight by height squared.

Calf circumference (CC, cm), hip circumference (cm) and arm circumference (cm) were measured using an extending tape measure (Sanny®, TR-4010, Brazil) and measured to the nearest 0.1 cm. CC was determined by measuring the largest perimeter (maximum measurement in the perpendicular plane to the longitudinal line of the calf), with the patient sitting with knees and ankles flexed at a 90° angle. Hip circumference was determined at the level of the pubic symphysis by measuring the widest girth of the hip. Arm circumference of the dominant arm was measured at the midpoint between the acromion and olecranon processes. Triceps skinfold thickness (TSF, mm) was assessed using a Lange® skinfold caliper (Cambridge Scientific Industries Inc.) and determined parallel to the longitudinal axis of the dominant arm, on the posterior surface, at the same point as used to measure arm circumference. Arm muscle area (AMA, cm) was obtained applying the equation proposed by Gurney & Jelliffe [16], using the arm circumference and TSF measurements. Corrected arm muscle area (CAMA, cm^2) was calculated by correcting the AMA for the bone area, according to the equation proposed by Heymsfield et al. [17].

5.1.2.5 Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution of the variables. Descriptive statistics (number / frequency [%], mean ± standard deviation, or median and interquartile interval, as appropriate) were used to depict the patients' characteristics. The differences between the included and not included patients were assessed by the Mann-Whitney U test for the continuous variables and by the chi-squared test for the categorical variables.

Part 1- Equation Development

The regression equation for estimating SM was developed by backward analysis using the SM obtained by CT as the dependent variable and anthropometric measurements (weight, height, BMI, CC, hip circumference, AMA, CAMA and TSF), as well as HGS, sex, age, race/skin color, primary tumor site, and tumor stage as the independent variables. Calibration

techniques (i.e., weighted linear regression) were used to assure coherence with the total number of patients evaluated in the study (n=1,694), according to sex, age (<60 years vs. □60 years), self-reported race/skin color (white, black, and mixed) and primary tumor site. The coefficient of determination (adjusted R^2) and root mean squared error (RMSE) were used to compare different models and determine the most precise model to be used for the prediction.

Part 2 - Validation tests

For the validation of the predictive equation, the bootstrap cross-validation method was employed to randomly resampled (with replacement - 10,000 replications) the individuals included in the study and recompute the model on these resamples. The model validity was assessed by comparing the average adjusted R^2 values from the resampled data to the original data. Additionally, the RMSE of the new equation was assessed with the same size of the dataset. Of note, since the bootstrap method is a resampling technique, the new data sets are produced with the same number of cases as the original data [18].

As part of the validation assessment, the ICC was used to test the reproducibility of the prediction equation in comparison to CT-measured SM. The equations' performance in different groups according to sex, age, race/skin color, primary tumor site, and health care setting was also evaluated. Values lower than 0.50, between 0.50 and 0.75, between 0.75 and 0.90, and greater than 0.90 were used as indicative of poor, moderate, good, and excellent reproducibility, respectively [19]. Pearson's association test was used to investigate the univariate association between of the prediction equation and the CT-measured SM. The Bland-Altman plots analysis were used to identify the bias and the concordance between the measured and estimated SM, with the limits of concordance being defined as the mean of the differences ±1.96 standard deviation. The statistical analyses were conducted using R, version 4.0.1 (The R-Project for Statistical Computing) and SPSS, version 21.0 (SPSS, Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

5.1.3 Results

The comparison of the baseline characteristics between included and not included patients are shown in **Table 1**. The group of the included patients was younger, had a higher proportion of females and gynecological tumors and a lower proportion of patients who self-identified themselves as white race/skin color than the group of not included patients. No difference was observed between the groups regarding performance status and anthropometric measurements. The included patients had median age within the 6th decade of life and about 70% of the sample were outpatients. The most common tumor site was those located in the gastrointestinal tract, followed by gynecological and breast tumors sites.

Results for the reproducibility to predict SM by the equation is presented in **Table 2**. The final model included the variables sex, race/skin color, body weight, CAMA and HGS, with the adjusted R^2 of 0.60 and the RMSE of 5.82. Of note, the model was not improved by the inclusion of age as continuous or dichotomized variable. The best predictive equation was:

Muscle mass (cm^2) = 57.37 + weight (kg) x 0.38 + CAMA (cm) x 1.17 + HGS (kg) x 0.65 + 7.75 (black race/skin color) – 22.96 (female)

The bootstrapping cross-validation analysis based on 10,000 replications showed that the predicted equation showed a good validity, given that the R square (0.59) and the root mean square error (5.90) were very similar to those found in linear regression models.

The ICC was indicative of good degree of reproducibility between the predicted and the CT-measured SM for the overall group (ICC=0.85; 95% CI: 0.81-0.88) and according to the groups of sex, age, race/skin color, tumor site, and health care setting (**Table 3**). The average difference for the overall group was of 0.73 cm^2 (1.96 SD= -29.88; 30.34), with a

maximum value of 30% in relation to the CT-measured SM. In addition, the predicted SM values from the proposed equation were correlated with those from CT measurements (Pearson's r=0.580, p<0.001 for male; Pearson's r=0.617, p<0.001 for female).

Individual agreement between the predicted and the CT-measured SM was evaluated using the Bland-Altman plot (**Figure 1**). There was a positive association between the difference (y-axis) and the average (x-axis) of the measured and the prediction equations (r= 0.519; p<0.001), indicating that the difference increased as individual average increased. The individual variability shown in these graphs indicates the existence of patients whose SM values were both underestimated and overestimated, even though the average difference was positive.

Table 1 - Baseline characteristics of the included and not included study patients.

Variables	**Included (n=272)**	**Not included (n=1,422)**
Age (years)[a]	60 (50-67)	63 (55-72)*
Age (≥ 60 years)[b]	138 (50.7)	898 (63.2)*
Female sex [b]	185 (68.0)	806 (56.7)*
Race/skin color[b]		
White	97 (35.7)	640 (45.1)*
Black	54 (19.9)	254 (17.9)
Mixed	121 (44.5)	528 (37.1)*
Health care setting (outpatient)[b]	198 (72.8)	1082 (76.1)
Tumor site [b]		
Gastrointestinal tract[c]	93 (34.2)	413 (29.0)
Gynecology[d]	76 (27.9)	217 (15.3)*
Breast	29 (10.7)	167 (11.7)
Lung	24 (8.8)	148 (10.4)
Bone and connective tissue	28 (10.3)	110 (7.7)
Others[e]	22 (8.1)	367 (25.8)*
Tumor stage		
Locally recurrent	37 (13.7)	223 (15.7)
Metastatic	235 (86.3)	1199 (84.3)
KPS[a]	50 (40-60)	50 (40-60)
Body weight (kg)[a]		
Male	60.6 (53.0;71.0)	58.9 (51.8;69.0)
Female	57.6 (47.7;68.9)	56.0 (46.0;65.8)
Height (cm)[a]		
Male	169 (163;175)	168 (163;172)
Female	157 (152;161)	155 (151;160)
BMI (kg/m²)[a]		
Male	21.9 (19.2;24.6)	21.0 (18.4;24.5)
Female	23.8 (19.7;27.9)	22.8 (19.0;26.8)
Calf circumference (cm)[a]		
Male	32.6 (30.5;34.5)	32.0 (29.0;35.0)
Female	32.0 (29.5;35.8)	31.0 (29.0;34.4)
Arm muscle area (cm)[a]		
Male	22.4 (20.7;24.1)	21.7 (19.7;23.9)
Female	20.3 (18.3;22.8)	20.1 (18.0;22.7)
Corrected arm muscle area (cm^2)[a]		
Male	30.2 (24.3;37.3)	27.8 (21.2;36.3)
Female	26.5 (20.5;34.6)	25.8 (19.4;34.5
Triceps skinfold (mm)[a]		
Male	8.0 (5.6;13.0)	8.3 (5.3;12.3)
Female	16.0 (10.5;23.5)	15.3 (9.6;22.0)
Hip circumference (cm)[a]		
Male	92.0 (88.5;96.0)	91.0 (86.0;97.0)
Female	94.0 (87.0;105.2)	94.0 (85.5;102.0)
Handgrip strength (kg)[a]		
Male	25.0 (20.0;31.0)	24.0 (18.0;30.0)

Table 1 - Baseline characteristics of the included and not included study patients.

Variables	Included (n=272)	Not included (n=1,422)
Female	15.0 (10.5;18.0)	14.0 (10.0;19.2)

Note: BMI= body mass index; KPS= Karnofsky Performance Status; n= number of observations.
[a]Median/interquartile ranges (p25-p75); Mann-Whitney; [b]Number of observation/frequency; chi-square; [c]upper and lower gastrointestinal tract; [d]cervix, uterus, endometrium, ovary and vulva; [e]head and neck, kidney and urinary tract, male reproductive system. *Statistically different from included patients

Table 2 - Equations for prediction skeletal muscle mass estimated by computed tomography in patients with incurable cancer (n=272).

Variable	Coefficient	95% CI
Intercept	57.37	45.46;73.00
Female sex	– 22.96	-26.28;-17.00
Black race/skin color	7.75	0.80;10.17
Body weight (kg)	0.38	0.18;0.51
Corrected arm muscle area (cm^2)	1.17	0.43;2.05
Handgrip strength (kg)	0.65	0.46;0.99

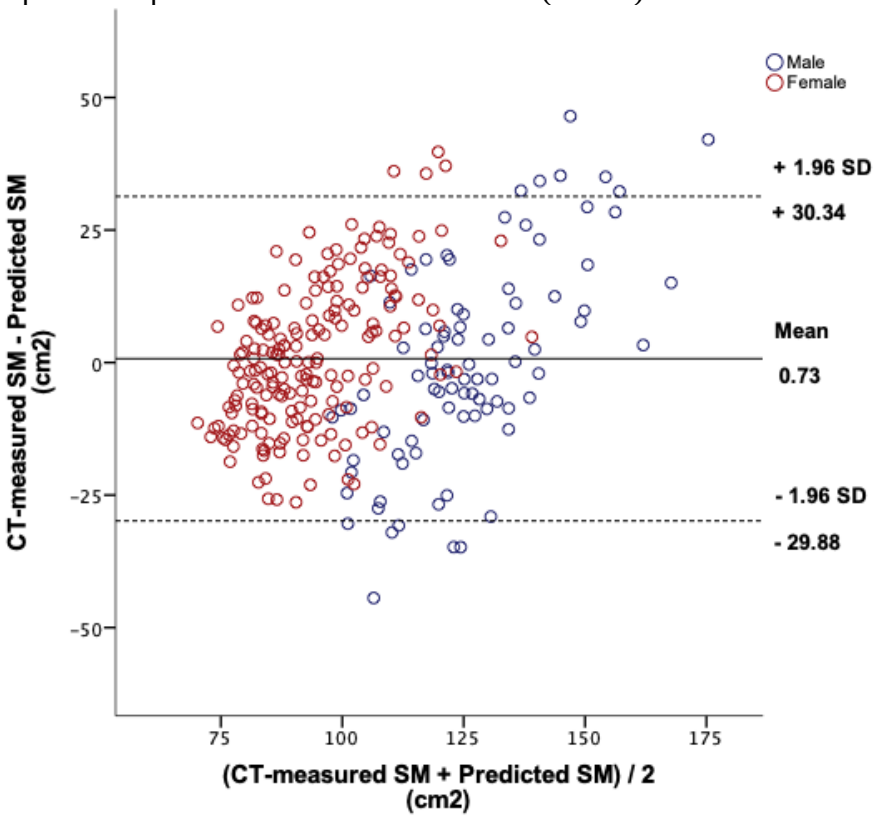
Note: CI= confidence interval. Equation R^2= 0.60

Table 3 - Concordance between CT-measured skeletal muscle mass and predict equations in the entire sample and according to groups by sex, age, primary tumor site and health care setting in patients with incurable cancer (n=272).

Equation	Delta (CT-measured – Predicted SM) cm^2	1.96 SD	ICC	95% CI
Overall sample	0.73	-29.88;31.34	0.85	0.81;0.88
Sex				
Male	-0.04	-37.71;37.63	0.79	0.69;0.88
Female	1.09	-25.66;27.84	0.82	0.73;0.89
Age (years)				
<60	1.96	-27.10;31.02	0.88	0.83;0.91
□60	-0.46	-31.44;30.52	0.82	0.74;0.87
Race/skin color				
White	-0.75	-31.05;30.55	0.82	0.73;0.88
Black	-1.09	-31.86;30.68	0.89	0.82;0.94
Mixed	2.77	-26.51;32.05	0.84	0.78;0.89
Primary tumor site				
Gastrointestinal tract	3.01	-25.70;30.72	0.88	0.82;0.92
Gynecological	-0.54	-29.52;28.44	0.83	0.77;0.93
Breast	2.05	-23.76;27.86	0.76	0.62;0.88
Lung	-1.51	-31.12;28.10	0.84	0.80;0.88
Bone and connective tissue	-1.57	-33.94;30.80	0.85	0.81;0.89
Others	3.92	-26.46;32.30	0.81	0.61;0.91
Health care setting				
Outpatient	0.52	-30.82;31.86	0.84	0.79;0.88
Inpatient	1.28	-27.49;30.05	0.87	0.80;0.92

Note: CI= confidence interval; CT= computed tomography; ICC= intraclass correlation coefficient; SD= standard deviation; SM= skeletal muscle mass

Figure 1 - Bland-Altmann plot analysis between skeletal muscle mass measured by computed tomography and predicted by equation in patients with incurable cancer (n=272).



Note: CT= computed tomography; SM= skeletal muscle mass (cm2); SD= standard deviation

5.1.4 Discussion

In this study, were developed and validated a simple equation with the use of anthropometry and HGS to predict SM in patients with incurable cancer using adequate statistical approaches. Given the feasibility and low-cost of anthropometric and HGS measures in clinical and epidemiological setting, we proposed an equation based on the variables sex, race/skin color, body weight, CAMA and HGS, which were able to explain about 60% of the variance in SM. Overall, the proposed equation had moderate predictive ability to assess muscle mass.

Several studies have already developed anthropometric prediction equations for estimating SM [13,14,15]. However, to the best of our knowledge, there are no published studies in which this has been explored in patients with incurable cancer. The routine assessment of SM in the oncological setting is of great importance since its reduction plays an important role in predicting clinical outcome [5,7,8]. In this sense, equations based on anthropometric and HGS measurements are suitable since it can be done quickly, requires modest training, and can be easily implemented in routine of most outpatient clinics and hospitals. [14,20,21]. Despite its advantages, one should be alert with factors that can influence the results coming from anthropometric measurements such as the presence of edema, lymphedema, ascites, and extensive tumor growth [22].

The model developed in this study included measurements for general body assessment (body weight) and localized distribution of SM (CAMA and HGS), in addition to sex and race/skin color. The latter two are especially important for the estimation of SM due to the considerable variations in body composition between different sex and race/skin color groups [5,12]. Interestingly, SM did not vary according to age. Although advanced age is the main independent factor related to sarcopenia in healthy people, in the current the study it is possible that the age range was not very wide to a point of exerting an influence on SM.

The use of CAMA for assessing SM in patients with cancer has been already documented in scientific literature [23,24] and the international consensus of cancer cachexia state AMA as an alternative SM measurement [6]. A previous study in patients with colorectal cancer identified that compared to CT, the M [25]. In addition, a cohort of patients with gynecological cancer reported that AMA significantly correlated with SM evaluated by total-body potassium [24 23].

The usage of HGS in prediction equation to estimate SM has already been documented in non-cancer individuals [26]. This technique is frequently used and validated method of easy applicability that allows to make predictions regarding key health indicators, such as functional capacity and integrity, especially in patients with cancer where malnutrition and cachexia are prevalent [27,28]. Noteworthy, the SM is recognized as a feature that impact muscle strength in patients with cancer [9] and the inclusion of HGS and CAMA in combination in the same equation may represent a good strategy for assessing localized muscle mass, that is, SM compartment.

In the present study, the coefficient of determination obtained for the predictive equation presented a moderate accuracy of 60%. However, a low R squared may not necessarily indicate poor performance, once the RMSE can also provide information of

estimated accuracy [29]. Of note, this study reported a relatively modest prediction error of ± 5.82 and the RMSE across the 10,000 bootstrapped samples was nearly identical to those for the regression results, which represents a good validity of the model. Furthermore, the ICC indicated a good reliability of the predict equation for the overall sample and also when analyzed separately for the different groups of sex, age, race/skin color, primary tumor site, and health care setting. Altogether, these results are of clinical relevance as they provide a validated and reproducible method that, although of moderate accuracy for predicting SM, can be routinely used in clinical practice without the need for any imaging assessment or specialized equipment. The only necessary instruments include scale, measuring tape, skinfold caliper and HGS that combined in a single equation can provide a better estimate of the patient's SM than the use of the of anthropometric measurements separately.

One strength of this study is the use of CT as the reference measurement to assess SM, a method recognized for its high accuracy to evaluated body composition in patients with cancer. To our knowledge, this is the first study in patients with cancer to develop an equation using a precise technique of CT as a reference method. In addition, although only patients with CT available from the trunk for analysis at L3 were evaluated in the study, which favored the inclusion of those with gastrointestinal and gynecological tumors, the sample were weighted according to the primary tumor site to assure coherence with the total number of patients assessed in the study. Limitations of the study includes the sample size and the lack of studies that estimating SM using anthropometry in cancer individuals, which limited our capacity to compare equations. In addition, measurements such as waist and thigh circumferences, which may contribute to the explain SM variability, were not evaluated.

5.1.5 Conclusion

In conclusion, a simple equation including sex, race/skin color, body weight, CAMA and HGS was developed and cross-validated to predict SM for patients with incurable cancer, providing a reproducible method for inferring SM that will be of significant value for clinical use, despite its moderate accuracy. The commonly measured variables included in the model are much cheaper and easier to collect then imaging or other specializes methods, been as alternative that requiring only proper training. Other studies testing its reproducibility in other similar groups are warranted.

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5.2 Artigo 2: Factors associated with skeletal muscle radiodensity in patients with incurable cancer

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Abstract

Background & aims: Investigating the characteristics associated with skeletal muscle radiodensity (SMD) in patients with cancer is important due to its association with adverse outcomes in distinct tumors. Therefore, the aim of this study was to explore the factors associated with SMD including cancer-related ones, in patients with incurable cancer. **Methods:** This study included 393 patients (median age 61 years, 69.7% female) who had abdominal or pelvic computed tomography (CT) scans up to 30 days before inclusion in the study. SMD was evaluated from CT by averaging the Hounsfield HU value obtained from the total muscle area. Body composition cross-sectional areas (cm^2) were also assessed by CT and normalized for square height (m^2) to obtain skeletal muscle index (SMI), visceral adipose tissue index (VATI), subcutaneous adipose tissue index (SATI) and total adipose tissue index (TATI). In addition, age, sex, race/skin color, disease characteristics, comorbidities,

inflammatory markers, handgrip strength (HGS) and body mass index (BMI) were also recorded and evaluated in a linear regression analysis to identify characteristics associated with low SMD. **Results:** Multivariate explanatory models were performed and included BMI (Model 1, r^2= 0.668), TATI (Model 2, r^2= 0.682) or VATI and SATI (Model 3, r^2= 0.673) in addition to age, white race/skin color, serum albumin, HGS and SMI. For all models, lower SMD was associated with higher age and adiposity measurements, white race/skin color and lower serum albumin, HGS, and SMI. The primary tumor site also contributed to changes in SMD in all three models, specifically those located in the gastrointestinal tract (directly associated), gynecological and bone and connective tissue (inversely associated). **Conclusion:** SMD was associated to age, race/skin color, albumin levels, HGS and body composition markers. In addition, gastrointestinal tract, gynecological and bone and connective tissue tumor sites further explained the variation in SMD. These findings can be used to target therapeutic interventions on the modifiable risk factors in order to maintain SMD as cancer stages advances.

Keywords: incurable cancer; muscle mass; computed tomography, skeletal muscle radiodensity

5.2.1 Introduction

Myosteatosis is the process of lipid infiltration into both inter- and intramyocellular compartments that is inferred as the presence of reduced skeletal muscle density (SMD) [1-4]. Low SMD is a common finding in patients with incurable cancer and has been recognized as an important factor underlying muscle quality [1,5]. Previous studies have shown an association between low SMD and adverse outcomes in distinct tumors, such as decline in muscle function and reduced survival, with independent prognostic value from skeletal muscle mass [1,6-9].

Muscle biopsy is the gold standard method for assessing SMD; however, as this is an invasive procedure, alternative methods are preferred, even in the context of clinical research [2,10]. In this regard, computed tomography (CT) confers some advantages. Although it exposes the patient to radiation, CT is a non-invasive technique based on high-resolution images that exhibit good accuracy to infer muscle lipid content by assessing the SMD in Hounsfield units (HU), where low values of SMD indicate greater intramuscular lipid infiltration [2,10-14]. Moreover, CT can be used as an opportunistic tool in oncologic patients that have these images for diagnostic purposes and / or for treatment follow-up [13, 14].

In non-cancer patients, several factors are associated with low SMD, including advanced age [15], obesity [16], insulin resistance [17], and sedentarism [18]. In patients with cancer, low SMD has been associated with increasing age [22,23], Caucasian ethnicity [22], low physical function [24,25], and altered systemic inflammatory response [23,25,26]. However, cancer intrinsic factors, such as the primary tumor site and the presence of metastatic disease have not yet been well investigated in the context of factors affecting SMD. If we consider the novelty aspects of SMD in oncologic patients, observational studies

exploring factors associated with this measurement are important to provide a better understanding of alterations in this body composition parameter. Hence, the purpose of the present study is to examine the factors associated with SMD in patients with incurable cancer.

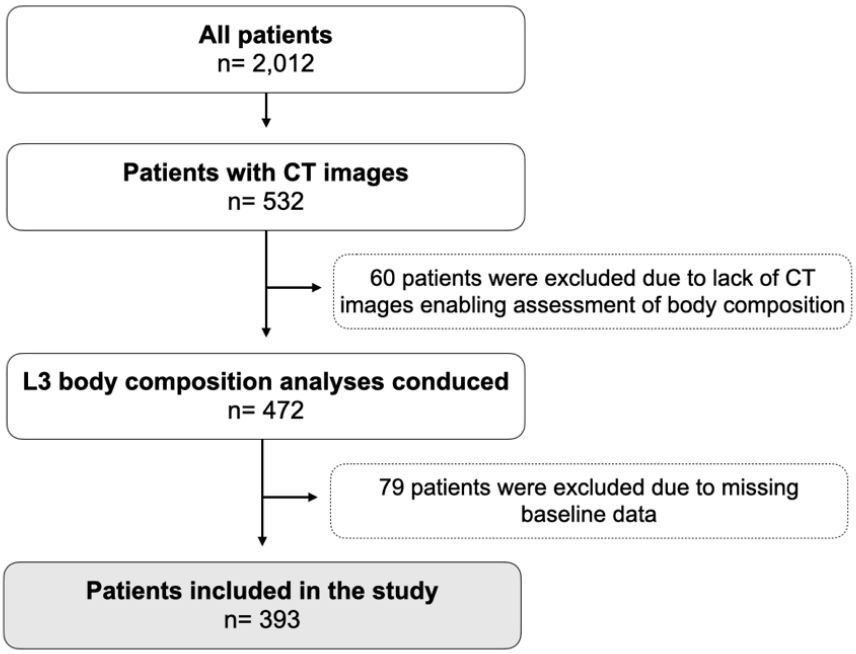
5.2.2 Methods

5.2.2.1 Patients and data collection

The Research Ethics Committee of the National Cancer Institute (protocol number 1.407.458 of 2016) approved the study, and all subject provided informed consent before inclusion. From July 2016 to October 2019, all consecutive inpatients or outpatients referred to the palliative care unit of the National Cancer Institute (Rio de Janeiro, Brazil) were registered. The inclusion criteria were as follows: 1) incurable cancer, defined as locally recurrent or distant metastatic cancer proven by histological, cytological, or radiological evidence, and not receiving any antineoplastic treatment with curative intent; 2) aged 20 years or older; 3) Karnofsky Performance Status (KPS) ≥30% and; 4) abdominal or pelvic CT scans up to 30 days before the initial assessment. From a total of 2,012 patients (63.3% female; median age of 62 [Interquartile range, IQR 52-69]) registered during the study period, 532 performed CT scans. Of these, 60 patients were excluded due to lack of CT images enabling assessment of body composition, and 79 due to missing baseline data. Therefore, 393 patients had complete data and were included for the current analysis (**Figure 1**). The baseline characteristics of the included and not included patients were compared. The included patients were younger and had a higher proportion of females and gynecological tumors than the not included patients, but no difference was observed between the groups regarding metastatic disease, inflammatory response (C-Reactive protein (CRP), albumin and neutrophil/lymphocyte ratio) and performance status evaluated by KPS and ECOG-PS (Eastern Cooperative Oncology Group Performance Status) (data not shown).

Information on sex, age and race/skin color were collected in interviews. The primary tumor site, the extent of the disease (locally recurrent or distant metastasis), and comorbidities (diabetes mellitus and systemic arterial hypertension) were obtained from medical charts. The primary tumor site was categorized as gastrointestinal tract, gynecological, bone and connective tissues, lung, breast, and others (less prevalent tumor sites, which includes head and neck, kidney and urinary tract and male reproductive system).

Figure 1 - Patients flow chart



Note: CT= computed tomography; L3= third lumbar vertebra

5.2.2.2 Body composition

The CT images obtained for diagnostic or staging purposes from the abdomen and pelvis were used to evaluate body composition, including skeletal muscle (SM), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and intermuscular adipose tissue (IMAT) (cm^2). Images were analyzed at the third lumbar vertebra (L3) using Slice-OMatic Software, version 5.0 (Tomovision™, Canada) by a trained examiner who was blinded to the outcome assessment. CT Hounsfield unit (HU) thresholds for each tissue were −29 to 150 for SM, −150 to −50 for VAT, and −190 to −30 for SAT and IMAT. Total adipose tissue (TAT) was calculated as the sum of the cross-sectional area of VAT, SAT and IMAT. The interobserver coefficient variations of SM, VAT, SAT and IMAT were 0.5%, 0.1%, 0.2% and 1.3%, respectively.

The sum of overall SM area (cm^2) obtained by the L3 scan was normalized for height (m^2) and reported as lumbar SM index (SMI) (cm^2/m^2). Similarly, the adipose tissue measurements were also normalized for height to calculate VAT index (VATI), SAT index (SATI) and TAT index (TATI). SMD was evaluated from CT by averaging the HU value of the total muscle cross-sectional area, derived from the density of each pixel of the total skeletal muscle area. SMI and adipose tissue measures were divided into tertiles for statistical analysis.

5.2.2.3 Anthropometric measurements

Body weight (kg) was measured using a Wiso® Digital portable scale (W905, Brazil, 180 kg capacity). For hospitalized and bedridden patients, a bed-scale system (Stryker®, model Go Bed II, USA) was used. The percentage of weight loss (%WL) in 6 months was calculated based on current measured body weight and patient-reported body weight from the past 6 months. Values >5% were recorded as high %WL [24]. For height (m), an inextensible tape fixed to the wall was used. When the patient was unable to stand, height was taken from the medical records or as reported by the patient. Body mass index (BMI; kg/m^2) was calculated as the body weight divided by the height in square meter (m^2). Values of BMI <20.0 kg/m^2 were recorded as underweight; 20.0 to 24.9 kg/m^2, normal weight; and □25.0 kg/m^2, overweight/obese.

5.2.2.4 Muscle strength

Muscle strength was assessed by handgrip strength (HGS) in dominant and nondominant hand with a Jamar® handheld hydraulic dynamometer (Baseline, Fabrication Enterprises, Inc, Elmsord, NY, USA). During testing, subjects were instructed to sit comfortably with shoulder adducted and neutrally rotated fore arm, elbow flexed at 90 degrees and, in sequence, perform a maximal isometric contraction to first one hand and then the other. The measurements were repeated three times in each hand, with a 1-minute rest interval, and the highest one was recorded for the study. HGS values were divided into tertiles for statistical analysis.

5.2.2.5 Laboratory assessments

A routine laboratory analysis was performed on the day of enrollment at the palliative care unit. A single intravenous blood sample was collected to analyze serum albumin, CRP, and a complete blood cell count. The neutrophil/lymphocyte ratio (NLR) was calculated by dividing the absolute count of neutrophils by the number of lymphocytes in the complete blood count. The cutoff value used to define presence of systemic inflammation was defined as CRP >10 mg/L, albumin <3.5 mg/dL [25,26], and NLR > 5 [27].

5.2.2.6 Statistical analysis

Kolmogorov-Smirnov test was performed to assess the variable's distribution normality. Descriptive statistics [count/frequency (%), means ± standard deviation (SD), or median and interquartile ranges (IQR), as appropriate] were used to depict the studied variables. Differences in SMD mean values between groups were tested by independent *t* test (up to two independent groups) or ANOVA (more than two independent groups). Univariate analysis was performed to identify the factors associated with SMD and the variables with a p value ≤ 0.20 were included in the multivariate analysis. Twelve final models were derived through multiple regression analysis using the stepwise procedure. To assess the presence of association between independent variables, the collinearity test was used with variance inflation factors (VIFs); VIFs greater than 5 was used to identified strong collinearity [28]. Statistical analysis was processed using the SPSS software version 21.0 (SPSS, Chicago, IL, USA). A p value <0.05 was considered statistically significant.

5.2.3 Results

A total of 393 patients were included in this study and the main characteristics are described in **Table 1**. In general, the median age was in the 6th decade of life, female (69.7%) was more prevalent than male (30.3%), gastro-intestinal (32.3%) and gynecologic (31.8%) tumors were the most prevalent ones and 70% of the sample were outpatients. **Table 2** describes the SMD according to demographic, clinical and body composition variables in the entire sample and according to sex. As can be seen, for both sexs, the SMD was significantly lower in patients older than 60 years, with white race/skin color, with metastatic tumor stage and diabetes (borderline significance for male). When analyzing the body composition markers, SMD was lower in the tertiles indicating higher adiposity markers, but higher in the higher HGS and SMI tertiles. Regarding the clinical characteristics, SMD was lower in patients with hypoalbuminemia and with high CRP levels. These differences were maintained when SMD was assessed according to sex.

Univariate linear regression analyses showed that patients with gastrointestinal tract tumor had on average 4.678 HU (r^2=0.070; p<0.001) greater SMD compared to the other tumor sites, while gynecological and BCT had on average 3.771 HU (r^2=0.045; p<0.001) and 3.065 HU (r^2=0.048; p<0.028) lower SMD, respectively. Breast (p=0.210) and lung (p=0.375) tumors were not associated with SMD. The variables with a p value ≤ 0.20 in the univariate

analysis that were included in the multivariate analysis were age, sex, race/skin color, distant metastasis, diabetes, hypertension, CRP, serum albumin, HGS, BMI, adipose tissue measurements (TATI, VATI and SATI) and SMI (**Table 3**).

The multiple regression analysis models are depicted on **Table 4**. As adipose tissue markers (BMI, TATI, VATI and SATI) showed collinearity with each other, three models were tested – Model 1 for BMI, Model 2 for TATI and Model 3 for VATI and SATI. First, the three models were tested not including the tumor site. The three models had similar r^2 regardless of adiposity marker tested and explained about 67% of variation in the SMD. The tumor site was then added to each model and further explained an additional variation in the SMD of about 2 to 3%. For all models, age, white race/skin color, markers of adiposity and gynecological and BCT tumor were inversely associated with SMD. The opposite was observed with albumin levels, HGS, SMI and with gastrointestinal tumor.

Table 1 - Characteristics of the patients with incurable cancer (n =393)

Variables	**Total**
Age (years)*	61 (50; 67)
Sex, female (n; %)	274 (69.7)
Race/skin color (n; %)	
White	139 (35.4)
Black	73 (18.6)
Mixed-race	181 (46.1)
Tumor site (n; %)	
Gastrointestinal tract [a]	127 (32.3)
Gynecological[b]	125 (31.8)
BCT	44 (11.2)
Breast	40 (10.2)
Lung	29 (7.4)
Others[c]	28 (7.1)
Tumor stage (n; %)	
Locally recurrent	89 (22.6)
Metastatic	304 (77.4)
Health care setting (n; %)	
Inpatient	118 (30.0)
Outpatient	275 (70.0)
Comorbidities (n; %)	
Diabetes mellitus	83 (21.1)
Hypertension	107 (27.2)
KPS <50% (n; %)	209 (53.2)

Note: n= number of observations; %= frequency; BCT= Bone and connective tissue; KPS= Karnofsky Performance Status
[a]Upper and lower GI tract; [b]Cervix, uterus, endometrium, ovary and vulva; [c]head and neck, kidney and urinary tract, male reproductive system
*Median/interquartile ranges (p25–p75)

Table 2 - Skeletal muscle radiodensity according to demographic, clinical and body composition variables in patients with incurable cancer (n=393).

Variables	**SMD (Mean±SD)**					
	Total n=393 (100%)	**p-value[a]**	**Male** n=119 (30.3%)	**p-value[a]**	**Female** n=274 (69.7%)	**p-value[a]**
Age (years)						
<60	29.4 (8.2)	**<0.001**	33.1 (9.3)	**0.030**	28.3 (7.6)	**<0.001**
>=60	26.3 (8.0)		29.7 (7.5)		24.4 (7.6)	
Race/skin color						
Black	30.1 (8.1)	**0.003**	33.3 (8.7)	**0.041**	29.0 (7.6)	**0.001**
Mixed	28.2 (8.1)		31.2 (8.0)		27.0 (7.9)	
White	26.2 (8.3)		30.1 (8.7)		24.2 (7.3)	
Health care setting						
Outpatient	28.2 (8.1)	0.208	31.2 (7.9)	0.687	26.9 (7.8)	0.098
Inpatient	27.0 (8.7)		30.6 (9.4)		25.2 (7.7)	
Tumor site						
Gastrointestinal tract	31.0 (8.2)	**<0.001**	33.2 (8.0)	**0.011**	29.1 (8.6)	**0.022**
Gynecological	25.1 (7.3)		-		25.2 (7.3)	
BCT	25.0 (8.0)		26.4 (9.3)		23.9 (6.7)	
Breast	26.2 (8.1)		-		26.2 (8.1)	
Lung	29.1 (8.4)		29.1 (8.6)		27.9 (8.0)	
Tumor stage						
Locally recurrent	30.8 (7.7)	**0.037**	32.4 (6.3)	**0.040**	28.6 (8.0)	**0.036**
Metastatic	27.2 (8.3)		30.6 (8.9)		25.8 (7.7)	
Hypertension						
No	28.1 (8.3)	**0.103**	31.1 (8.7)	**0.825**	26.8 (7.8)	**0.044**
Yes	26.2 (8.1)		30.6 (7.0)		24.2 (7.8)	
Diabetes mellitus						
No	28.9 (8.5)	**<0.001**	32.0 (9.0)	**0.089**	27.6 (7.9)	**<0.001**
Yes	25.6 (7.3)		29.2 (6.9)		23.8 (6.9)	
HGS (kg)						
Tertile 1 (<20.6 male; <14.0 female)	24.0 (8.0)	**0.004**	29.3 (9.3)	**0.004**	24.0 (8.0)	**0.004**
Tertile 2 (≥20.6 and <27.2 male; ≥14.0 and <18.0 female)	26.5 (7.8)		29.4 (73.)		26.5 (7.8)	
Tertile 3 (≥27.2 male; ≥18.0 female)	28.0 (7.3)		34.9 (7.7)		28.0 (7.3)	
BMI (kg/m^2)						
<20	32.5 (8.1)	**<0.001**	36.9 (7.3)	**<0.001**	30.6 (7.7)	**<0.001**

20-24.9	27.6 (8.0)		30.0 (8.2)		26.8 (7.5)	
≥25	25.1 (7.2)		28.0 (6.9)		24.3 (7.1)	
TATI (cm^2/m^2)						
Tertile 1 (<51.0 male; <69.8 female)	31.6 (7.6)	**<0.001**	35.8 (7.1)	**<0.001**	29.7 (7.2)	**<0.001**
Tertile 2 (≥51.0 and <103.9 male; ≥69.8 and <154.9 female)	26.7 (7.9)		31.8 (6.8)		24.5 (7.4)	
Tertile 3 (≥103.9 male; ≥154.9 female)	25.3 (7.9)		25.9 (8.1)		25.1 (7.9)	
VATI (cm^2/m^2)						
Tertile 1 (<16.2 male; <15.8 female)	31.2 (7.6)	**<0.001**	34.9 (8.3)	**<0.001**	29.6 (6.7)	**<0.001**
Tertile 2 (≥16.2 and <48.3 male; ≥15.8 and <47.6 female)	26.9 (8.3)		31.3 (7.0)		25.0 (8.0)	
Tertile 3 (≥48.3 male; ≥47.6 female)	25.4 (7.9)		27.1 (8.1)		24.7 (7.7)	
SATI (cm^2/m^2)						
Tertile 1 (<24.6 male; <45.0 female)	31.7 (7.7)	**<0.001**	36.5 (6.8)	**<0.001**	29.6 (7.1)	**<0.001**
Tertile 2 (≥24.6 and <45.1 male; ≥45.0 and <85.6 female)	26.2 (7.8)		29.6 (7.9)		24.8 (7.3)	
Tertile 3 (≥45.1 male; ≥85.6 female)	25.6 (7.9)		27.1 (7.5)		24.9 (8.0)	
SMI (cm^2/m^2)						
Tertile 1 (<41.3 male; <35.1 female)	25.2 (7.4)	**0.022**	27.5 (8.9)	**0.005**	25.8 (9.1)	**0.022**
Tertile 2 (≥41.3 and <48.7 male; ≥35.1 and <41.3 female)	25.8 (8.1)		32.1 (7.2)		25.2 (7.4)	
Tertile 3 (≥48.7 male; ≥41.3 female)	28.2 (6.6)		33.2 (8.1)		28.2 (6.6)	
Albumin (g/dL)						
<3.5	26.0 (8.1)	**0.013**	29.3 (8.2)	**0.004**	25.0 (7.9)	**0.045**
≥3.5	29.1 (8.4)		33.8 (8.1)		27.9 (7.7)	
C-reactive protein (mg/L)						
≥10	26.2 (7.5)	**0.017**	28.5 (9.1)	**0.049**	25.2 (6.8)	**0.040**
<10	29.8 (8.5)		31.9 (8.0)		28.5 (8.2)	
Neutrophil/lymphocyte ratio						
>5	27.6 (7.8)	0.708	30.5 (8.0)	0.155	26.5 (8.1)	0.519
≤5	28.0 (8.9)		32.0 (8.8)		25.7 (7.5)	

Note: BCT= Bone and connective tissue; BMI= body mass index; HGS= handgrip strength; SATI= subcutaneous adipose tissue index; SMI= skeletal muscle index; TATI = total adipose tissue index VATI= visceral adipose tissue index

[a]Independent T test or ANOVA as appropriate.

Table 3 - Association by simple linear regression models between skeletal muscle radiodensity (HU) and demographic, clinical and body composition variables in patients with incurable cancer (n=393).

Variables	**SMD**			
	R^2	**B**	**95% IC**	**p**
Age (year)	0.164	-0.127	-0.186; -0.068	**<0.001**
Sex (female as reference)	0.065	-4.609	-6.344; -2.874	**<0.001**
Black race/skin color	0.047	2.800	0.698; 4.903	**0.009**
White race/skin color	0.121	-2.543	-4.250;-0.837	**0.004**
Mixed race/skin color	0.001	0.635	-1.018; 2.289	0.450
Health care setting (Inpatient)	0.004	-1.152	-2.948; 0.643	0.208
Gynecological tumor	0.045	-3.771	-5.501; -2.040	**<0.001**
Gastrointestinal tract tumor	0.070	4.678	2.977; 6.379	**<0.001**
Bone and connective tissue tumor	0.042	-3.065	-5.807; -0.324	**0.029**
Lung tumor	0.002	1.424	-1.729; 4.575	0.375
Breast tumor	0.004	-1.737	-4.459;0.985	0.210
Metastatic disease	0.014	-2.886	-5.323; -0.449	**0.020**
Diabetes mellitus	0.033	-3.241	-4.975; -1.507	**<0.001**
Hypertension	0.007	-1.862	-4.102; 0.378	0.103
HGS (kg)	0.275	0.368	0.269; 0.466	**<0.001**
BMI (kg/m^2)	0.284	-0.394	-0.529; -0.259	**<0.001**
TATI (cm^2/m^2)	0.160	-0.020	0.028;-0.012	**<0.001**
VATI (cm^2/m^2)	0.116	-0.017	-0.030; -0.004	**0.013**
SATI (cm^2/m^2)	0.176	-0.043	-0.057;-0.028	**<0.001**
SMI (cm^2/m^2)	0.179	0.286	0.189;0.383	**<0.001**
% Weight loss in 6 mo	0.002	0.039	-0.055; 0.134	0.414
Albumin (g/dL)	0.162	1.678	0.662; 2.693	**0.001**
C-reactive protein (mg/L)	0.014	-0.065	-0.172; 0.043	0.140
Neutrophil/lymphocyte ratio	0.001	-0.326	-2.002; 1.350	0.703

Note: BCT= Bone and connective tissue; BMI= body mass index; CI: confident interval; HGS= handgrip strength; SATI= subcutaneous adipose tissue index; SMI= skeletal muscle index; TATI = total adipose tissue index VATI= visceral adipose tissue index

Table 4 - Association by multiple linear regression models between skeletal muscle radiodensity (HU) and demographic, clinical and body composition variables in patients with incurable cancer (n=393).

Model 1	**R^2= 0.668**	**Coefficient**	**95% IC**	**p**
Age (years)		-0.173	-0.227; -0.118	<0.001
White race/skin color		-1.993	-3.456;-0.530	0.008
Albumin (g/dL)		1.731	0.789; 2.673	<0.001
HGS (kg)		0.233	0.140; 0.325	<0.001
SMI (cm^2/m^2)		0.359	0.265; 0.453	<0.001
BMI (kg/m^2)		-0.624	-0.746; -0.495	<0.001
+ Gastrointestinal tract tumor	**r^2=** 0.694	3.682	2.146; 5.217	<0.001
+ Gynecological tumor	**r^2=** 0.682	-2.913	-4.562; -1.264	0.001
+ BCT	**r^2=** 0.694	-5.355	-7.685; -3.025	<0.001
Model 2	**r^2= 0.682**	**Coefficient**	**95% IC**	
Age (years)		-0.140	-0.191; -0.090	<0.001
White race/skin color		-1.781	-3.188;-0.373	0.013
Albumin (g/dL)		2.210	1.294; 3.126	<0.001
HGS (kg)		0.190	0.101; 0.280	<0.001
SMI (cm^2/m^2)		0.319	0.230; 0.408	<0.001
TATI (cm^2/m^2)		-0.060	-0.071;-0.050	<0.001
+ Gastrointestinal tract tumor	**r^2=** 0.699	2.946	1.432; 4.459	<0.001
+ Gynecological tumor	**r^2=** 0.691	-2.231	-3.797; -0.665	0.005
+ BCT	**r^2=** 0.700	-4.535	-6.802; -2.267	<0.001
Model 3	**r^2= 0.673**	**Coefficient**	**95% IC**	**p**
Age (years)		-0.142	-0.195; -0.089	<0.001
White race/skin color		-1.794	-3.232;-0.356	0.015
Albumin (g/dL)		2.173	1.244; 3.103	<0.001
HGS (kg)		0.194	0.102; 0.286	<0.001
SMI (cm^2/m^2)		0.323	0.232; 0.413	<0.001
VATI (cm^2/m^2)		-0.066	-0.099;-0.034	<0.001
SATI (cm^2/m^2)		-0.058	-0.078;-0.039	<0.001
+ Gastrointestinal tract tumor	**r^2=** 0.692	3.061	1.532; 4.589	<0.001
+ Gynecological tumor	**r^2=** 0.684	-2.387	-3.985; -0.788	0.004
+ BCT	**r^2=** 0.696	-4.904	-7.210; -2.598	<0.001

Note: BCT= Bone and connective tissue; BMI= body mass index; HGS= handgrip strength; SATI= subcutaneous adipose tissue index; SMI= skeletal muscle index; TATI = total adipose tissue index VATI= visceral adipose tissue index

5.2.4 Discussion

In this cross-sectional study, we examined the factors associated with SMD in patients with incurable cancer, using CT images obtained as part of the routine oncologic care. Multivariate explanatory models were studied, and the characteristics related with low SMD were older age, white race/skin color, lower serum albumin, HGS and SMI and higher adiposity, the last one evaluated by BMI, TATI or VATI and SATI. Additionally, the primary tumor site contributed to changes in SMD, particularly those located in the gastrointestinal tract, gynecological and bone and connective tissue.

For many decades, the interest in studying body composition in patients with cancer were focused on the assessment of muscle mass and adipose tissue markers. However, over the last decade there has been increasing interest in studying also SMD, which according to recent publications also plays an important role in predicting clinical outcome [1,6-9].

The pathophysiological mechanisms underlying changes in SMD in patients with cancer are not yet fully understood. In this context, an interesting finding of the present study was the role of the primary tumor site influencing SMD. In agreement with our findings, Xiao et al [19] reported different likelihood of showing low SMD according to the tumor location, with patients with rectal cancers having lower odds ratio for low SMD as compared to those with colon cancer. In the current study, the higher SMD found in patients with tumor located in the gastrointestinal tract and the lower SMD in those with gynecological cancer may be partially explained by an association with adiposity markers, as previously shown [19,22,29]. On the other side, patients with bone and connective tissue tumors usually have physical limitations, which negatively influence physical function and, as a consequence, decrease SMD [30]. Longitudinal studies are warranted to elucidate the relationship between different tumor sites and muscle lipid infiltration in patients with cancer.

Our findings regarding the inverse association between SMD and age confirms previous studies in the general population [15] and in oncologic patients [19], since muscle lipid infiltration is a physiological feature of aging [2,15]. Furthermore, the finding that patients with white race/skin color had on average lower SMD than black and mixed race/skin color is aligned with a previous study with patients with colorectal cancer reporting that caucasians had lower mean SMD and were more likely to have low SMD compared with African Americans and Asians [19], although contrary to a previous study in noncancer individuals [31]. It is possible changes in body composition by ethnicity arise in the context of the disease changes.

Regarding body composition measurements, BMI, TATI, VATI and SATI were inversely associated with SMD, a finding already documented in non-cancer population [32], but also reported in cancer cohorts [19,22,29]. The explanation to this finding is that high concentrations of circulating free fatty acid observed in overweigh individuals impair mitochondrial function, with a reduction in muscle lipid oxidation and, therefore, an increase in muscle lipid content [2,32,33,34]. These results can be used to support the hypothesis that interventions aiming to ameliorate nutritional status in early cancer stages may result in better preservation of muscle density as cancer stages advances.

As expected, HGS was directly associated with SMD, a finding also observed in a previous study in patients with cancer [7]. Muscle disuse has been suggested to decrease the capacity for lipid oxidation, and these non-metabolized lipids accumulates in the atrophied muscle [30]. On the other hand, low SMD has been recognized as an important determinant of muscle function in patients with cancer, regardless of muscle area. The proposed mechanism explaining this finding goes beyond the skeletal muscle quantity. The muscle lipid accumulation induces a transition of muscle fibers from type II to type I, which results in a muscle with impaired contractile capacity and decreased power, negatively influencing muscle performance [3,35]. This suggests a bidirectional association between SMD and muscle strength [19,30], especially patients' groups with advanced cancer who are normally sedentary and even in bedridden.

Regarding the association between low SMD and hypoalbuminemia, the results of the present study are in line with those already documented in patients with cancer [19,36,37], indicating that a nutritional intervention aiming to downregulate the systemic inflammation may likely prevent muscle lipid infiltration. Although in our study only albumin was associated with SMD in the multivariate model, patients with elevated CRP had lower mean SMD.

Altogether, the results of the present study clarify and represent an advance in understanding factors related to SMD in patients with incurable cancer. Features such as age, race/skin color and the primary tumor site are non-modifiable risk factors related to SMD. However, systemic inflammatory response, muscle strength, the amount of SMI, as well as the degree of adiposity, are modifiable risk factors that, if identified early, allows for a timely therapeutic intervention in order to prevent the muscle lipid infiltration.

This study has some limitations. First, the fact that only patients with CT available from the trunk for analysis at L3 favored the inclusion of those with gastrointestinal and gynecological tumors. Second, the cross-sectional design of the analysis does not allow causal-effect explanations. An important strength of this study is that the statistical analyses were performed with SMD as a continuous variable, which eliminates bias related to cutoff points. In addition, CT is recognized for its high accuracy for SMD assessment in patients with cancer.

In conclusion, SMD was associated with age, albumin, race/skin color, HGS and body composition markers. In addition, gastrointestinal, gynecological and bone and connective tissue tumor sites further explained variation in SMD. Considering that muscle lipid infiltration is an important component of the overall body composition analysis, combined

with the beginning of an era with studies investing this field, our findings contribute to better understand the factors explaining SMD in patients with incurable cancer. Future studies are warranted to further investigate the pathophysiology of the association between the identified risk factors and SMD.

Statement of authorship
Larissa Calixto-Lima: project conception; development of the overall research plan; analysis and interpretation of data; drafting the article and had primary responsibility for final content. **Emanuelly Varea Maria Wiegert, Livia Costa de Oliveira and Gabriela Villaça Chaves**: project conception, development of research plan; critical revision of important intellectual content. **Flavia Fioruci Bezerra and Carla Maria Avesani:** project conception, development of the overall research plan; interpretation and editing; critical revision of important intellectual content. **All authors:** read and approved the final version submitted.

Conflict of interest
None declared.

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5.3 Artigo 3: Altered skeletal muscle phenotypes are associated with functional impairment, worse systemic inflammatory response, and reduced survival in patients with incurable cancer

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Abstract

Background & aims: The factors associated with the simultaneous occurrence of low muscle mass and myosteatosis are unclear. This study investigated whether different skeletal muscle phenotypes are associated with systemic inflammatory response, functional impairment, and survival in patients with incurable cancer. **Methods:** Three hundred and twenty-six patients (median age 60 years, 67.5% female) with incurable cancer who had abdominal or pelvic computed tomography (CT) scans up to 30 days before the initial assessment were enrolled for the study. The CT images were used for the assessment of skeletal muscle index (SMI) and skeletal muscle radiodensity (SMD) and optimal stratrification analysis were used to derive cohort-specific cutpoints to define low SMI (<45.0 cm^2/m^2 for males and <44.0 cm^2/m^2 for females) and myosteatosis (SMD <*34* HU for males and <30 HU for females). Based on these cutoffs, participants were classified into four phenotypes: high SMI + non-myosteatotic*;* low SMI + non-myosteatotic; high SMI + myosteatotic*;* and low SMI + myosteatotic. **Results:** The phenotypes with low SMI or myostatosis, and especially the combination of both conditions, were associated with low handgrip strength (HGS), poor performance status, altered C-reactive protein and albumin concentrations, and worse inflammation-based

prognostic scores. The phenotypes with myosteatosis, regardless of high SMI (HR: 1.74; 95% CI: 1.05-2.88) or low SMI (HR: 1.99; 95% CI: 1.29-3.05) were associated with a higher 90-days mortality, with high SMI + non-myosteatotic as the reference group. **Conclusion:** In patients with incurable cancer, the phenotype groups with low SMI and myosteatosis, particularly in combination, were correlated with worse functional impairment and altered inflammatory response. Moreover, the presence of myosteatosis increased the mortality risk.
Keywords: muscle mass, myosteatosis, incurable cancer, inflammation, physical function, survival.

5.3.1 Introduction

In oncology, sarcopenia is characterized by severe muscle mass depletion, which is associated with increased chemotherapy toxicity, surgical complications, and reduced overall survival [1,2,3]. Approximately 40% of pre-treatment patients with cancer have sarcopenia, a level that can increase to 74% in the advanced stages of the disease [4,5]. More recently, in addition to skeletal muscle mass (SM), muscle fat infiltration (i.e., myosteatosis) has been considered a component of sarcopenia rather than just a distinct entity [6,7,8,9]. The term muscle quality has been used to describe micro- and macroscopic aspects of muscle architecture and composition [9] and among the characteristics related to muscle quality, the SM quantity and the degree of myosteatosis are included [10]. Myosteatosis is also associated with adverse cancer-specific outcomes [3,11,12], which underlines the importance of investigating this compartment in patients susceptible to muscle wasting, such as those in advanced stages of cancer.

The precise quantification of SM and the assessment of myosteatosis can be evaluated by computed tomography (CT), with myosteatosis being estimated by low skeletal muscle radiodensity (SMD). As CT scans are usually performed in patients with cancer for diagnostic and staging purposes, these images could be opportunistically used to assess body composition with high precision and without extra exposure to radiation [13,14].

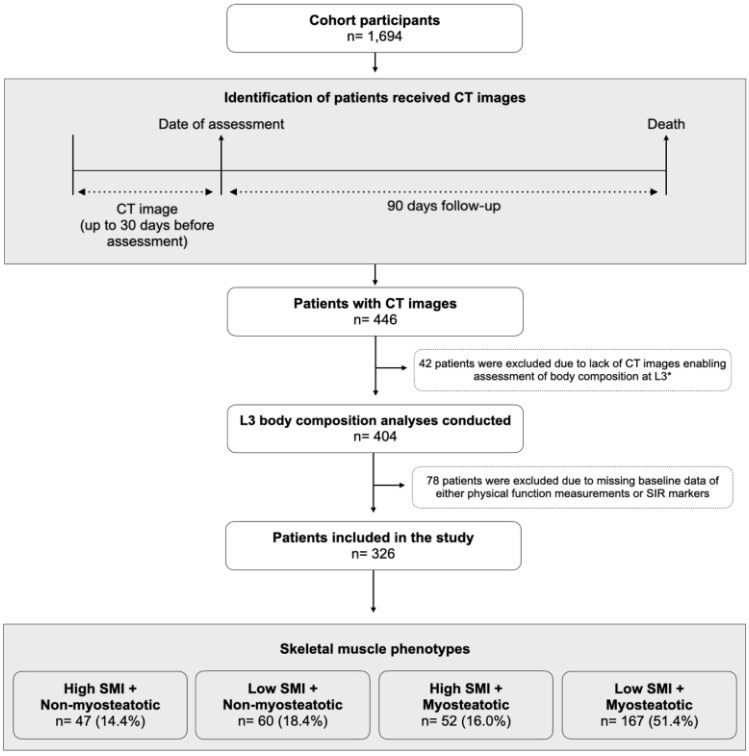
The association between low muscle mass / myosteatosis and clinical variables in patients with incurable cancer has scarcely been investigated. It is likely that the simultaneous occurrence of these two muscle abnormalities have a worse effect on physical function, inflammatory response, and survival than they have when occurring in isolation. In view of these considerations, we hypothesize that the combined occurrence of low SM and myosteatosis may predict worse clinical outcomes than either condition alone. Thus, the aim of the present study was to investigate whether distinctive skeletal muscle phenotypes based on low or high SM and absence or presence of myosteatosis are associated with systemic inflammatory response, functional impairment, and survival in patients with incurable cancer.

5.3.2 Materials and Methods

5.3.2.1 Study design and population

This study included a subsample of a prospective cohort comprised of patients with incurable cancer referred to the palliative care unit (PCU) of the National Cancer Institute in Rio de Janeiro, Brazil. Patients were included between July 2016 and March 2019 and were followed up for survival analysis purposes until 90 days after inclusion. Incurable cancer was defined as metastatic cancer (based on histological, cytological, or radiological evidence) or locally recurrent cancer in patients who were not receiving any antineoplastic treatment with curative intent. All consecutive inpatients and outpatients were evaluated at their first appointment at the PCU by trained researchers. The inclusion criteria were: age ≥ 20 years old, Karnofsky Performance Status (KPS) ≥ 30%, and ability to answer the research questionnaires. Data regarding the primary cancer site, extent of the disease, and date of death were obtained from the medical records. The cohort that constituted this sample included 1,694 patients, most of whom were female (n=991; 58.5%) with a median age of 63 (interquartile range, IQR 54-72) years. For the current study, only those who had abdominal or pelvic CT scans up to 30 days before the date of the initial assessment (n=326) were included (**Figure 1**). The baseline characteristics of the included and not included patients were compared (Supplementary Table). The included patients were younger and had a higher proportion of females and gynecological tumors than the not included patients, but no difference was observed between the groups regarding metastatic disease, performance status (KPS and PS), and inflammatory response (C-reactive protein [CRP] and albumin). The Research Ethics Committee of the National Cancer Institute (protocol number 1.407.458 of 2016) approved the study, and all the patients provided informed consent before inclusion.

Figure 1 - Patient flow chart



Note: CT= computed tomography; L3= third lumbar vertebra; SIR= systemic inflammatory response; SMI= skeletal muscle index
*****Significant artifacts or missing region of interest (n=9), poor quality of the CT images (n=29), or whole cross-sectional area not included in the images (n=4).

Supplementary table - Baseline characteristics of the included and not included patients

Variables	**Patients not included (n=1,368)**	**Patients included (n=326)**	**p-value**
Age (years)*	63 (55-73)	60 (50-67)	**<0.001**
Age (>60years)**	868 (63.5%)	168 (51.5%)	**<0.001**
Females**	771 (56.3%)	220 (67.5%)	**<0.001**
Current medical situation (outpatient)**	1046 (76.4%)	234 (71.8%)	0.081
Tumor site**			
GI Tract[a]	415 (30.3%)	111 (34.0%)	0.069
Gynecology[b]	195 (14.3%)	98 (30.1%)	**<0.001**
Breast	164 (12.0%)	32 (9.8%)	0.114
Lung	144 (10.5%)	28 (8.6%)	0.174
BCT	107 (7.8%)	31 (9.5%)	0.186
Others[c]	343 (25.1%)	26 (8.0%)	**<0.001**
Metastatic disease**	1137 (83.1%)	285 (87.4%)	0.077
KPS <50%**	666 (48.6%)	147 (45.1%)	0.163
BMI (kg/m²)*	21.4 (18.0-25.1)	22.5 (19.2-26.0)	0.103
Albumin (g/dL)***	3.2 (±0.8)	3.1 (±0.8)	0.970
CRP (mg/L)**	4.3 (1.0-9.7)	4.5 (1.4-10.5)	0.801

Note: %= frequency; BCT= bone and connective tissue; BMI= body mass index; CRP= C-reactive protein; GI= gastrointestinal; n= number of observations; KPS= Karnofsky Performance Status;
[a]upper and lower GI tract; [b]cervix, uterus, endometrium, ovary and vulva; [c]head and neck, kidney and urinary tract, male reproductive system and hematological.
*Median/interquartile ranges (p25-p75); Mann-Whitney
**Number of observation/frequency; chi-square.
***Mean/± standard deviation; *t*-test

5.3.4 Body composition

Body composition analysis was performed using abdominal and pelvic CT scans performed routinely for staging purposes up to 30 days before the patients' inclusion. Slices located at the third lumbar vertebra (L3) were evaluated by a trained examiner who was blinded to the outcome assessment using sliceOmatic, version 5.0 (Tomovision™, Canada). Different tissues were identified based on their anatomical features and quantified using pre-established tissue-specific Hounsfield unit (HU) ranges: −29 to 150 for SM, −150 to −50 for visceral adipose tissue, and −190 to −30 for subcutaneous and intermuscular adipose tissue. Scans with significant artifacts or missing region of interest were not considered for inclusion in the study. The sum of overall SM area (cm^2) obtained by the L3 scan was normalized for stature (m^2) and reported as lumbar skeletal muscle index (SMI) (cm^2/m^2). SMD was also evaluated from CT scans and was derived by averaging the HU value of the total muscle area. Low muscle mass and myosteatosis were identified using sex-specific threshold values for SMI and SMD, respectively, both defined by an optimal stratification approach derived from our own cohort [11,15,16]. This method identifies a cutoff point for a continuous variable from a fixed set of possible values that best discriminates the patients' risk with a specific outcome, in this case, survival [16]. Patients were then categorized as "low SMI" or "high SMI" based on the cutoff values <45.0 cm^2/m^2 for male and <44.0 cm^2/m^2 for female. Patients were also classified as "non-myosteatotic*" or "*myosteatotic*"* according to the cutoff values for SMD of <34 HU for males and <30 HU for females.

5.3.5 Skeletal muscle phenotypes

Based on the SMI and SMD categories previously described, four muscle phenotype groups were created: high SMI + non-myosteatotic; low SMI + non-myosteatotic; high SMI + myosteatotic; and low SMI + myosteatotic.

5.3.6 Physical function

Physical function was evaluated by means of muscle strength and performance status. Muscle strength was assessed by handgrip strength (HGS) using a Jamar® hydraulic hand dynamometer. Each participant was instructed to sit in a chair, comfortably arrange the instrument in his/her hand, and apply as much strength as possible in each measurement.

During the exam, the upper limb assessed was placed alongside the body with the elbow flexed at 90° and the contralateral limb was relaxed on the thigh. Three trials were performed per hand in an alternating manner with a one-minute rest interval between trials. Since no statistical difference was observed between the measurements from the dominant and non-dominant hands (p=0.113), the maximum strength obtained from the six measurements was defined to represent HGS. Low muscle strength was defined as an HGS value below the 10th percentile (<P10) of an age, sex, and arm-side-matched population-based normative table of healthy individuals living in Rio de Janeiro, Brazil [17].

Performance status was evaluated by KPS, which is a percentage scale (0%-100%) that classifies patients based on their ability to perform active work and care for themselves, as well as their need for regular medical care due to greater disease severity. This scale has 11 categories and is scored with increments of 10, where the lower the score, the lower the function. The cutoff value for functional impairment was set at <50% (30% or 40% *versus* ≥50%). Performance status was also assessed using the Portuguese-validated version of the Patient-Generated Subjective Global Assessment Short Form (PG-SGA SF) (©FD Ottery, 2005, 2006, 2015), available from Ottery at Pt.Goblal.org. This tool consists of four boxes, the fourth of which focuses on functional status (maximum score of 3), evaluated by the Eastern Cooperative Oncology Group Performance Status (ECOG PS). The higher the score, the worse the functional status. The cutoff value for functional impairment was set at >2 (0 to 2 *versus* 3).

5.3.7 Systemic inflammatory response (SIR)

On the day of enrollment to the study, a blood sample was drawn for the analysis of serum CRP, albumin, and complete blood cell count. Cancer-related inflammatory response was assessed according to CRP, albumin, and four inflammation-based prognostic scores:

1) modified Glasgow Prognostic Score (mGPS), varying from 0 to 2, with 0 = CRP <10 mg/L, 1 = albumin ≥3.5 mg/dL and CRP □10 mg/L, and 2 = albumin <3.5 mg/dL and CRP □10 mg/L;

2) Prognostic Nutrition Index (PNI), obtained by the sum of serum albumin and 5 x lymphocyte count;

3) neutrophil-lymphocyte ratio (NLR); and

4) platelet-lymphocyte ratio (PLR).

Based on previous evidence, SIR was defined as fulfilling at least one of the following criteria: CRP □10 mg/L [18], albumin <3.5 mg/dL [18], mGPS □1 [18], PNI ≤45 [19], NLR >3 [20,21], and PLR >150 [21,22].

Survival

Patient overall survival was defined as the time interval in days between the date of inclusion in the study and the date of death from any cause. Patients who were alive after follow-up period (90 days) were censored for survival analysis.

Statistical analysis

The Kolmogorov-Smirnov test was performed to assess distribution symmetry. Descriptive statistics (count/frequency [%], means ± standard deviation [SD], or median and IQR, as appropriate) were used to describe patient characteristics. Differences between muscle phenotype groups were tested by one-way analysis of variance followed by Bonferroni post-hoc test for the normally distributed variables and by the Kruskal-Wallis test followed by Dunn's post-hoc test for the non-normally distributed variables. For categorical variables, the Chi-square test was used to compare the groups.

The association between the variables and muscle phenotypes was explored by the performance of nine logistic multiple regressions (one for each selected variable of physical function and SIR), with adjustments for age, sex, primary tumor site, and presence of metastatic disease. As muscle phenotypes were categorized into four groups, four dummy variables (D1, D2, D3, and D4) were inserted into each of the regression equations. The high SMI + non-myosteatotic group, represented by D1, was used as the reference category.

The Kaplan-Meier curves were used to evaluate survival probability and the log-rank test was used to compare the difference between the muscle phenotype groups. Additionally, the Cox proportional hazards model adjusted by age, sex, primary tumor site, presence of metastatic disease and health care setting was used to verify the hazard ratios (HRs) of the muscle phenotype groups that were able to predict overall survival, taking the high SMI + non- myosteatotic group as the reference.

The cutoff points for low SMI and low SMD (both sex-specific) were defined using the optimal stratification method based on the maximum absolute value of the log-rank statistic test that best discriminated the patients' risk with respect to time to death. Statistical

analysis was processed using SPSS version 21.0 (SPSS, Chicago, IL, USA) and optimal stratification was conducted using Stata version 13.0 (Stata Corp., College Station, Texas, USA). Statistical significance was set at $p < 0.05$.

5.3.8 Results

A total of 326 patients were included in this study. There was a higher proportion of female patients (67.5%) and the median age was in the 6th decade of life. The most common tumor site was gastrointestinal tract (34%; upper and lower gastrointestinal tract, pancreas, gallbladder, and liver), followed by gynecological (30.1%; cervix, endometrium, ovarian, and vulva), breast (9.8%), bone and connective tissue (9.5%), lung (8.6%), and others sites (8.0%; head and neck, and urological).

Regarding clinical, functional, and inflammatory markers, our results demonstrated that most differences occurred between the groups showing no muscle abnormalities (high SMI + non-myosteatotic) and with both muscle abnormalities (low SMI + myosteatotic). Compared to the high SMI + non-myosteatotic group, the patients in the high SMI + *m*yosteatotic and low SMI + myosteatotic phenotype groups were older and had a higher proportion of female. The patient groups with at least one muscle alteration (low SMI, myosteatosis, or both) had lower HGS and higher prevalence of ECOG PS >2 than the high SMI + non-myosteatotic group. The SIR markers indicated a worse inflammatory status in patients with low SMI + myosteatotic group as compared with those with no muscle alteration (high SMI + non-myosteatotic) (**Table 1***).*

The logistic regression analysis demonstrated a significantly higher risk of functional impairment and altered inflammatory response in the groups with at least one muscle alteration as compared with the group with no alteration (high SM + non-myosteatotic) (**Table 2**).

In regard to 90-day survival, the median survival of the entire group was 47 (95% CI: 41–52) days, and 229 (70.2%) deaths occurred. The Kaplan-Meier analysis showed that the survival curves were different among the phenotypes, with the group with high SMI + non-myosteatotic showing the highest survival (72; 95% CI: 12–90 days) and those in the low SMI + myosteatotic the lower survival (32; 95% CI: 22–41 days) (**Figure 2**). These results remained significant when evaluated in the Cox multiple regression analysis. As can be seen in **Table 3**, in the non-adjusted and adjusted analyses, when compared with the reference

group (high SM + non-myosteatotic*)*, the HR for mortality was significantly higher in the myosteatotic groups, but not in the groups with low SMI.

Table 1 - Clinical characteristics, functional impairment and systemic inflammatory response according to skeletal muscle phenotypes groups in patients with incurable cancer (n=326).

Variables	**Total n= 326 (100%)**	**High SMI + non-*myosteatotic* 47 (14.4%)**	**Low SMI + non-*myosteatotic* n=60 (18.4%)**	**High SMI + m*yosteatotic* n=52 (16.0%)**	**Low SMI + myosteatotic n=167 (51.2%)**
Female sex*	220 (67.5%)	8 (17.0%)	36 (60.0%)[a]	35 (67.3%)[a]	141 (84.4%)[a,b,c]
Age (years)**	60 (50-67)	57 (47-66)	58 (46-67)	62 (53-68)[a,b]	63 (52-69)[a,b,]
Health care setting (outpatient)*	234 (71.8%)	33 (70.2%)	45 (75.0%)	35 (67.3%)	121 (72.5%)
Distant metastasis*	285 (87.4%)	38 (80.9%)	52 (86.7%)	47 (90.4%)	148 (88.6%)
Functional impairment					
HGS (kg)**	18 (13-23)	27 (22-34)	19 (16-22)[a]	19 (12-23)[a]	16 (12-20)[a,b,c]
KPS (<50%)*	147 (45.1%)	17 (36.2%)	26 (43.3%)	26 (50.0%)	78 (46.7%)
ECOG PS (>2 pts)*	206 (63.2%)	16 (34.0%)	36 (60.0%)[a]	35 (67.3%)[a]	119 (71.3%)[a]
SIR markers					
CRP (mg/L)**	4.5 (1.4-10.5)	3.5 (0.8-8.0)[a]	4.2 (1.0-8.8)[a]	4.4 (0.7-10.0)[a]	4.9 (1.7-12.0)[a,b,c]
Albumin (g/dL)***	3.1 (±0.8)	3.5 (±0.8)	3.1 (±0.8)[a]	3.3 (±0.7)	3.0 (±0.8)[a,c]
mGPS (□1)*	109 (33.5%)	10 (21.2%)	18 (30.0%)[a]	13 (25.0%)[a]	68 (40.7%)[a,b,c]
PNI (≤45)*	236 (72.4%)	26 (55.3%)	46 (76.7%)[a]	39 (75.0%)[a]	125 (74.9%)[a]
NLR (>3)*	182 (55.7%)	22 (46.8%)	37 (61.7%)[a]	25 (48.1%)[b]	98 (58.7%)**[a,c]**
PLR (>150)*	278 (85.2%)	35 (74.5%)	49 (81.6%)	44 (84.6%)	150 (89.8%)**[a]**

Note: SMI= skeletal muscle index; HGS= hand grip strength; KPS= Karnofsky Performance Status; ECOG PS= Eastern Cooperative Oncology Group Performance Status; SIR= systemic inflammatory response; CRP= C-reactive protein; mGPS= modified Glasgow Prognostic Score; PNI= Prognostic Nutrition Index; NLR= neutrophil/lymphocyte ratio; PLR= platelet/lymphocyte ratio;

[a]Statistically different from high SMI + non-*myosteatotic*; [b]Statistically different from low SMI + non-*myosteatotic*; [c]Statistically different from high SMI + m*yosteatotic*

*Number of observation/frequency; chi-square.

**Median/interquartile ranges (p25-p75); Kruskal-Wallis, Dunn post hoc

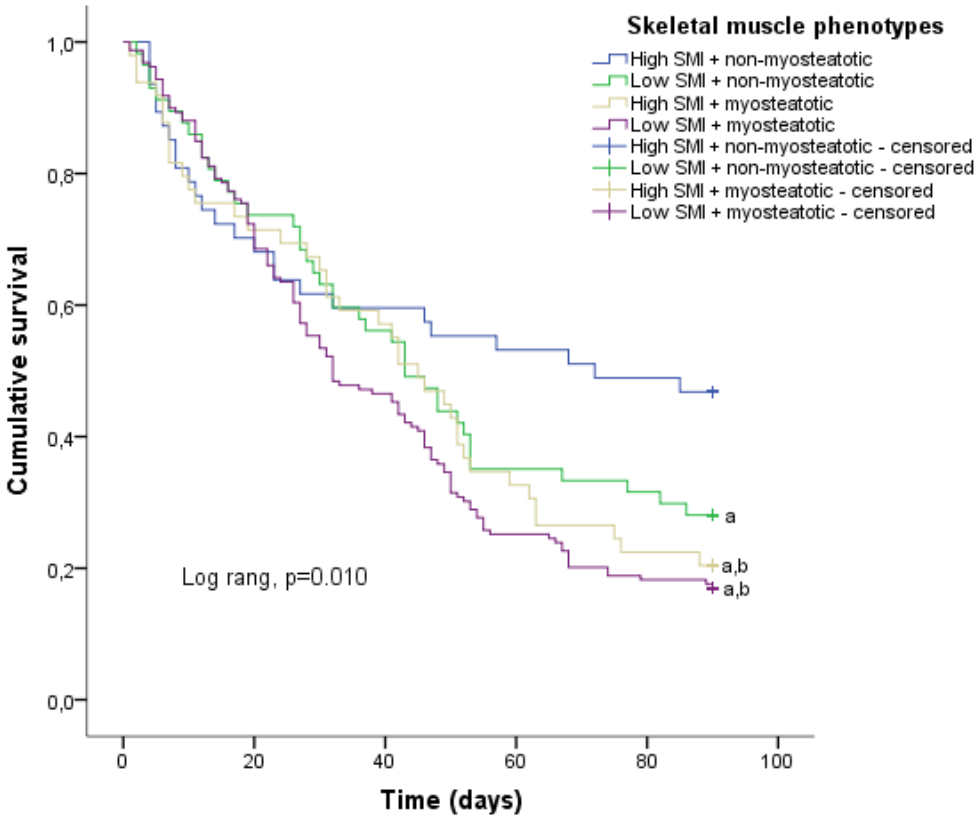
***Mean/± standard deviation; ANOVA, Bonferroni post hoc

Table 2 - Multiple logistic regression models for skeletal muscle phenotypes groups according to functional impairment and systemic inflammatory response in patients with incurable cancer (n=326).

Independent variables	Low SMI + non-myosteatotic n=60 (18.4%)		High SMI + *m*yosteatotic n=52 (16.0%)		Low SMI + myosteatotic n=167 (51.2%)	
	OR (95% CI)*	**p-value**	**OR (95% CI)***	**p-value**	**OR (95% CI)***	**p-value**
Functional impairment						
Low HGS (<P10)	4.06 (1.39-11.83)	0.010	6.26 (1.54-25.34)	0.010	11.14 (3.20-38.69)	<0.001
KPS <50%	2.37 (0.60-9.32)	0.214	1.54 (0.38-6.26)	0.542	3.64 (1.18-11.24)	0.025
ECOG PS >2 pts	5.17 (1.88-14.26)	0.001	4.96 (1.57-15.67)	0.006	6.75 (2.53-18.01)	<0.001
SIR Markers						
CRP □10.0 mg/L	2.24 (1.04-4.48)	0.036	2.30 (1.13-4.02)	0.046	2.94 (1.01-8.50)	0.047
Albumin <3.5 g/dL	8.50 (1.90-38.50)	0.005	2.28 (0.82-6.30)	0.111	10.6 (2.94-38.5)	<0.001
mGPS□1	2.31 (1.08-3.94)	0.045	0.93 (0.32-2.69)	0.905	2.98 (1.05-8.47)	0.040
PNI ≤45	7.53 (1.68-33.66)	0.008	4.91 (1.60-15.04)	0.005	8.51 (2.44-29.68)	0.001
NLR>3	2.66 (1.01-6.98)	0.046	0.96 (0.29-3.12)	0.949	4.75 (1.64-13.72)	0.004
PLR >150	1.47 (0.43-5.05)	0.535	2.21 (0.74-6.62)	0.154	4.14 (1.17-14.54)	0.027

Note: ECOG PS= Eastern Cooperative Oncology Group Performance Status; mGPS= modified Glasgow Prognostic Score; CRP= C-reactive protein; HGS= handgrip strength; KPS= Karnofsky Performance Status; NLR= neutrophil/lymphocyte ratio; PLR= platelet/lymphocyte ratio; PNI= Prognostic Nutrition Index; SIR= systemic inflammatory response; SMI= skeletal muscle index.
*Logistic regression adjusted for age as continuous variable, female sex and primary tumor site and metastatic disease.

Figure 2 - Kaplan-Meier curve for 90-days survival according to the skeletal muscle phenotypes proposed.



Note: SMI= skeletal muscle index.
[a]Statistically different from High SMI + Non-myosteatotic; [b]Statistically different from Low SMI + Non-myosteatotic

Table 3 - Cox regression hazard ratios of associations between skeletal muscle phenotypes and mortality in patients with incurable cancer (n=326).

Independent variables	**Unadjusted**		**Adjusted***	
	HR (95% CI)	***p***	**HR (95% CI)**	***p***
Skeletal muscle phenotypes				
High SMI + non-myosteatotic	Ref	-	Ref	-
Low SMI + non-myosteatotic	1.51 (0.90-2.54)	0.115	1.49 (0.90-2.45)	0.115
High SMI + myosteatotic	2.19 (1.27-3.78)	0.004	1.74 (1.05-2.88)	0.031
Low SMI + myosteatotic	2.33 (1.43-3.79)	0.001	1.99 (1.29-3.05)	0.002

Note: CI= confident interval; HR= hazard ratio; Ref= reference; SMI= skeletal muscle index.
*Adjusted for age as continuous variable, female gender, primary tumor site, metastatic disease and and health care setting

5.3.9 Discussion

This study demonstrated that skeletal muscle phenotypes were associated with functional impairment and altered SIR. Low SMI and myostatosis, particularly in

combination, were associated with low HGS, poor performance status (KPS and ECOG-PS), altered CRP and albumin concentrations as well as the inflammation prognostic scores studied, mGPS, PNI, NLR and PLR. Moreover, the muscle phenotypes where myosteatosis was present had a higher HR for mortality. To the best of our knowledge, this is the first study examining muscle phenotypes considering skeletal muscle mass and myosteatosis (both assessed by CT) individually and combined in a sample of patients with incurable cancer.

Our findings regarding the association between the phenotypes of abnormal muscularity and functional impairments are aligned with previous studies with similar objectives, where low muscle mass and myosteatosis were found to have a negative impact on physical function evaluated by HGS [10] and ECOG-PS [23]. However, other studies have found no association between low muscle mass [3] or myosteatosis [24] and impaired physical function. Although these studies used the same method to assess body composition (i.e., CT scans), the divergent results can be attributed to the use of different cutoff points to define myosteaosis and low muscle mass. It is worth noting that the optimal stratification approach used to define the thresholds for low muscle mass and myosteaosis is increasingly accepted for patient risk stratification in the oncology setting [11,14,15]. In addition, the aforementioned studies were done on samples of patients with different characteristics from ours, who were exclusively patients with incurable cancer.

The relationship between skeletal muscle mass and impaired functional capacity in patients with cancer has not been fully elucidated. Although muscle mass is often recognized as a major contributor of strength, there is a growing body of evidence that in patients with cancer myosteatosis is an important feature that negatively impacts physical function [3,10]. One hypothesis is that changes in muscle mass and muscle density are caused by different molecular factors originating from the tumor [4,7]. According to this theory, both muscle abnormalities do not necessarily correlate with each other and might have independent effects on physical function, but when combined indicate a worse functional capacity than when appearing in isolation. This is consistent with our findings.

Regarding the association between skeletal muscle phenotypes and inflammation in patients with cancer, the results of the present study are in agreement with others, also using CT to evaluate body composition, in which low muscle mass [25,26,27] and myosteatosis [20,21,26,28] were associated with increased inflammatory response. Due to the cross-sectional design of these studies, it is not possible to draw conclusions about the causal associations between low SMI/SMD and the presence of systemic inflammation. Although longitudinal studies examining the correlation between inflammation and myosteatosis in

patients with cancer are lacking, studies in type II diabetes patients found a lipotoxic effect of myosteatosis as a driver in the development of insulin resistance, which can increase systemic inflammation [29,30]. On the other hand, given that in cancer the etiology of muscle alterations involves metabolic impairments that include insulin resistance [31,32], it is likely that the inflammatory nature of cancer leads to the development of myosteatosis, which in turn exacerbates SIR, thus suggesting a bidirectional association between these conditions. However, further studies are required to elucidate the causal effect of this association. In relation to skeletal muscle quantity, data from longitudinal studies have demonstrated that increased SIR at baseline is associated with lower muscle mass in the follow-up [33,34].

Our results concerning survival analysis showed that even when not occurring concorrently with low muscle mass, myosteatosis was still associated with a higher risk of mortality. Our findings are supported by a meta-analysis including 21,222 patients with cancer at different disease stages, which demonstrated that those classified as having myosteatosis had 75% greater mortality risk than the non-myosteatotic patients. Eleven of the 40 articles selected for inclusion in this study were exclusively of advaced patients with advanced cancer, four of which showed no association between myosteatosis and mortality [12]. Also in agreement, a recent systematic review demonstrated that low muscle mass was not associated with shorter survival in patients with incurable cancer in most of the studies included [5]. The reasons for the differences in the prognostic value of low muscle mass and myosteatosis are not clear. It is possible that the increase in muscle fat infiltration occurs before the decline SM. Thus, myosteatosis is detected earlier than the decrease in SMI.

Finally, the limitations and strengths of the current study deserve attention. Due to the cross-sectional design of the analysis, we cannot draw inferences regarding the causal relationship between skeletal muscle phenotypes, functional impairment, and altered SIR. Secondly, the inclusion criteria could have induced a selection bias, since only patients with CT scans available were included. On the other hand, one strength of this study is that it used CT for the assessment of muscle abnormalities, which is recognized for its high accuracy in identifying muscle mass and myosteatosis, the latter of which has not been widely investigated in patients with incurable cancer. In addition, we collected a representative and relatively large sample of patients with incurable cancer and followed up for mortality.

In conclusion, low SMI and myosteatosis, particularly when combined, correlated with functional impairment and the presence of an increased SIR in patients with incurable cancer, whereas only those with myosteatotic phenotypes correlated with a higher mortality risk. These findings suggest that low SMI and myostatosis are both important indicators of

poor clinical outcomes in advanced-stage patients with cancer, and their co-occurrence is a predictor of worse clinical outcomes, confirming our primary hypothesis.

Statement of authorship
Larissa Calixto-Lima: project conception; development of the overall research plan; analysis and interpretation of data; drafting the article and had primary responsibility for final content. **Emanuelly Varea Maria Wiegert, Livia Costa de Oliveira and Gabriela Villaça Chaves**: project conception, development of research plan; critical revision of important intellectual content. **Flavia Fioruci Bezerra and Carla Maria Avesani:** project conception, development of the overall research plan; interpretation and editing; critical revision of important intellectual content. **All authors:** read and approved the final version submitted.

Conflict of interest
None declared.

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CONSIDERAÇÕES FINAIS

A depleção muscular e a mioesteatose são condições frequentes em pacientes com câncer, particularmente na fase avançada da doença. Particularmente a massa muscular, além de consistir em um importante componente para identificação da caquexia, é o principal fator associado a redução da qualidade de vida e mortalidade. Por essa razão, todos os pacientes com câncer devem ser avaliados regularmente quanto a sua composição corporal. Considerando a inviabilidade da utilização de medidas consideradas padrão-ouro para avaliação da massa muscular na prática clínica, tais como a TC e a RM, a presente tese propõe uma equação de predição como alternativa simples e de baixo custo para avaliação da massa muscular de pacientes com câncer avançado utilizando medidas antropométricas e a força de preensão manual.

Em pacientes com câncer, além da baixa massa muscular, a mioesteatose também está associada à desfechos desfavoráveis. A avaliação da mioesteatose por meio da TC parte da premissa que o valor de radiodensidade do músculo reduz com o aumento da quantidade de TA intramuscular. A presente tese demostrou os fatores associados a radiodensidade do músculo esquelético, destacando-se a associação entre este e a localidade de alguns tumores. Embora características como idade, raça/cor da pele e o local do tumor primário sejam fatores não modificáveis relacionados a radiodensidade do músculo esquelético, a resposta inflamatória sistêmica, a força muscular, a quantidade de massa muscular, bem como o grau de adiposidade são fatores de risco modificáveis cujas alterações, se identificadas precocemente, poderão permitir uma intervenção terapêutica oportuna de forma a prevenir a infiltração de gordura no músculo.

Por fim, demostramos em nosso estudo que fenótipos de baixa massa muscular e mioesteatose, particularmente quando combinados, se associaram à pior função física e maior resposta inflamatória sistêmica avaliada por meio de diferentes indicadores inflamatórios. Estes achados sugerem que as duas anormalidades musculares – baixa massa muscular e mioesteatose – representam fenótipos clínicos distintos e, quando combinadas, podem conferir efeito adicional no prognóstico clínico do paciente com câncer.

Futuros estudos são necessários para testar a reprodutibilidade da equação de predição proposta em grupos semelhantes, bem como para avaliar se esta ferramenta é eficaz para o acompanhamento do estado nutricional de pacientes com câncer avançado, propiciando, assim, intervenções mais oportunas e individualizadas. De forma similar, estudos futuros que investiguem os fatores associados a radiodensidade do músculo esquelético, especialmente aqueles relacionados ao câncer, ajudarão na melhor compreensão da fisiopatologia da mioesteatose no câncer. Conhecer estes fatores podem possibilitar intervenções precoces nos estágios iniciais da doença objetivando a preservação da “qualidade muscular” a medida que os estágios da doença avançam.

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APÊNDICE A - Termo de consentimento livre e esclarecido

Título do projeto: Diagnóstico nutricional diferencial e qualidade de vida de pacientes com câncer avançado em cuidados paliativos

Você está sendo convidado a participar de uma pesquisa porque possui atendimento nesta instituição e teve diagnóstico de câncer. Para que você possa decidir se quer participar ou não, precisa conhecer os benefícios, os riscos e as consequências pela sua participação.

Este documento é chamado de Termo de Consentimento Livre e Esclarecido e tem esse nome porque você só deve aceitar participar desta pesquisa depois de ter lido e entendido este documento. Leia as informações com atenção e converse com o pesquisador responsável e com a equipe da pesquisa sobre quaisquer dúvidas que você tenha. Caso haja alguma palavra ou frase que você não entenda, converse com a pessoa responsável por obter este consentimento, para mais esclarecimentos. Converse com os seus familiares, amigos e com a equipe médica antes de tomar uma decisão. Se você tiver dúvidas depois de ler estas informações, entre em contato com o pesquisador responsável.

Após receber todas as informações, e todas as dúvidas forem esclarecidas, você poderá fornecer seu consentimento por escrito, caso queira participar.

PROPÓSITO DA PESQUISA

Esta pesquisa tem como objetivo avaliar o estado nutricional dos pacientes com câncer avançado. O estado nutricional é a quantidade de energia e de massa muscular que uma pessoa possui armazenada, o que dependerá, principalmente, do tipo e da quantidade de alimento que ela ingere, e se ela pratica exercício físico ou não.

PROCEDIMENTOS DA PESQUISA

Neste estudo iremos:

- Avaliar o seu estado nutricional por meio de medidas do seu corpo que incluem pesar, medir a altura, passar uma fita ao redor do seu braço e da sua panturrilha e ver a gordura do seu braço. Com ajuda de um aparelho chamado bioimpedância, vamos conhecer a quantidade de músculo e de gordura do seu corpo. Para fazer a bioimpedância você vai ficar deitado e colocaremos uns sensores iguais ao do aparelho de eletrocardiograma. É um exame que não fura, não tem radiação e não acarreta nenhum tipo de complicação. Iremos também avaliar sua força manual utilizando um aparelho chamado dinamômetro.
- Avaliar algumas questões relacionadas à sua alimentação, ao seu estado nutricional e a sua qualidade de vida utilizando perguntas que serão feitas em um único momento (nas consultas ambulatoriais ou durante internação hospitalar na instituição). Estes questionários normalmente são respondidos em no máximo 20 minutos.
- Fazer um exame de sangue que irá avaliar se você tem algum grau de inflamação, bem como seu estado nutricional. A coleta de sangue é a que habitualmente você já realiza no seu tratamento e será realizada por um profissional de saúde que empregará todos os procedimentos adequados. Entretanto, alguns sinais e sintomas relacionados à coleta de sangue podem aparecer, como dor e infecção no local de punção ainda que pouco frequentes e temporários.

BENEFÍCIOS

Você não será remunerado por sua participação e esta pesquisa não poderá oferecer benefícios diretos a você. Se você concordar com o uso de suas informações, é necessário esclarecer que você não terá quaisquer benefícios ou direitos financeiros sobre eventuais resultados decorrentes desta pesquisa.

O benefício principal da sua participação é possibilitar que, no futuro, com os resultados alcançados com esta pesquisa, o diagnóstico e o tratamento para esse tipo de câncer beneficiem outros pacientes. Ao participar deste estudo, você terá a oportunidade de realizar uma avaliação mais completa e detalhada da sua saúde, além de identificar se você precisa de um reforço nutricional específico. Você terá acesso a todos os seus resultados se assim desejar. Com o resultado dessa pesquisa poderemos conhecer melhor o estado nutricional da população dos indivíduos com câncer avançado no Brasil.

RISCOS

Não existem riscos físicos adicionais a você pela sua participação nesta pesquisa. É importante que você entenda que nenhum procedimento médico adicional será realizado. O seu tratamento será exatamente o mesmo caso você participe ou não deste estudo.

CUSTOS

Você não terá quaisquer custos ou despesas (gastos) pela sua participação nessa pesquisa. Você não pagará por qualquer procedimento ou teste exigido como parte desta pesquisa.

CONFIDENCIALIDADE

Se você optar por participar desta pesquisa, as informações sobre a sua saúde e seus dados pessoais serão mantidas de maneira confidencial e sigilosa. Seus dados somente serão utilizados depois de anonimizados (ou seja, sem sua identificação). Apenas os pesquisadores autorizados terão acesso aos dados individuais, resultados de exames e testes bem como às informações do seu registro médico. Mesmo que estes dados sejam utilizados para propósitos de divulgação e/ou publicação científica, sua identidade permanecerá em segredo.

TRATAMENTO MÉDICO EM CASO DE DANOS

Todo e qualquer dano decorrente do desenvolvimento desta pesquisa, e que necessite de atendimento médico, ficará a cargo da instituição. Seu tratamento e acompanhamento médico independem de sua participação nesta pesquisa.

BASES DA PARTICIPAÇÃO

A sua participação é voluntária e a recusa em autorizar a sua participação não acarretará quaisquer penalidades ou perda de benefícios aos quais você tem direito, ou mudança no seu tratamento e acompanhamento médico nesta instituição. Você poderá retirar seu consentimento a qualquer momento sem qualquer prejuízo. Em caso de você decidir interromper sua participação na pesquisa, a equipe de pesquisadores deve ser comunicada e os procedimento referentes à pesquisa será imediatamente interrompido.

ACESSO AO RESULTADOS DE EXAMES

Você pode ter acesso a qualquer resultado relacionado à esta pesquisa. Estes resultados serão enviados ao seu médico e ele os discutirá com você. Se você tiver interesse, você poderá receber uma cópia dos mesmos.

GARANTIA DE ESCLARECIMENTOS

A pessoa responsável pela obtenção deste Termo de Consentimento Livre e Esclarecido lhe explicou claramente o conteúdo destas informações e se colocou à disposição para responder às suas perguntas sempre que tiver novas dúvidas. Você terá garantia de acesso, em qualquer etapa da pesquisa, sobre qualquer esclarecimento de eventuais dúvidas e inclusive para tomar conhecimento dos resultados desta pesquisa. Neste caso, por favor, ligue para uma das seguintes nutricionistas, no horário entre 8hs e 20hs: EmanuellyVarea Maria Wiegert - (21) 97577-0548; Larissa Calixto Lima (21) 99172-9948; e Livia Costa de Oliveira - (21) 98644-8650.

Esta pesquisa foi aprovada pelo Comitê de Ética em Pesquisa (CEP) do INCA, que está formado por profissionais de diferentes áreas, que revisam os projetos de pesquisa que envolvem seres humanos, para garantir os direitos, a segurança e o bem-estar de todos as pessoas que se voluntariam a participarem destes. Se tiver perguntas sobre seus direitos como participante de pesquisa, você pode entrar em contato com o CEP do INCA na Rua do Resende N°128, Sala 203, de segunda a sexta de 9:00 a 17:00 hs, nos telefones (21) 3207-4550 ou 3207-4556, ou também pelo e-mail: cep@inca.gov.br.

Este termo está sendo elaborado em duas vias, sendo que uma via ficará com você e outra será arquivada com os pesquisadores responsáveis.

CONSENTIMENTO

Li as informações acima e entendi o propósito da solicitação de permissão para o uso das informações contidas no meu registro médico e de parte de meu tumor e/ou meu sangue obtidos durante o atendimento nesse hospital. Tive a oportunidade de fazer perguntas e todas foram respondidas. Ficaram claros para mim quais são procedimentos a serem realizados, riscos e a garantia de esclarecimentos permanentes. Ficou claro também que a minha participação é isenta de despesas e que tenho garantia do acesso aos dados e de esclarecer minhas dúvidas a qualquer tempo. Entendo que meu nome não será publicado e toda tentativa será feita para assegurar o meu anonimato.

Concordo voluntariamente em participar desta pesquisa e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidade ou prejuízo ou perda de qualquer benefício que eu possa ter adquirido.

Eu, por intermédio deste, dou livremente meu consentimento para participar nesta pesquisa.

	/ /
Nome e Assinatura do participante	Data
	/ /
Nome e Assinatura do Responsável Legal/Testemunha Imparcial (quando pertinente)	Data

Eu, abaixo assinado, expliquei completamente os detalhes relevantes desta pesquisa ao paciente indicado acima e/ou pessoa autorizada para consentir pelo mesmo. Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste paciente.

	/ /
Nome e Assinatura do Responsável pela obtenção do Termo	Data

ANEXO A - Aprovação do Comitê de Ética em Pesquisa

INSTITUTO NACIONAL DE CÂNCER/ INCA/ RJ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Diagnóstico nutricional diferencial e qualidade de vida de pacientes com câncer avançado em cuidados paliativos

Pesquisador: LIVIA COSTA DE OLIVEIRA

Área Temática:

Versão: 2

CAAE: 52396416.4.0000.5274

Instituição Proponente: Hospital do Câncer IV

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.407.458

Apresentação do Projeto:

Conforme Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016.

Objetivo da Pesquisa:

Conforme Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016.

Avaliação dos Riscos e Benefícios:

Conforme Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016.

Comentários e Considerações sobre a Pesquisa:

Conforme Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016.

Considerações sobre os Termos de apresentação obrigatória:

Conforme Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016.

Recomendações:

Conforme parecer 1.396.382.

Endereço: RUA DO RESENDE, 128 - SALA 203
Bairro: CENTRO **CEP:** 20.231-092
UF: RJ **Município:** RIO DE JANEIRO
Telefone: (21)3207-4550 **Fax:** (21)3207-4556 **E-mail:** cep@inca.gov.br

Conclusões ou Pendências e Lista de Inadequações:

Trata-se da análise das respostas às pendências apontadas no Parecer CConsubstanciado do CEP-INCA de número 1.396.382, datado de 27 de Janeiro de 2016:

Pendência: No item 4.2 do Projeto, sugere-se alterar título "Sujeitos da Pesquisa" por "Participantes da Pesquisa", atendendo à recomendação de Resolução 466/2012.

Resposta: Atendido. O título foi alterado para Participantes da Pesquisa (página 15, linha 450).

Análise: Pendência atendida.

Pendência: Esclarecer, acerca de tamanho da amostra, que figura como "0" na PB, estimativa de participantes a serem envolvidos ao longo de período assinalado, em que pese definição de amostragem de conveniência, tendo em vista abrangência de objetivos propostos e variedade de medidas que se pretende inferir dos participantes.

Resposta: O item foi atendido e mesmo se tratando de uma amostragem não probabilística de conveniência, foi realizada uma estimativa de captação mensal de participantes para a pesquisa de acordo com o número médio anual de atendimentos de primeira vez do HCIV e o texto reformulado.

Novo texto (página 15, linha 435): Será realizado um estudo clínico, observacional de coorte, com amostragem não probabilística de conveniência, com pacientes com câncer avançado atendidos na unidade de cuidados paliativos (HC IV) do INCA, na cidade do Rio de Janeiro/RJ. Estima-se de acordo com o número médio anual de atendimentos de primeira vez que o número de captação de participantes será de 80 pacientes/mês.

Análise: Pendência atendida.

Pendência: Quanto ao Orçamento apresentado nestes arquivos como em documento anexo, definido na PB como de financiamento próprio, pede-se ratificar tal condição em vista de valor considerável apresentado, esclarecendo se há (ou não) presença dos equipamentos referidos na instituição e, se necessário, acerca de sua autorização de uso.

Resposta: A condição foi ratificada com o esclarecimento a cerca da disponibilidade dos equipamentos necessários para a realização da pesquisa.

Novo texto (página 25, linha 736): O INCA não possui disponibilidade dos equipamentos supracitados, de forma que estes serão requeridos por meio de auxílio à pesquisa (FAPERJ - Auxílio ao Pesquisador Recém-contratado – ARC). Caso não ocorra concessão do mesmo, os pesquisadores responsáveis pelo estudo disponibilizarão equipamentos próprios.

Análise: Pendência atendida.

Pendência: Quanto aos instrumentos empregados, fica compreendido que são utilizados na rotina do Serviço, igualmente para pacientes de ambulatório como aqueles internados de que se compõem a amostra do presente estudo (com exceção, ao que parece, de questionário de Qualidade de Vida), validando a ausência de custos (de transporte, por exemplo) prevista em outro documento (TCLE). Pede-se confirmar.

Resposta: O item foi atendido.

Novo texto (página 25, linha 740): Os participantes do estudo serão avaliados no decurso da consulta ambulatorial rotineira ou durante internação hospitalar, não necessitando de deslocamento adicional. Desta forma, não haverá nenhum dispêndio financeiro por parte do paciente.

Análise: Pendência atendida.

Pendência: Solicita-se descrever melhor etapa de treinamento "teórico/prático" a ser realizada previamente ao estudo piloto e coleta de dados, incluindo a previsão de possíveis novos egressos à equipe de pesquisa ao longo do estudo.

Resposta: Demais esclarecimentos foram escritos a respeito do treinamento teórico/prático.

Novo texto (página 25, linha 740): Os entrevistadores participarão de um treinamento realizado no

INCA sob supervisão do coordenador da pesquisa, com duração total de 24 horas. Tal atividade constará de uma etapa teórica com o estudo de manuais e publicações científicas pertinentes aos questionários utilizados e as técnicas de avaliação antropométrica descritas por Gordon et al. (LOHMAN et al.,1988) e Frisancho (1974). Em seguida, os entrevistadores serão padronizados para aplicação dos questionários e aferição das medidas antropométricas de acordo com a proposta de Habicht et al. (1974), sendo avaliados segundo exatidão e precisão. A entrada de novos entrevistadores será condicionada a realização de novos treinamentos.

Análise: Pendência atendida.

Pendência: No item 4.6 de Projeto, é mencionado que resultados "serão utilizados para elaboração de duas teses de doutorado e um trabalho de conclusão de Residência", o que se verifica ainda no preenchimento de documentação (Formulário de Submissão), entretanto não há vínculos institucionais de ensino explícitos ao longo de proposta de investigação, e quanto à equipe de pesquisa apresentada nela não constam residentes. Esclarecer.

Resposta: Embora haja pretensão de que os dados possam ser utilizadas em futuras teses de doutorado das pesquisadoras auxiliares Emanuelly Varea Maria Wiegert e Larissa Calixto Lima, ainda não há vínculos institucionais de ensino estabelecidos e por isso não foram explicitados no formulário de submissão. Caso isso ocorra, os projetos das referidas propostas de teses serão submetidos a reapreciação do CEP. Portanto, esta informação foi retirada do Formulário de Submissão e do projeto.
Entretanto, foi incluso no Formulário de Submissão a proposta de pesquisa do Trabalho de Conclusão de Residência Multiprofissional em Oncologia bem como o nome da discente como integrante da equipe de pesquisa. Poderá haver inclusão de novos pesquisadores na coleta de dados no decorrer do estduo condicionada a comunicação prévia a este CEP e posterior treinamento.

Análise: Pendência atendida.

Pendência: Em relação ao Questionário de Qualidade de Vida a ser utilizado no estudo, é mencionada autorização para seu emprego no texto, solicitando-se confirmação por meio de mensagens trocadas com editor responsável. E alerta-se para rever cabeçalho deste instrumento

em anexo, onde constam iniciais de paciente, o que deve dar lugar a um padrão de codificação numérica que assegure anonimato de participantes.

Resposta: Item foi atendido. Anexo comprovação solicitada.
Item foi acatado. Substituída versão anexada pela enviada pelo editor responsável pelo questionário (página 45).

Análise: Pendência atendida.

Considerações Finais a critério do CEP:
Diante do exposto, o Comitê de Ética em Pesquisa do Instituto Nacional de Câncer (CEP-INCA), de acordo com as atribuições definidas na Resolução CNS 466/12 e na Norma Operacional Nº 001/2013 do CNS, manifesta-se pela aprovação do projeto de pesquisa proposto.
Ressalto o(a) pesquisador(a) responsável deverá apresentar relatórios semestrais a respeito do seu estudo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_651351.pdf	05/02/2016 15:47:50		Aceito
Outros	FORMULARIO_DE_SUBMISSAO.pdf	05/02/2016 14:26:00	Larissa Calixto Lima	Aceito
Outros	QLQ_C15_AUTORIZACAO_DE_USO.pdf	05/02/2016 14:24:02	Larissa Calixto Lima	Aceito
Outros	RESPOSTA_DO_PARECER.doc	04/02/2016 20:43:02	Larissa Calixto Lima	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_DE_PESQUISA_COM_CORRECOES_CEP.doc	04/02/2016 20:40:49	Larissa Calixto Lima	Aceito
Folha de Rosto	Folha_de_rosto.pdf	12/01/2016 13:52:55	Larissa Calixto Lima	Aceito
Orçamento	Orcamento.docx	11/01/2016 15:36:54	Larissa Calixto Lima	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	11/01/2016 15:35:12	Larissa Calixto Lima	Aceito
Declaração de Pesquisadores	Declaracao_de_pesquisadores.doc	11/01/2016 15:34:27	Larissa Calixto Lima	Aceito

INSTITUTO NACIONAL DE CÂNCER/ INCA/ RJ

Continuação do Parecer: 1.407.458

Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_de_manuseio_material_biologico.doc	11/01/2016 15:33:26	Larissa Calixto Lima	Aceito
Cronograma	Cronograma.docx	11/01/2016 15:26:58	Larissa Calixto Lima	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 12 de Fevereiro de 2016

Assinado por:
Carlos Henrique Debenedito Silva
(Coordenador)

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ANEXO B – Comprovante de submissão *Clinical Nutrition*

Clinical Nutrition

Altered skeletal muscle phenotypes are associated with functional impairment, worse systemic inflammatory response, and reduced survival in patients with incurable cancer

--Manuscript Draft--

Manuscript Number:	
Article Type:	Full Length Article
Keywords:	muscle mass, myosteatosis, incurable cancer, inflammation, physical function, survival
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Abstract:	Background & aims: The factors associated with the simultaneous occurrence of low muscle mass and myosteatosis are unclear. This study investigated whether different skeletal muscle phenotypes derived from a combination of low/high muscle mass and absence/presence of myosteatosis are associated with systemic inflammatory response, functional impairment, and survival in incurable cancer patients. Methods: Three hundred and twenty-six patients (median age 60 years, 67.5% female) with incurable cancer who had abdominal or pelvic computed tomography (CT) scans up to 30 days before the initial assessment were enrolled for the study. The CT images were used for the assessment of skeletal muscle index (SMI) (low SMI <45.0 cm2/m2 for males and <44.0 cm2/m2 for females) and for skeletal muscle radiodensity(SMD), which was used to evaluate myosteatosis (<34 HU for males and <30 HU for females). Based on these cutoffs, participants were classified into four phenotype groups: high SMI + non-myosteatotic; low SMI + non-myosteatotic; high SMI + myosteatotic; and low SMI + myosteatotic. Results: The phenotypes with low SMI or myostatosis, and especially the combination of both conditions, were associated with low handgrip strength (HGS), poor performance status, altered C-reactive protein and albumin concentrations, and worse inflammation-based prognostic scores. The phenotypes with myosteatosis, regardless of high SMI (HR: 1.74; 95% CI: 1.05-2.88) or low SMI (HR: 1.99; 95% CI: 1.29-3.05) were associated with a higher mortality risk, with high SMI + non-myosteatotic as the reference group. Conclusion: In patients with incurable cancer, the phenotype groups with low SMI and myosteatosis, particularly in combination, were correlated with worse functional impairment and altered inflammatory response. Moreover, the presence of myosteatosis increased the mortality risk.
Opposed Reviewers:	