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ORIGINAL RESEARCH

Leptin as a surrogate immune-metabolic marker to predict impact of anti-cachectic therapy: results of a prospective randomized trial in multiple solid tumors

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Description of the work: Leptin is a reliable predictive and surrogate marker of the efficacy of multitargeted treatment of cancer cachexia.

Purpose: To the best of our knowledge, no study has assessed the predictive role of biomarkers in establishing the effectiveness of anti-cachectic treatment, which remains a complex issue. Herein, we aimed to find a marker that can detect early response to anti-cachectic treatment.

Patients and methods: From January 2012 to December 2022, all consecutive eligible advanced cancer patients with cachexia were prospectively enrolled in an exploratory and validation cohort according to eligibility criteria. All patients received a combined anti-cachectic treatment consisting of megestrol acetate plus celecoxib plus L-carnitine plus antioxidants that showed efficacy in a previous phase III randomized study. Primary endpoints were an increase in lean body mass (LBM), a decrease in resting energy expenditure (REE), a decrease in fatigue, and improvement in global quality of life.

Results: A total of 553 consecutive patients were recruited. Twenty patients dropped out, equally distributed over the exploratory (11 patients) and validation (9 patients) cohorts, for early death due to disease progression. Then, 533 patients were deemed assessable. Leptin level changes inversely correlated with circulating levels of inflammatory mediators and reflected the improvement of body composition, energy metabolism, functional performance, and quality of life. At multivariate regression analysis, at week 8, leptin change was an independent predictor of LBM, skeletal muscle index (SMI), grip strength increase, and REE; at week 16, leptin change was an independent predictor of the same parameters and improvement in Eastern Cooperative Oncology Group performance status. The ability of leptin to predict changes in LBM, SMI, REE, and grip strength was superior to that of other inflammatory markers when comparing the receiver operating curves. Moreover, increasing delta leptin values were associated with significantly better outcomes in LBM, SMI, REE, grip strength, and fatigue.

Conclusions: Leptin is a reliable predictive marker for multitargeted anti-cachectic treatment outcomes. Thus, it can be an ideal candidate for monitoring and predicting the effects of anti-cachectic treatment and a surrogate marker of the immune-metabolic actions of the selected drugs.

Key words: cachexia, quality of life, inflammation, leptin, combined approach, sarcopenia

INTRODUCTION

Cancer cachexia is a multifactorial multiorgan disorder spanning the skeletal muscle, adipose tissue, liver, brain, endocrine, respiratory, gastrointestinal, and cardiac systems; a plethora of symptoms including anorexia, nausea, weight loss with reductions in lean body mass (LBM) and adipose tissue, anemia, and fatigue characterize it.[1,2]

Cancer cachexia is evidence of the evolution of the neoplastic disease,[3] resulting from metabolic changes induced by cancer during its development and progression and by the immune cells involved in the phases of resistance and tolerance that characterize the immune response to cancer.[4-6] Such metabolic changes, including reduced

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energy intake, elevated catabolism, and excess resting energy expenditure (REE), with specific changes in glucose, lipid, and protein metabolism, are likely caused by the direct action of immune mediators, i.e. proinflammatory cytokines, that play a key role in the pathogenesis and progression of cancer cachexia.[4,6-8] Proinflammatory cytokines operate within the tumor microenvironment and interact with other body tissues, generating a systemic chronic inflammatory response.[6]

Accordingly, numerous studies, including our own,[9-16] have demonstrated that cancer cachexia is associated with high levels of proinflammatory cytokines and acute-phase proteins [fibrinogen and C-reactive protein (CRP)], proportional to its severity. Conversely, the circulating levels of leptin, a key parameter of nutritional status, belonging to the adipokine family, exhibit an inverse correlation with cancer cachexia.[17,18] Leptin plays a crucial role in conveying important information about energy storage to the brain and regulating appetite and energy metabolism.[19] Defects in energy efficiency, especially glucose, hamper leptin synthesis, independent of changes in total body and adipose tissue mass.[20] Consistently, in muscle wasting and sarcopenia, low leptin levels reflect a state of persistent energy depletion and are directly related to reduced muscle mass and function.[21]

Cancer cachexia syndrome affects >50% of patients with metastatic cancer,[22] leading to a progressive decline in physical functioning,[22] reduced quality of life (QoL), diminished tolerance and response to anticancer treatment, and ultimately decreased survival.[23-26] Therefore, attempts are undertaken to identify cancer cachexia as a therapeutic target to alleviate patients' symptoms and improve their QoL and survival.

Despite the deep knowledge of cancer cachexia pathogenesis, no licensed or approved treatment or standard of care has been established yet.[27] Multiple clinical trials assessing unimodal treatments have failed to reach meaningful endpoints or gain regulatory approval. Indeed, there is a growing consensus that the management of cancer cachexia should reflect its multisystem and multifactorial nature using a multimodal approach.[28] This evidence led us to test multitarget treatments, demonstrating positive results.[29-32] In particular, a pharmacological approach[32] consisting of megestrol acetate (MA), celecoxib, L-carnitine, and antioxidants supported the merits of combination therapy in improving several main endpoints. It was inserted as an evidence basis for American Society of Clinical Oncology (ASCO) guidelines recommendations published in 2020[33] and in National Comprehensive Cancer Network (NCCN) guidelines in 2023.[34]

To enhance anti-cachectic treatment, researchers acknowledge the need for a reliable parameter that can reflect the various alterations of cachexia and adequately monitor and predict the treatment's outcomes.[35,36] Several nutritional/inflammatory tools have been increasingly applied in cancer cachexia and are emerging as important prognostic and predictive parameters.[37-43] None of them, however, have been tested to predict the effectiveness of anti-cachectic approaches.

Considering this, we hypothesized that leptin could be used as a marker of cancer cachexia linked to its main changes and symptoms. Therefore, in the present study, we aimed to prospectively explore the role of leptin—in predicting the efficacy of a combined anti-cachectic treatment in patients with advanced cancers at different sites affected by cachexia.

PATIENTS AND METHODS

Study design and patients

Between April 2006 and January 2011, we conducted a phase III, open-label, controlled, prospective, randomized study to evaluate the effectiveness of a combination treatment comprising MA along with L-carnitine, celecoxib, and antioxidants (α-lipoic acid and carboxycysteine) in improving key parameters and symptoms of cachexia in patients with advanced gynecological cancers. The study involved 144 patients. The combination treatment was superior to MA alone in improving LBM, REE, fatigue, QoL, and appetite.[32] Following these positive results, from January 2012 to December 2022, an independent cohort of new consecutive advanced cancer patients (external to the previous randomized study) was prospectively enrolled in the combination arm into an exploratory and temporally split validation cohort according to the eligibility criteria and were included in the present analysis. Detailed inclusion and exclusion criteria are listed in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103738. The detailed treatment protocol is reported in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.103738. The patients were recruited at the Department of Gynecologic Oncology, Businco Hospital, ARNAS G. Brotzu, Cagliari; the Medical Oncology Unit at the Azienda Ospedaliero Universitaria, Cagliari; and the Medical Oncology Unit, "N.S. Bonaria" Hospital, San Gavino, Italy. The study protocol was approved by the Institutional Ethics Committee of the "Azienda Ospedaliero Universitaria" of Cagliari (protocol number 237/10/CE) and was conducted following the guidelines of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all patients. All patients provided informed consent for data publication.

Assessments

All patients were evaluated at baseline before treatment initiation. The following clinical data were collected: anthropometric parameters [age, sex, weight, height, and body mass index (BMI)], tumor histology and stage, current chemotherapy regimen, and Eastern Cooperative Oncology Group performance status (ECOG PS). Stage and extent of measurable disease were evaluated using total body computed tomography (CT) carried out no more than 1 month before treatment initiation. The weight lost within

the past 6 months was obtained from patients upon admission and used for analysis. BMI-adjusted weight loss grade (WLGS) was assessed according to the protocol described by Martin et al.[44] According to Fearon et al.,[2] the diagnostic criteria for cachexia are $\geq$5% weight loss over the past 6 months, or $\geq$2% weight loss in individuals with BMI $<$20 kg/m^2, or skeletal muscle mass consistent with sarcopenia.[14] The laboratory tests included the following: blood cell count; chemical profile; main parameters related to inflammation and nutritional status [hemoglobin, absolute neutrophil count, absolute lymphocyte count, neutrophil-to-lymphocyte ratio (NLR), CRP, and serum albumin]; modified Glasgow Prognostic Score (mGPS)[40]; serum leptin; serum proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α; blood levels of reactive oxygen species (ROS).

Efficacy endpoints

To establish the efficacy of anti-cachectic treatment and the predictive role of leptin in treatment outcomes, the following primary endpoints, as per the original design of the randomized phase III trial, were considered: an increase in LBM and skeletal muscle index (SMI),[45] a decrease in REE, a decrease in fatigue,[46] and an improvement in global QoL assessed by an appropriate questionnaire. The study also considered secondary endpoints: improvement of appetite, increase in grip strength, and ECOG PS. The study endpoints were selected from the defining variables of cachexia, as identified by international consensus guidelines.[2] The endpoints were assessed before treatment (T0), at 8 (T1), and 16 (T2) weeks after treatment onset, and the delta value, that is the difference between the T1, T2, and T0 values, was calculated. The study also evaluated the changes in the main inflammatory/nutritional parameters associated with the clinical course of cachexia, such as IL-6, TNF-α, CRP, NLR, ROS, hemoglobin, albumin, and mGPS during treatment. The Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2024.103738 provides detailed methods for assessing the parameters above.

Statistical analyses

Statistical analysis and sample size calculation are detailed in Supplementary material, available at https://doi.org/10.1016/j.esmoop.2024.103738.

RESULTS

Baseline clinical, nutritional, and laboratory characteristics of enrolled patients

From January 2012 to December 2022, 553 patients with advanced stage IV cancers were recruited. A total of 20 patients dropped out, equally distributed over the exploratory (11 patients) and external validation (9 patients) cohorts, for early death due to disease progression. Then, 533 patients were deemed assessable (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.103738, CONSORT Diagram). The clinical characteristics of the enrolled patients are shown in Table 1. Supplementary Table S3 (ex Table 2), available at https://doi.org/10.1016/j.esmoop.2024.103738 displays body composition, metabolic, nutritional, and inflammatory data for patients in both cohorts. Of the total population, 219 patients (41.1%) had an mGPS of 1, and 194 patients (36.3%) had an mGPS of 2. This indicates that most patients suffered from an inflammatory condition linked with a weakened nutritional status. By grouping patients according to cachexia severity (mild, i.e. weight loss $<$10%, or severe, i.e. weight loss $>$10%), we found a significant difference in LBM, SMI, REE, leptin, CRP, IL-6, and hemoglobin (Supplementary Table S3, ex Table 2, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Association between leptin levels and parameters of body composition, energy expenditure, inflammation, and nutritional status at baseline

Leptin levels at baseline were positively correlated with BMI, fat mass, and SMI. Conversely, a significant inverse correlation was found between baseline leptin levels, BMI-adjusted WLGS, REE, CRP, IL-6, and mGPS (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Leptin levels were significantly lower in patients with sarcopenia than those without sarcopenia both in the exploratory ($P = 0.006977$; P for trend $= 0.00700$) and validation cohort ($P = 0.029674$; P for trend $= 0.01030$) (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.103738). Logistic regression analysis revealed a significant inverse correlation between baseline leptin levels and sarcopenia, both in the exploratory [$r = -0.031492$; odds ratio (OR) $= 0.9690$; 95% confidence interval (CI) 0.9444-0.9943; $P = 0.0165$] and validation cohort ($r = -0.039504$; OR $= 0.9613$; 95% CI 0.9053-0.9758; $P = 0.0314$) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Treatment endpoints

Consistent with the results of our phase III randomized study,[32] we found that combined anti-cachectic treatment resulted at 8 weeks in a significant increase in LBM and SMI, a significant decrease in REE, and a significant improvement in fatigue and overall QoL; at 16 weeks in a significant increase of total body weight, LBM, SMI, a significant decrease in REE, a significant improvement of fatigue and QoL both in the exploratory and validation cohort. Among the secondary endpoints, significant improvements were observed in the exploratory and validation cohort at 8 and 16 weeks in appetite and ECOG PS (Table 2). Such improvements in the primary and secondary endpoints were accompanied by a significant increase in leptin and a significant decrease in CRP, IL-6, and mGPS both at 8 and 16 weeks, and a decrease in ROS at 16 weeks, both in the exploratory and validation cohort (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Table 1. Patients anthropometric and clinical characteristics at baseline

	Exploratory cohort	Validation cohort	*P* value
Enrolled patients (No.)	315	218	
Male/female (%)	184/131 (58/42)	125/93 (57/43)	0.8185
Age, years: mean ± SD (range)	62.6 ± 10.6 (29-85)	63.7 ± 11.1 (31-86)	0.2536
Weight, kg: mean ± SD (range)	56.9 ± 11.9 (30-91)	56.6 ± 10.5 (33-86)	0.7643
Height, cm: mean ± SD (range)	163 ± 9.1 (140-196)	162.8 ± 9 (144-190)	0.8022
BMI: mean ± SD (range)	21.2 ± 3.9 (12.3-33.3)	20.9 ± 5.5 (12.8-33)	0.4615
Under-weight, No. (%)	73 (23.2)	51 (23.4)	
Norm-weight, No. (%)	203 (64.4)	141 (64.7)	
Over-weight, No. (%)	32 (10.2)	21 (9.6)	
Obese, No. (%)	7 (2.2)	5 (2.3)	0.9977
Pre-illness body weight, kg: mean ± SD (range)	65.9 ± 12.4 (39-98)	66.2 ± 14.5 (36-99)	0.7980
Weight loss at baseline, kg: mean ± SD (range)	−7 ± 5.1 (−27 to −2)	−7.3 ± 5.3 (−37 to −2)	0.5114
Weight loss at baseline: No. (%)			
<5%	47 (14.9)	32 (14.6)	
5-10%	136 (43.2)	93 (42.7)	
>10%	132 (41.9)	93 (42.7)	0.9849
BMI-adjusted WLGS: No. (%)			
1	29 (9.2)	20 (9.2)	
2	42 (13.3)	29 (13.3)	
3	123 (39.1)	86 (39.4)	
4	121 (38.4)	83 (38.1)	0.9998
Sarcopenia at baseline: No. (%)			
No	55 (17.5)	32 (14.7)	
Yes	260 (82.5)	186 (85.3)	0.3934
Tumor site: No. (%)			
Lung	74 (23.5)	51 (23.4)	
Gynecological	59 (18.7)	41 (18.8)	
Colorectal	49 (15.6)	34 (15.6)	
Pancreas, biliary tract, HCC	38 (12.1)	27 (12.4)	
Stomach	35 (11.1)	24 (11)	
Head and neck	32 (10.2)	22 (10.1)	
Breast	23 (7.3)	16 (7.3)	
Others	5 (1.5)	3 (1.4)	1.000
Stage: No. (%)			
IV	315 (100)	218 (100)	1.000
ECOG PS: No. (%)			
0	12 (3.8)	9 (4.1)	
1	84 (26.7)	58 (26.6)	
2	165 (52.4)	114 (52.3)	
3	54 (17.1)	37 (17)	0.9982
mGPS: No. (%)			
0	71 (22.5)	49 (22.5)	
1	129 (41)	90 (41.3)	
2	115 (36.5)	79 (36.2)	0.9969
Concomitant palliative chemotherapy: No. (%)			
Yes	229 (72.7)	158 (72.5)	
No	86 (27.3)	60 (27.5)	0.9551

P value between exploratory and validation cohort. Results were considered significant for $P < 0.05$.
BMI, body mass index; CRP, C-reactive protein; ECOG PS, Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma; mGPS, modified Glasgow Prognostic Score [mGPS = 2, both elevated CRP (≥10 mg/l) and low serum albumin levels (<3.5 g/dl); mGPS = 1, elevated CRP levels only; mGPS = 0, normal CRP levels (<10 mg/l)]; SD, standard deviation; WLGS, weight loss grade.

Correlation between changes in leptin levels and changes in the primary and secondary treatment endpoints and laboratory parameters of inflammation and nutritional status

Changes in leptin levels were positively correlated with changes in LBM, SMI, and grip strength after 8 and 16 weeks of treatment in both cohorts. Conversely, changes in leptin levels were inversely correlated with changes in REE and ECOG PS after 8 and 16 weeks of treatment in both cohorts. Changes in leptin levels were also positively correlated with changes in total body weight and BMI, but only after 16 weeks of treatment in both cohorts (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2024.103738). Our study found a significant correlation between changes in leptin levels and laboratory parameters of inflammation and nutritional status. This correlation was inverse with CRP, IL-6, changes in TNF-α, and changes in mGPS, and positive with changes in albumin levels. These results were observed at 8 and 16 weeks of treatment in both the exploratory and validation cohorts (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Table 2. Changes of primary and secondary endpoints during anticachectic treatment

Primary endpoints	Baseline		After 8 weeks of treatment				After 16 weeks of treatment			
	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	P^a	P^b	Exploratory cohort	Validation cohort	P^a	P^b
Total body weight, kg	56.7 ± 11.7	56.6 ± 10.5	56.9 ± 11.8	56.8 ± 11.6	0.6427	0.5896	57.9 ± 10.2	57.6 ± 11	**0.0120**	**0.0143**
CT										
LBM (kg)	40.5 ± 8.2	40.8 ± 8.8	42.5 ± 7.6	42.6 ± 7.1	**0.0042**	**0.0091**	44 ± 8.6	44.3 ± 8.1	**0.0004**	**0.0002**
Fat mass, kg	19.1 ± 5.1	19.3 ± 8	18.3 ± 5.4	17.9 ± 6.3	0.5506	0.4425	18 ± 6.2	18.1 ± 7.6	0.6030	0.5197
SMI, kg	40.9 ± 8.0	41.3 ± 7.9	41.6 ± 7.6	42.1 ± 7.5	**0.0025**	**0.0054**	43.4 ± 7.3	43 ± 7	**0.0002**	**0.0003**
REE										
Resting energy expenditure by indirect calorimetry, kcal	1243 ± 276.	1280 ± 280	1098.5 ± 276.3	1120 ± 299	**0.0078**	**0.0327**	1084 ± 311	1142 ± 345	**0.0072**	**0.0010**
Quality of life										
EORTC-QLQ-C30	57.3 ± 17.8	56.8 ± 18.6	62.1 ± 19.1	62 ± 16.5	**0.0108**	**0.0356**	65 ± 15.4	64.5 ± 17	**0.0053**	**0.0124**
Fatigue										
MFSI-SF	27.7 ± 12.1	26.5 ± 13	26.7 ± 11.6	25.3 ± 9.5	**0.0254**	**0.0120**	23.5 ± 9.5	22.9 ± 9.1	**0.0065**	**0.0049**
Secondary endpoints										
Appetite, VAS	5.0 ± 2.5	5.1 ± 2.4	6.0 ± 2.6	5.6 ± 2.5	**0.0090**	**0.0230**	6.6 ± 2.1	6.0 ± 2.5	**0.0067**	**0.0187**
Grip strength	25.5 ± 10.1	25.4 ± 9.2	25.6 ± 9.5	25.3 ± 8.6	0.1877	0.1776	26.9 ± 9.6	26.7 ± 9.3	0.0775	0.0854
ECOG PS	1.75 ± 0.5	1.8 ± 0.4	1.4 ± 0.6	1.5 ± 0.4	**0.035**	**0.029**	1.3 ± 0.7	1.4 ± 0.5	**0.012**	**0.016**

P value was calculated by Student's *t*-test or Wilcoxon test for paired data versus baseline values for the exploratory (P^a) and the validation cohort (P^b). Significant *P* values are indicated in bold.

CT, computed tomography; ECOG PS, Eastern Cooperative Oncology Group performance status; EORTC-QLQ, European Organisation For Research and Treatment of Cancer Quality of Life Questionnaire; LBM, lean body mass; MFSI-SF, Multidimensional Fatigue Symptom Inventory-Short Form; REE, resting energy expenditure; SMI, skeletal muscle index; VAS, visual analog scale.

Regression analysis between response to anti-cachectic treatment and the changes in leptin levels and other laboratory variables of inflammation and nutritional status

In both the exploratory and validation cohorts, logistic regression analysis showed a positive association between changes in leptin levels at week 8 and an increase in LBM, SMI, grip strength, and a decrease in REE. At week 16, changes in leptin levels showed a positive association with an increase in LBM, SMI, grip strength, a decrease in REE, and an improvement in ECOG PS. Changes in leptin levels, however, did not significantly affect fatigue, QoL, or appetite (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Among other laboratory parameters, we found significant negative correlations at week 8 between the following: an increase in LBM and SMI with changes in IL-6 and CRP levels, as well as between a decrease of REE and changes in ROS in both the exploratory and validation cohorts (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2024.103738). At week 16, we found a negative correlation between LBM and changes in IL-6, CRP, and mGPS, as well as between SMI and changes in IL-6, CRP, and mGPS. Additionally, we observed a negative correlation between a decrease in REE and changes in ROS, a decrease in fatigue and changes in NLR, an increase in QoL and changes in mGPS, and an increase in grip strength and changes in mGPS (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2024.103738). Moreover, we found a significant positive association between improvement in SMI and changes in albumin levels, as well as between increased grip strength and changes in albumin levels at 16 weeks, in both cohorts (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2024.103738). At multivariate regression analysis, leptin change at week 8 was an independent predictor of LBM increase, SMI increase, REE decrease, and increase in grip strength, in both cohorts. The significant independent predictive value of leptin change in terms of LBM increase, SMI increase, and REE decrease remained significant after being weighted for tumor site in both exploratory ($P = 0.0002$, $P < 0.0001$, $P = 0.0283$, $P = 0.0035$, respectively) and validation cohorts ($P = 0.0202$, $P < 0.001$, $P < 0.0001$, $P = 0.0088$, respectively). At week 16, leptin change was an independent predictor of LBM increase, SMI increase, REE decrease, increase in grip strength, and improvement in ECOG PS, in both cohorts (Table 3).

Among other laboratory parameters, change in the NLR at week 16 was an independent predictor of a decrease in fatigue, and change in mGPS at week 16 was an independent predictor of an increase in QoL in both cohorts (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2024.103738).

Receiver operating curve analysis

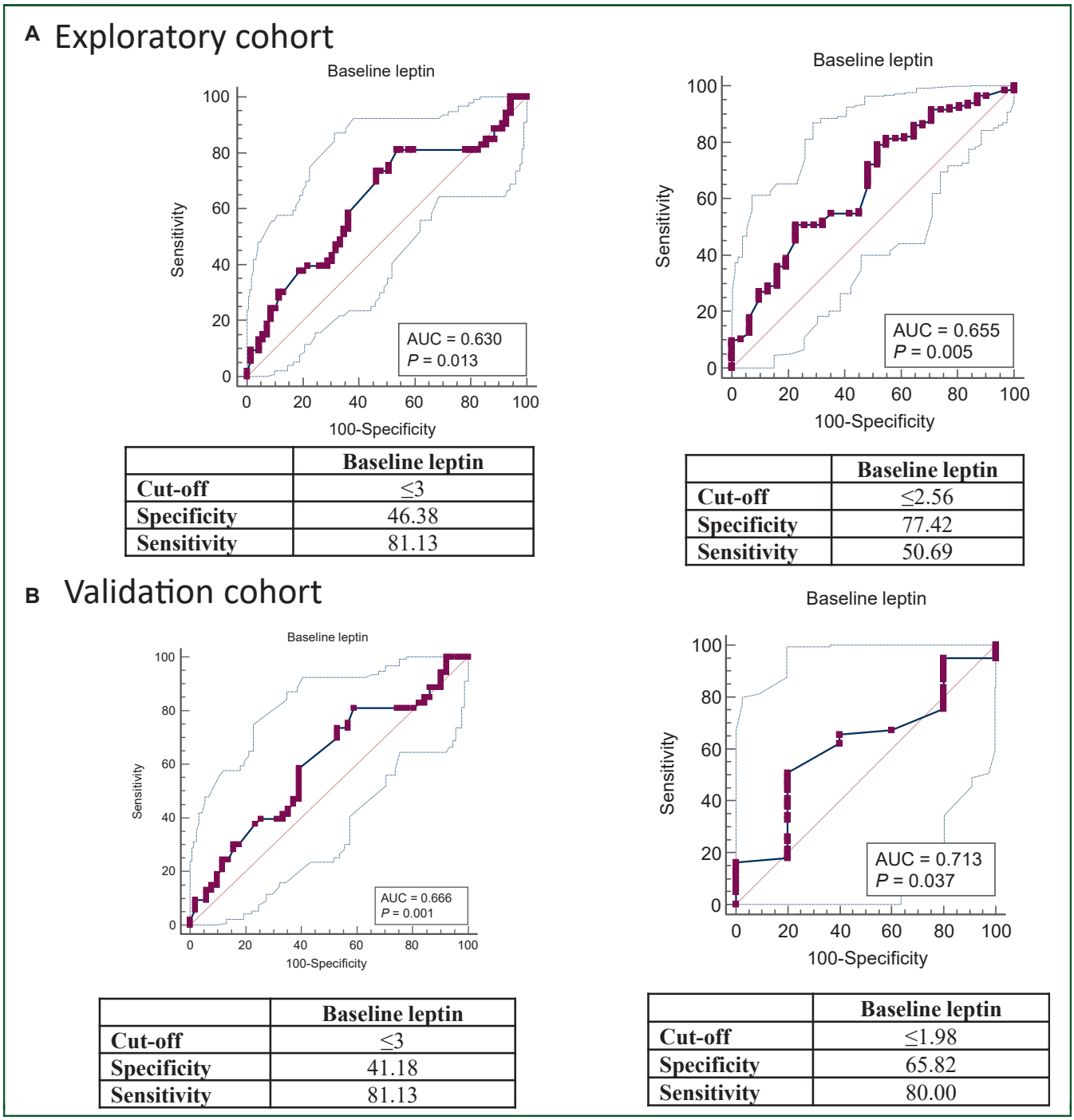
Leptin levels were analyzed using the receiver operating curve (ROC) to identify the severity of weight loss and the presence of sarcopenia. The exploratory cohort showed area under the curve (AUCs) of 0.630 ($P = 0.0130$) and 0.655 ($P = 0.0047$), while the validation cohort showed AUCs of 0.666 ($P = 0.001$) and 0.713 ($P = 0.037$), respectively (Figure 1). Other laboratory variables were not significant in predicting weight loss severity and sarcopenia at baseline.

Table 3. Multivariate logistic regression analysis of the predictive role of delta value of leptin and other laboratory variables of inflammation (i.e. IL-6, TNF, CRP, NLR, Fbg, GPS) of response to anticachectic treatment (responders/not responders in terms of LBM, SMI, REE, appetite, grip strength, fatigue, overall QoL)

Primary endpoints	Regression coefficient		OR		95% CI		*P* value	
Delta LBM	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort
Multivariate at week 8								
Delta leptin	1.28514	1.32949	3.6512	3.7791	1.6978-7.6977	1.7587-8.1205	**0.0009**	**0.0007**
Excluded variables								
Delta IL-6	−0.0021763	−0.023655	0.9978	0.9766	0.9162-1.0868	0.9050-1.0540	0.9602	0.5430
Delta CRP	−0.0049283	−0.017724	0.9951	0.9824	0.9435-1.0495	0.9243-1.0443	0.8561	0.5692
Multivariate at week 16								
Delta leptin	1.01790	2.18487	2.7674	8.8895	1.2842-5.9638	1.4982-52.7466	**0.0094**	**0.0162**
Excluded variables								
Delta IL-6	−0.0024154	−0.027280	0.9976	0.9371	0.9128-1.0902	0.8945-1.0586	0.9575	0.5256
Delta CRP	−0.018196	−0.048670	0.9280	0.9525	0.9125-1.0567	0.8539-1.0625	0.6268	0.3827
Delta mGPS	−0.57204	−0.61362	0.7719	0.8471	0.5969-2.2600	0.5646-2.0427	0.3028	0.3102
Delta SMI	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort
Multivariate at week 8								
Delta leptin	1.83631	1.91891	6.2733	6.8136	1.9231-20.4643	1.9960-23.2588	**0.0023**	**0.0022**
Excluded variables								
Delta IL-6	−0.013594	−0.075903	0.9865	0.9269	0.8473-1.1486	0.8213-1.0461	0.8610	0.2186
Delta CRP	−0.015669	−0.005041	0.9158	0.9950	0.8460-1.0908	0.9138-1.0834	0.6664	0.9076
Multivariate at week 16								
Delta leptin	1.80780	1.55508	6.0970	4.7355	1.8490-20.1049	1.3411-16.7209	**0.0030**	**0.0157**
Excluded variables								
Delta IL-6	−0.14039	−0.14321	0.8690	0.8666	0.4423-1.7072	0.7072-1.0618	0.6836	0.1671
Delta CRP	−0.026204	−0.15038	0.9741	0.8604	0.8435-1.1250	0.6756-1.0958	0.7213	0.2229
Delta mGPS	−0.89154	−0.95636	0.9583	0.9277	0.0414-4.9448	0.5334-3.1537	0.2352	0.1060
Delta albumin	2.31552	1.93408	1.8967	1.6746	0.3570-7.2147	0.3328-6.1193	0.3034	0.1892
Delta REE	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort
Multivariate at week 8								
Delta leptin	0.82814	0.71039	2.2891	1.1167	1.0385-4.6024	1.0467-3.3172	**0.0367**	**0.0450**
Excluded variables								
Delta ROS	−0.031126	−0.011621	0.9694	0.9884	0.8984-1.0460	0.9768-1.0003	0.4225	0.0550
Multivariate at week 16								
Delta leptin	0.85231	0.659340	2.3451	1.9335	1.1258-4.8846	1.0282-4.0277	**0.0228**	**0.0482**
Excluded variables								
Delta ROS	−0.097091	−0.086754	0.8675	0.9780	0.7580-1.0967	0.7654-1.1985	0.8970	0.9980
Secondary endpoints								
Delta grip strength	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort	Exploratory cohort	Validation cohort
Multivariate at week 8								
Delta leptin	0.72464	0.85769	2.0640	2.3577	1.3244-3.2165	1.1788-4.7158	**0.0014**	**0.0153**
Multivariate at week 16								
Delta leptin	0.84069	1.11118	2.3180	3.0380	1.2298-4.3689	1.0633-8.6795	**0.0193**	**0.0380**
Excluded variables								
Delta mGPS	−0.041628	−0.41987	0.9425	0.6571	0.3657-2.9717	0.2951-1.4636	0.9379	0.3041
Delta albumin	2.24834	0.63004	9.4720	1.8777	0.4170-15.1285	0.3391-10.3976	0.3582	0.4706

Results were considered significant for $P < 0.05$. Significant *P* values are reported in bold.
CI, confidence interval; CRP, C-reactive protein; Fbg, fibrinogen; IL, interleukin; LBM, lean body mass; mGPS, modified Glasgow Prognostic Score; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; QoL, quality of life; REE, resting energy expenditure; ROS, reactive oxygen species; SMI, skeletal muscle index; TNF, tumor necrosis factor.

ROC analysis on early delta values of laboratory variables at week 8 to predict changes in anti-cachectic treatment endpoints are reported in Table 4. By comparing ROC curves, delta leptin showed the highest AUC (0.928; 95% CI 0.815-0.982) for detecting an improvement in LBM, followed by delta IL-6 (AUC: 0.845; 95% CI 0.712-0.933) and



	Baseline leptin
Cut-off	≤3
Specificity	46.38
Sensitivity	81.13

	Baseline leptin
Cut-off	≤2.56
Specificity	77.42
Sensitivity	50.69

	Baseline leptin
Cut-off	≤3
Specificity	41.18
Sensitivity	81.13

	Baseline leptin
Cut-off	≤1.98
Specificity	65.82
Sensitivity	80.00

Figure 1. ROC and AUC of baseline leptin for cachexia severity (left side) and sarcopenia (right side) in the exploratory (A) and validation (B) cohort.
AUC, area under the curve; ROC, receiver operating curve.

delta CRP (AUC: 0.790, 95% CI 0.649-0.894), with a significant difference between the leptin and CRP ROC curves ($P = 0.0372$). For identifying an improvement in SMI, delta leptin showed the highest AUC (0.962; 95% CI 0.857-0.996) followed by delta CRP (AUC: 0.909, 95% CI 0.785-0.974), delta IL-6 (AUC: 0.907; 95% CI 0.783-0.973), delta mGPS (AUC: 0.782, 95% CI 0.634-0.891), and delta albumin (AUC: 0.710, 95% CI 0.543-0.844), with a significant difference between delta leptin and delta mGPS ($P = 0.0111$), and between delta leptin and delta albumin ($P = 0.100$) ROC curves. For detecting an improvement in REE, delta leptin had the highest AUC (1.000; 95% CI 0.805-1.000), followed by delta IL-6 (AUC: 0.967, 95% CI 0.750-1.000), delta ROS (AUC: 0.917, 95% CI 0.521-1000), delta CRP (AUC: 0.817, 95% CI 0.558-0.959), delta mGPS (AUC: 0.867, 95% CI 0.616-0.980), without a significant difference between ROC curves. The AUC for identifying improvements in fatigue was 0.762 for delta NLR ($P = 0.0036$) (Table 4). AUCs of other laboratory variables were not significant in predicting fatigue improvement after treatment.

Table 4. Performance of early changes of different variables in predicting the outcomes of anti-cachectic treatment

Primary endpoints							
Delta LBM	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut-off	*P* value
Week 8							
Delta leptin	0.921 (0.869-0.957)	87.13 (79.0-93.0)	89.06 (78.8-95.5)	92.6 (86.2-96.2)	81.4 (72.4-88.0)	>0.5	**<0.0001**
Delta IL-6	0.740 (0.665 to 0.805)	83.80 (75.1-90.5)	69.84 (57.0-80.8)	81.0 (74.3-86.3)	71.0 (61.0-79.3)	≤−0.188	**<0.0001**
Delta CRP	0.806 (0.672 to 0.902)	94.12 (80.3-99.3)	66.67 (41.0-86.7)	84.2 (73.4-91.2)	85.7 (60.1-96.0)	≤−0.2	**<0.0001**
Delta SMI	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut-off	*P* value
Week 8							
Delta leptin	0.935 (0.886 to 0.968)	96.19 (90.5-99.0)	84.75 (73.0-92.8)	91.8 (86.0-95.3)	92.6 (82.6-97.0)	>0.11	**<0.0001**
Delta IL-6	0.761 (0.687 to 0.825)	83.33 (74.7-90.0)	74.14 (61.0-84.7)	85 (78.4-89.8)	71.7 (61.5-80.0)	<−0.68	**<0.0001**
Delta CRP	0.901 (0.785 to 0.967)	91.67 (77.5-98.2)	80 (51.9-95.7)	91.7 (79.9-96.8)	80 (56.8-92.4)	<−0.2	**<0.0001**
Delta mGPS	0.735 (0.601 to 0.843)	92.7 (80.1-98.5)	37.5 (15.2-64.6)	89.5 (68.9-97.0)	36.8 (29.8-44.5)	≤0	**0.0007**
Delta albumin	0.721 (0.580 to 0.835)	85.71 (69.7-95.2)	55.56 (30.8-78.5)	78.9 (68.7-86.5)	66.7 (44.6-83.3)	>−0.16	**0.0034**
Delta REE	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut-off	*P* value
Week 8							
Delta leptin	0.789 (0.659 to 0.887)	91.43 (76.9-98.2)	71.43 (47.8-88.7)	84.2 (72.9-91.4)	83.3 (62.1-93.8)	>−0.54	**<0.0001**
Delta IL-6	0.756 (0.626 to 0.858)	84.21 (68.7-94.0)	71.43 (47.8-88.7)	84.2 (72.8-91.4)	71.4 (53.3-84.5)	≤−1.81	**0.0009**
Delta CRP	0.856 (0.612 to 0.974)	66.7 (38.4-88.2)	100 (29.2-100.0)	100	37.5 (22.7-55.1)	≤−5	**0.0007**
Delta ROS	0.785 (0.597 to 0.913)	55 (31.5-76.9)	100 (69.2-100.0)	100	52.6 (40.6-64.3)	≤−69	**0.0008**
Delta mGPS	0.875 (0.636 to 0.981)	62.5 (35.4-84.8)	100 (15.8-100.0)	100	25 (15.-38.6)	≤−1	**0.0004**
Delta fatigue	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut-off	*P* value
Week 8							
Delta NLR	0.762 (0.609 to 0.877)	96.77 (83.3-99.9)	53.85 (25.1-80.8)	83.3 (73.5-90.0)	87.5 (48.8-98.1)	≤2.53	**0.0036**
Secondary endpoints							
Delta grip strength	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Cut-off	*P* value
Week 8							
Delta leptin	0.732 (0.646 to 0.806)	76.39 (64.9-85.6)	68.42 (54.8-80.1)	75.3 (67.1-82.1)	69.6 (59.4-78.3)	>0.84	**<0.0001**
Delta CRP	0.706 (0.557 to 0.828)	85.29 (68.9-95.0)	64.29 (35.1-87.2)	85.3 (73.9-92.2)	64.3 (42.3-81.6)	≤−0.2	**0.0298**
Delta mGPS	0.690 (0.548 to 0.810)	94.44 (81.3-99.3)	35.29 (14.2-61.7)	75.6 (68.3-81.6)	75 (40.3-93.0)	≤0	**0.0138**

Results were considered significant for $P < 0.05$. Significant *P* values are reported in bold.
AUC, area under curve; CI, confidence interval; CRP, C-reactive protein; IL, interleukin; LBM, lean body mass; mGPS, modified Glasgow Prognostic Score; NLR, neutrophil-to-lymphocyte ratio; NPV, negative predictive value; PPV, positive predictive value; REE, resting energy expenditure; ROS, reactive oxygen species; SMI, skeletal muscle index; TNF, tumor necrosis factor.

Among secondary parameters, on comparison of ROC curves to identify an improvement in grip strength, delta leptin showed the highest AUC (0.853; 95% CI 0.717-0.940) followed by delta CRP (AUC: 0.699, 95% CI 0.546-0.825), and delta mGPS (AUC: 0.690; 95% CI 0.536-0.818), with a significant difference in delta leptin ROC curve compared with delta CRP ($P = 0.0446$) and delta mGPS ($P = 0.0293$).

Logistic regression of association between early leptin changes and treatment endpoints in the validation cohort

Based on the LBM endpoint, the standardized log-rank statistic determined the optimal delta leptin cut-off value to be >0.5 ng/ml. Logistic regression analysis showed that higher delta leptin at week 8 was associated with significantly better outcomes in term of LBM (OR = 50.6020; 95% CI 19.2434-133.0624; $P < 0.0001$), SMI (OR = 76.4844; 95% CI 24.3128-240.6086; $P < 0.001$), REE (OR = 8.0000; 95% CI 2.3070-27.7413; $P = 0.0010$), fatigue (OR = 2.5; 95% CI 1.1055-5.6537; $P = 0.0277$), and grip strength (OR = 6.0; 95% CI 2.7683-13.0044; $P < 0.0001$). The association remained significant after being weighted for tumor site for LBM ($P < 0.0001$), SMI ($P < 0.0001$), REE ($P < 0.0001$), and grip strength ($P < 0.0001$). Furthermore, by dividing delta leptin value by quartile, we confirmed an association with increasing changes in leptin value and endpoint weight ($P = 0.0002$, test for trend $P < 0.0001$), LBM ($P < 0.0001$, test for trend $P < 0.0001$), SMI ($P < 0.0001$, test for trend $P < 0.001$), REE ($P < 0.0001$, test for trend $P < 0.0001$), overall QoL ($P = 0.0145$, test for trend $P = 0.3232$), fatigue ($P = 0.0491$, test for trend $P = 0.2871$), and grip strength ($P < 0.0001$, test for trend $P < 0.001$). No association was found between the quartiles of delta leptin levels and appetite. The associations and trends also remained statistically significant in different tumor site subgroups for endpoint LBM, SMI, REE, and grip strength.

DISCUSSION

Detecting responsiveness to anti-cachectic treatment is a complex challenge due to various factors, such as tumor location, histotype, antineoplastic therapy administration, different stages of cachexia, and the crucial role of inflammation with related mediators that influence the outcomes. Given this intricate web of factors, our team has been working to show that a multitargeted treatment can yield the best clinical results,[29-32] significantly impacting the QoL of patients navigating a complex phase of their cancer. Identifying the most specific marker that could validate the early effectiveness of anticachectic therapy, however, is yet a challenging critical issue and represents the aim of the present study.

The present study confirmed that a combined treatment approach consisting of MA, celecoxib, L-carnitine, and antioxidants effectively improved the key features of cachexia and related QoL. Within this framework, we established that leptin is the most sensitive parameter capable of reflecting the effects of the anti-cachectic approach on the main

treatment endpoints. Changes in leptin levels have been found to be an early predictive marker of treatment outcomes, outperforming inflammatory mediators, and reflected the amelioration of body composition, muscle mass, energy metabolism, functional performance, and overall QoL achieved through the multitargeted anti-cachectic approach. In detail, yet after 8 weeks of anti-cachectic treatment, an increase in leptin was associated with improvement of LBM, SMI, and muscle strength as well as a decrease of systemic inflammation markers and mGPS and was shown to be an independent predictive factor of their early improvement at week 8. This result underscores the ability of leptin to reflect as reliable surrogate markers the main immune-metabolic changes of cachexia and the effective anti-cachectic actions of a combined targeted approach.

Several experimental and clinical studies have clarified that leptin production is closely related not only to body weight and fat content, but also to glucose utilization[47-49] and metabolic changes in adipocytes.[50,51] Insulin indirectly increases leptin production by enhancing glucose utilization and oxidative glucose metabolism in adipocytes,[52] thus influencing ATP production, cellular redox status, and pyruvate cycling.[53] Indeed, leptin production is regulated by pyruvate and by converting pyruvate to acetyl-CoA through aerobic glucose metabolism.[54-56] Thus, leptin levels appear to closely follow glucose energy metabolism, independent of adipose tissue mass. These observations led to the hypothesis that leptin may play a role as a marker of nutritional and metabolic perturbations in advanced cancer patients, particularly those with cachexia.[10,13]

Indeed, tumor growth produces a complex metabolic picture that finally leads to cachexia, characterized by impaired energy intake due to anorexia; increased energy expenditure due to the activation of futile energy cycles such as Cori cycle and gluconeogenesis; compromised glucose utilization because of hypoinsulinemia and peripheral insulin resistance; oxidative damage which affects the regulation of the main cellular anabolic and catabolic pathway.[4] These metabolic abnormalities are precipitated by the Warburg effect, i.e. the metabolic shift from the tricarboxylic acid pathway to 'aerobic glycolysis' in tumor cells.[57] Notably, Warburg et al. first observed this peculiar energetic behavior in activated macrophages,[58,59] indicating that macrophage-dependent activation involves additional energy expenditure. As a consequence of such high energy-consuming metabolic behavior, immune system activation greatly contributes to the increased energy metabolism typical of cachexia[60] and the associated weight loss. We recently clarified this particular role of the immune system by attributing a key role in the etiopathogenesis of neoplastic cachexia to the tolerance phase of the immune response, in which the role of nonspecific innate immunity is prominent.[4] Indeed, most of the main metabolic changes involved in the pathogenesis of cancer cachexia are directly driven by the chronic action of proinflammatory cytokines, mainly IL-6.[1,61]

Just studying the role of proinflammatory cytokines in inducing the nutritional and metabolic changes typical of cachexia, we were among the first to investigate the role of leptin in cancer cachexia. Our studies have shown that leptin levels are lower in patients with advanced cancer than in healthy individuals,[10,13] and were inversely correlated with the intensity of the inflammatory response (CRP, fibrinogen, IL-6, TNF-α), increased oxidative stress markers, poor performance status, and reduced survival.[10,13] Specifically, we confirmed such a correlation between low leptin levels and poor nutritional status to be associated with high circulating levels of inflammatory markers and oxidative stress in patients with cancer cachexia.[10-13] Similarly, other studies have demonstrated a correlation between low leptin levels and compromised nutritional status, cachexia, and prognosis in patients with advanced cancers.[17,18,39] Interestingly, in a prospective study on patients with ovarian cancer, we found that during the course of cancer, increasing levels of IL-6 were linked to a progressive decrease in leptin levels, with the lowest values observed near the time of death.[11] Leptin values during the disease trajectory closely mirrored changes in IL-6, with increasing values being associated with tumor response and decreasing values indicating tumor progression, even before significant weight loss occurred.[11]

Consistent with the aforementioned data, herein, we confirmed that both leptin levels at baseline and changes in leptin levels during treatment correlated inversely with markers of systemic inflammation, i.e. CRP, NLR, and proinflammatory cytokines. Leptin levels were also inversely correlated with increased REE and muscle wasting, measured by LBM and SMI, before anti-cachectic treatment. During anti-cachectic treatment, an increase in leptin levels correlated with an improvement in REE, LBM, and SMI. Notably, early leptin changes during treatment predicted the effectiveness of the anti-cachectic approach in ameliorating REE and SMI, even before a significant increase in body weight occurred. Thus, leptin changes paralleled the changes in energy metabolism, which preceded the increase in body weight and were modulated mainly by proinflammatory cytokines. Such ability of leptin to early reflect the immune-metabolic changes associated with cachexia may confer to this mediator a crucial role also in the context of the early stage of pre-cachexia, a phase where, according to international guidelines,[2] early inflammatory and metabolic signs precede severe wasting and occur without evident, or with only minimal, weight loss. Therefore, the assessment of leptin levels may help in establishing the indication for a timely and early anti-cachectic treatment, potentially able to prevent the evolution toward severe and refractory cachexia.

In the present study, the improvement in LBM and SMI was inversely associated with the decrease in IL-6, CRP, and mGPS. In multivariate regression analysis, however, only leptin change remained an independent predictive factor for multiple endpoints, i.e. an increase in LBM, SMI, and grip strength, and a decrease in REE. Leptin was superior to

IL-6, CRP, and mGPS in predicting the main outcomes of anti-cachectic treatment. Higher delta leptin values were associated with significantly better outcomes in terms of LBM, SMI, REE, grip strength, and fatigue in the validation cohort. Considering the mechanisms that regulate leptin levels, the increase in leptin levels during treatment appears to be indirect evidence of the ability of our combined approach to affect energy derangement, inflammation, and catabolic drivers typical of cancer cachexia.

The rationale of a multitargeted anti-cachectic treatment is to hamper the inflammatory cascade and inflammation-driven pathways and thus counteract the metabolic derangements responsible for cachexia.[4] Our combined approach included MA and celecoxib, which can reduce the expression of the pro-inflammatory cascade, thus inhibiting the main catabolic factors and drivers of metabolic derangements of cachexia.[62,63] Celecoxib also prevents the activation of glycolysis with lactate production and inhibits peripheral response to insulin and hepatic glycolysis mediated by the COX-2 activation in experimental models associated with muscle wasting.[64] Additionally, we included L-carnitine because it increases aerobic glycolysis and oxidative mitochondrial energy metabolism and exerts antioxidant effects.[65-67] By regulating these metabolic pathways, L-carnitine can alleviate the main symptoms of cachexia, muscle wasting, and fatigue.[68] Additionally, antioxidants alpha lipoic acid and carbocysteine, the most important precursors of cell-reduced glutathione, are crucial in maintaining glucose balance through oxidative phosphorylation and the pentose-phosphate pathway.[69-71]

Then, our approach targets the main pathways responsible for cachexia by suppressing the inflammation and restoring the metabolic alterations that lead to increased REE and muscle atrophy. This leads to improvements in body weight, function, and QoL.[72]

The association between the effectiveness of combined treatment and an increase in leptin levels suggests that leptin reflects the improvement in metabolic and energetic efficiency, inversely linked to changes in inflammation. These findings support the evidence that cachexia symptoms come from a common pathway that induces metabolic disruptions, modulating leptin signals. Activation of leptin feedback is a biological defense that attempts to help limit energy use when energy is scarce.[61] Low leptin levels should reduce metabolic rate and maintain normal eating habits to prevent weight loss. Neoplastic disease and related inflammation, however, counteract this mechanism. Therefore, benefits can be achieved only if the concomitant antineoplastic protocol is effective.

It is noteworthy that the present study found no correlation between leptin levels and loss of appetite before or during treatment. This is consistent with our previous findings[10] and supported by recent preclinical evidence.[73] Arora et al.,[73] in an animal model of cachexia, found that the pro-cachectic effect of leukemia inhibiting factor and IL-6 family members was counterbalanced by decreased leptin levels, resulting in adipose and muscle wasting without loss of appetite.

Overall, the results of the present study strongly highlight that leptin can monitor and predict anti-cachectic treatment outcomes and serve as a surrogate marker of the immune-metabolic actions of the selected drugs.

The clinical impact could be relevant because, although an increasing number of studies have assessed proinflammatory cytokines, NLR, acute phase proteins, albumin, and their combined score to define cachexia and its prognosis,[41,44,74-76] none of them have evaluated the role of these parameters in predicting the efficacy of anti-cachectic treatments.[38,41,77-79]

Few studies, including some of our previous works,[31,32,80,81] have assessed the changes in leptin during different pharmacological treatments for cachexia, with discordant results. In particular, our phase III trial on a combined approach for cachexia in patients with advanced gynecological cancer found that the superiority of the combination arm over the single-agent arm in achieving treatment endpoints was accompanied by a significant rise in leptin levels.[32]

Monitoring of leptin during supportive anti-cachectic treatment can also help in selecting the patients who are likely to benefit from novel immunotherapy-based anticancer treatments. A number of studies have examined the negative impact of cachexia on the efficacy of immunotherapies.[25,82-85] In such a setting, an increase in leptin levels could indicate an improvement in cachexia, which can improve the efficacy of immunotherapy. Additionally, increased leptin levels may directly boost immunity by regulating both innate and adaptive immunity; *vice versa*, leptin deficiency weakens immune responses and decreases T-cell function.[86]

The strengths of our study are its prospective design and sample size, including both an exploratory and validation cohort in new temporally split patients. Moreover, we identified a reliable, inexpensive, and sensitive marker that can be detected in blood and be used for early diagnosis of cachexia alongside conventional inflammatory markers. A limitation of our study is that we did not assess the serum leptin concentrations before cachexia diagnosis; therefore, we could not establish whether earlier changes in leptin levels were predictive of the onset of cachexia. Another limitation was the heterogeneity of cancer sites with different prognoses, although our cohort had similar treatment and disease outcomes among patients with different tumors, and the enrolled patients were united by a unifying underlying condition of cachexia. In this regard, assessing the role of leptin as a potential marker of response to anti-cachectic treatment among different tumor types was not a specific endpoint of our study. The results, however, remained significant after being weighted for the tumor site as a covariate. We aim to plan future updates and specifically designed analyses to establish with more accuracy the potential difference in the predictive role of leptin among different tumor sites.

Moreover, in the present study, we enrolled only metastatic cancer patients since cancer cachexia is, per definition, typical of an advanced stage of neoplastic disease, a

consequence of the associated cancer-derived chronic inflammation. Assessing the role of leptin as a parameter predictive of the efficacy of nutritional approaches in earlier cancer stages and conditions of simple malnutrition warrants future specifically designed studies in different patient settings.

Further studies are warranted to test leptin's sensitivity and specificity as a marker of metabolic alterations associated with cancer cachexia and its prognostic role.

Conclusions

Our results suggest a central role for leptin as an early marker of anti-cachectic outcomes, helpful in monitoring the effectiveness of treatment. Leptin assessment may allow for more timely and targeted supportive care and enhance the patient's well-being. Integrating leptin with inflammatory markers (such as CRP and IL-6) can help develop a cachexia signature that identifies the risk of cachexia development and its severity, optimizing the clinical application of anti-cachectic treatment. More clinical trials are needed, however, to explore these possibilities.

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DISCLOSURE

The authors have declared no conflicts of interest.

DATA SHARING

Original clinical, laboratory, and instrumental data can be found in the patient chart archived at the different clinical departments, and at the Data Management Service at the Department of Medical Oncology at University of Cagliari and are available on request from the corresponding author.

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