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Defining Cancer Cachexia in Head and Neck Squamous Cell Carcinoma

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Abstract

Purpose: Cancer cachexia is a devastating and understudied illness in patients with head and neck squamous cell carcinoma (HNSCC). The primary objective was to identify clinical characteristics and serum levels of cytokines and cachexia-related factors in patients with HNSCC. The secondary objective was to detect the occurrence of cytokine and cachexia-related factor gene expression in HNSCC tumors.

Experimental Design: For the primary objective, cross-sectional data were obtained from prospectively recruited patients identified as cachexia cases and matching cachexia-free controls. For the secondary objective, a retrospective cohort design with matched controls was used.

Results: Clinical characteristics associated with cancer cachexia in HNSCC were T_4 status ($P = 0.01$), increased C-reactive protein ($P = 0.01$), and decreased hemoglobin ($P < 0.01$). Exploratory multiplex analysis of serum cytokine levels found increased interleukin (IL)-6 ($P = 0.04$). A highly sensitive ELISA confirmed the multiplex result for increased IL-6 in cachectic patients ($P = 0.02$). Quality of life was substantially reduced in patients with cachexia compared with noncachectic patients ($P < 0.01$). All tumors of HNSCC patients both with and without cachexia expressed RNA for each cytokine tested and the cachexia factor lipid-mobilizing factor. There were no statistically significant differences between the cytokine and cachexia factor RNA expression of cachectic and noncachectic patients (each $P > 0.05$). No tumors expressed the cachexia factor proteolysis-inducing factor.

Conclusion: We have identified clinical characteristics and pathophysiologic mechanisms associated with cancer cachexia in a carefully defined population of patients with HNSCC. The data suggest that the acute-phase response and elevated IL-6 are associated with this complex disease state. We therefore hypothesize that IL-6 may represent an important therapeutic target for HNSCC patients with cancer cachexia.

Cachexia was first described by Hippocrates as a syndrome of irreversible wasting in the terminally ill. The term is derived from the Greek words kakos, meaning "bad things," and hexus, meaning "state of being." According to the National Cancer Institute, cancer cachexia is estimated to be the immediate cause of death in 20% to 40% of cancer patients.[6] The tumor is thought to directly secrete factors and cytokines, which induce cachexia. An important distinction between starvation and cachexia is that the latter cannot be reversed with appropriate nutritional supplementation as the sole intervention. Additionally, cachexia is characterized by a disproportionate loss of lean mass in the setting of increased resting energy expenditure; however, in starvation lean mass is better preserved, and energy expenditure is normal or decreased (1). The clinical definition of cancer cachexia requires an unintentional weight loss of at least 5% premorbid weight occurring over 3 to 6 months. Cachexia is also found in several inflammatory processes including chronic obstructive pulmonary disease, acquired immunodeficiency syndrome, and trauma. Anemia, emotional and mental fatigue, and markedly decreased quality of life are clinical characteristics associated with cachexia (1).

The complex, multifactorial pathogenesis of cancer cachexia is an active focus of investigation. Cytokines such as interleukin

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(IL)-1β, IL-6, IL-8, tumor necrosis factor-α, and IFN-γ are involved in cachexia pathogenesis with cancers of the esophagus, pancreas, lung, and prostate (2–6). The dysregulation of neuropeptides such as α-melanocyte stimulating hormone, neuropeptide Y, melanocortin, and corticotrophin-releasing factor is thought to cause altered orexigenic and anorexigenic effects leading to cachexia (2, 4, 7). Increased skeletal muscle wasting seems to be related to defects in the ubiquitin-proteasome system and the dystrophin glycoprotein complex (7).

Lipid-mobilizing factor (LMF) and proteolysis-inducing factor (PIF) are described as cachexia-specific proteins and are thought to be secreted directly by the tumor (7). These factors lead to direct catabolic effects on adipose and muscle tissue. Both LMF (8) and PIF (9) were first identified in murine tumor models as potential cachexia-causing factors. Subsequent studies used murine models to confirm the association of cachexia with PIF (10) and LMF (11). Human homologues of LMF and PIF have been identified in the urine of cachectic cancer patients (12–14). Studies have investigated whether PIF is expressed by the tumors of cachectic patients, and the results have been inconsistent (13, 15).

It is estimated that up to 80% of patients with upper gastrointestinal cancer and 60% of those with lung cancer have cachexia at the time of diagnosis (16). The presence of ulcerative tumors causing dysphagia in the upper aerodigestive tract can directly affect oral intake as a result of obstruction and pain. Therefore, cachexia and starvation may be occurring simultaneously in this population. Our group recently reviewed the existing clinical (1) and pathophysiologic (7) data related to cancer cachexia in head and neck cancer. Whereas advances are being made in the understanding of cancer cachexia pathogenesis, the disease is understudied in head and neck squamous cell carcinoma (HNSCC). Little is known about the clinical characteristics, the inflammatory pathogenesis, the presence of cachexia factors, or the effect of cachexia on treatment and survival in these patients.

The primary objective of this study was to more completely characterize cancer cachexia in HNSCC in terms of associated clinical variables, serum cytokines, measures of inflammation and anemia, and cachexia factors. The secondary objective was to investigate tumor cytokine and cachexia factor expression. Both objectives were achieved by comparing HNSCC patients with and without cachexia.

Materials and Methods

Patients

Approval for this study was obtained from the University of North Carolina School of Medicine Institutional Review Board, and informed consent was obtained from each subject. For the primary objective, cross-sectional data were obtained from prospectively recruited patients identified as cachexia cases and matching cachexia-free controls. The subjects in the control group met all inclusion and exclusion criteria. The patients were recruited through the University of North Carolina Head and Neck Tumor Board conferences and outpatient clinics. All subjects in the case and control groups had newly diagnosed or recurrent HNSCC of the oral cavity, oropharynx, hypopharynx, and larynx. All patients in the case and control groups who had undergone chemotherapy, radiation, or surgery in the 6 months before enrollment were excluded due to the effect of treatment on cachexia pathogenesis. Other exclusion criteria were treatment with oral corticosteroids, androgens, progestational agents, or appetite stimulants within 4 weeks before enrollment; daily nonsteroidal anti-inflammatory use for >2 weeks in the 6 weeks before enrollment except low dose (81 mg) aspirin; severe concomitant diseases that may effect cytokine production such as dialysis patients, New York Heart Association stage IV heart disease, uncontrolled autoimmune disease, known HIV-positive patients, or severe anemia with hemoglobin <7.0; pregnant or lactating women; and those with untreated hyperthyroidism or hypothyroidism.

Weight loss was assessed by surveying the patients' medical records and by an interviewer-administered questionnaire. After measuring their current weights and heights, the patients were given the M.D. Anderson Dysphagia Inventory (MDADI) and the Functional Assessment of Anorexia/Cachexia Therapy (FAACT) quality of life survey. Karnofsky performance scale scores were obtained by the study coordinator. Tumor stage was classified using the American Joint Committee on Cancer staging system, 6th edition.

For the secondary study objective, a retrospective cohort design was used. Cachexia cases and matching controls were identified by retrospective chart review. Real-time reverse transcription-PCR (RT-PCR) was done on tissues previously collected from these HNSCC patients. Consent had previously been obtained when the patients were about to undergo surgery and/or biopsy. The tissues had been collected through the Lineberger Comprehensive Cancer Center Tissue Procurement Facility and stored at -80°C until RNA extraction. The same inclusion and exclusion criteria described above were applied to this second group retrospectively. Medical records were reviewed to obtain records of weight loss and other clinical characteristics.

Clinical laboratory analysis

After phlebotomy, sera were collected by centrifuging whole blood at 5,000 rpm for 12 min at 5°C. C-reactive protein (CRP) was measured using a dry-slide method with the VITROS Fusion Series analyzer (Ortho Clinical Diagnostics, Inc.). Hemoglobin was measured using the ADVIA 120 Hematology System (Bayer Diagnostics). Thyroid stimulating hormone was measured with the ECI system, so that patients with untreated thyroid disease could be excluded (Olympic Diagnostic Systems).

Dysphagia evaluation

In the prospectively recruited cohort, dysphagia was evaluated by a speech pathologist through the MDADI results and the patients' clinical histories. Patients with severe dysphagia, as evidenced by MDADI scores below 50 ($n = 2$) or severe trismus ($n = 1$), were excluded due to the possibility that their weight loss was confounded by the dysphagia. Patients with g-tubes ($n = 4$) or no dysphagia at presentation ($n = 3$) who did not complete the MDADI were assigned a dysphagia score of 80, which correlates with having no dysphagia. The score is referred to as the adjusted MDADI.

Serum cytokine analysis by multiplex and ELISA

Aliquots of previously collected sera were stored in -80°C until multiplex or ELISA. Cytokines were measured in serum samples with R&D Systems multiplex assays. Multiplex analysis of serum cytokine levels was done using a Luminex 100 analyzer (R&D Systems) running on Bio-Plex software (Bio-Rad Laboratories). Data were analyzed using BeadView software version 5.0 (Upstate, Inc.). Inflammatory cytokines with known involvement in cancer cachexia (IL-1β, IL-6, IL-8, tumor necrosis factor-α, and IFN-γ) were analyzed in triplicates. Exploratory analyses were done in singlicates using the R&D Fluorokine MAP Multiplex Human Cytokine Panel A Kit. Cytokines showing statistical trends of difference in multiplex levels between patients with and without cachexia (IL-1β, IL-6, and granulocyte colony-stimulating factor) were measured in triplicates with a highly sensitive colorimetric sandwich ELISA (Quantikine HS, R&D Systems).

RT-PCR measure of cytokine mRNA expression by tumor tissue

RNA extraction. Total RNA was extracted from tumor tissue with RNeasy mini kit from Qiagen. The quality of RNA was evaluated using

Table 1. Clinical characteristics of cachectic and noncachectic HNSCC patients

	Cachectic (n = 11)*	Noncachectic (n = 13)*	P
Age (y)*	57 (12)	58 (9)	
Gender			
Male	73% (8/11)	85% (11/13)	
Female	27% (3/11)	15% (2/13)	
T_4 status	72% (8/11)	15% (2/13)	0.01
N_2-N_3 status	36% (4/11)	54% (7/13)	0.44
AJCC stage IV	82% (9/11)	62% (8/13)	0.39
Site			
Oral	27% (3/11)	8% (1/13)	
Oropharynx	46% (5/11)	54% (7/13)	
Hypopharynx	9% (1/11)	8% (1/13)	
Larynx	18% (2/11)	31% (4/13)	
Weight (kg)	67.6 (22.3)	81.6 (22.5)	
BMI (kg/m^3)	22.8 (6.3) †	27.0 (5.3)	
Weight loss over 3-6 mo (kg)	8.6 (6.1)	0.1 (0.5)	
Hemoglobin (g/dL)	12.4 (3.7)	14.7 (1.2)	<0.01
CRP (mg/dL)	3.0 (2.5) †	1.1 (1.7)	0.01

Abbreviations: AJCC, American Joint Committee on Cancer; BMI, body mass index.
*Values expressed as mean (SD).
† n = 10 nonmissing values.

the RNA 6000 Nano Lab Chip kit and an Agilent 2100 Bioanalyzer (Agilent Technologies).

Real-time RT-PCR. cDNA synthesis for real-time RT-PCR was done as follows. One microgram of total RNA was annealed with 0.5 μg of random hexamers at 70°C for 5 min. The following reagents were then added to each tube: 3 mmol/L $MgCl_2$, 0.5 mmol/L deoxynucleotide triphosphates, 1.25 units/μL reverse transcriptase, and 1× reaction buffer for a total volume of 20 μL in each tube. The tubes were heated at 40°C for 1 h and the reaction was terminated at 70°C for 15 min. Eighty microliters of nuclease-free H_2O were added to each sample to bring the total volume to 100 μL. The PCR reaction was done using 8 μL of the synthesized cDNA, 10 μL of TaqMan universal PCR master mix (PE Biosystems), and 1 μL of 20× assays-on-demand gene expression assay mix. Six cytokine gene expression assay mixes (IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor-α, and IFN-γ) and two cachexia factor assay mixes (LMF and PIF) were used. Water was added to make a total volume of 20 μL. PCR was done on AB 7900 using universal cycling parameters. The relative quantitation method of real-time RT-PCR was used, in which the C_t values for all samples are compared with the C_t value of one arbitrarily chosen calibrator sample. The resulting measurement is a relative quotient for each sample, with the calibrator sample having a relative quotient value of 1. An endogenous control, human β-glucuronidase, was used to normalize target sample variations (from Applied Biosystems). The control gene is constitutively expressed in all human cells. Expression of the control gene was measured for every sample. Incorporating human β-glucuronidase measurements into relative RT-PCR minimized variations in the amount of input cDNA among samples. It also served as a positive control for each sample, ensuring that each sample was present and capable of measurable mRNA production.

Statistical methods

For the primary and secondary analyses, the two data sets (prospectively enrolled patients and retrospectively enrolled patients) were analyzed separately. The study design specified a target sample size of at least 16 patients with cachexia and 16 patients without cachexia. These groups were to be further halved into those with and without severe dysphagia. However, after beginning enrollment, we were unable to enroll sufficient numbers of patients with severe dysphagia, which led to a decision to exclude patients with severe dysphagia from the study.

We obtained statistical estimates of cytokine levels from a previous study that compared IL-6 in cachectic and noncachectic patients with advanced prostate cancer (7). The IL-6 estimated coefficient of variation was 0.57. In the context of our study, if mean IL-6

Table 2. Serum multiplex tests of cachectic and noncachectic HNSCC patients

Cytokine (pg/mL)	Cachectic (n = 10), (pg/mL)	Noncachectic (n = 12), (pg/mL)	P
IL-1α	0 (0.0)	0 (0.0)	1.00
IL-1β	3.6 (1.9)	2.2 (2.3)	0.15
IL-1a	0 (0.0)	0 (0.0)	1.00
IL-2	1.9 (3.0)	1.1 (2.1)	0.53
IL-4	27.1 (7.1)	24.2 (3.3)	0.37
IL-5	0 (0.0)	0 (0.0)	1.00
IL-6	18.6 (16.2)	9.6 (8.9)	0.04
IL-8	103.3 (21.6)	98.1 (25.8)	0.64
IL-10	0 (0.0)	0.7 (2.5)	0.36
IL-17	29.4 (5.0)	28.3 (1.3)	0.52
TNF-α	24.9 (2.2)	25.6 (2.0)	0.35
IFN-γ	31.8 (13.1)	33.2 (12.5)	0.56
VEGF	152.1 (37.7)	145.6 (79.7)	0.32
G-CSF	57.5 (21.3)	45.1 (10.7)	0.11
GM-CSF	31.9 (1.6)	32.8 (3.6)	0.84
MCP-1	224.3 (92.3)	216.9 (95.5)	0.97
MIP-1α	187.2 (17.9)	175.0 (21.8)	0.25
MIP-1β	156.7 (38.3)	149.0 (56.1)	0.41
RANTES	57,061 (54,626)	79,887 (50,736)	0.26
Tpo	592.6 (93.6)	641.5 (119.1)	0.41
ENA-78	1,308.5 (853.5)	3,536.3 (5,492.5)	0.43
FGF	37.3 (1.7)	37.3 (1.6)	0.67

NOTE: Values in table expressed as mean (SD).
Abbreviations: TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein; MIP-1α and MIP-1β, macrophage inflammatory proteins 1α and 1β; RANTES, regulated on activation, normal T-cell expressed, and secreted; Tpo, thrombopoietin; ENA-78, epithelial-neutrophil activating peptide; FGF, fibroblast growth factor.

Table 3. Serum ELISA results of cachectic and noncachectic HNSCC patients

Cytokine (pg/mL)	Cachectic (n = 10), (pg/mL)	Noncachectic (n = 12), (pg/mL)	P
IL-1β	—	—	—
IL-6	25.3 (26.9) [6.1-44.5]	9.6 (12.4) [1.7-17.4]	0.02
G-CSF	65.6 (58.2) [24.0-107.3]	33.5 (19.1) [21.4-45.6]	0.26

NOTE: — indicates all values were below the lower limit of detection. Values in table expressed as mean (SD) [95% confidence interval].

concentration was 2.3 times greater in the 16 cases than in the 16 controls, we calculated a 90% chance of rejecting the null hypothesis of no difference between cases and controls in each group obtained by prospective screening of, at most, 50 patients and retrospective chart review of, at most, 50 patients. No interim analyses were scheduled. A Wilcoxon rank-sum test procedure was used in the comparisons of patient groups in terms of continuous-scale measures such as various cytokine levels. Similarly, Fisher's exact test procedure was applied to the binary outcomes. For each test done, $P < 0.05$ was considered statistically significant. No adjustments were made for multiple comparisons. All statistical calculations were done with STATA version 8.0.

Results

For the primary objective, 27 patients with HNSCC were enrolled to complete the analysis of CRP and hemoglobin, serum cytokine analysis, dysphagia inventory, and quality of life questionnaire. The patients were enrolled before undergoing any treatment for either new or recurrent HNSCC. Three patients were excluded due to trismus or severe dysphagia, as diagnosed using the MDADI. Weight loss in those patients was assumed to be associated with starvation. Clinical variables associated with cachexia were evaluated for the remaining 24 patients. Forty-six percent (11 of 24) were cachectic as defined by the current clinical criterion (i.e., they experienced weight loss exceeding 5% of the premorbid body weight over 3-6 months). In comparing those with and without cachexia, no statistically significant differences were detected with regard to age, gender, or site (see Table 1). Patients with cachexia had larger tumors ($P < 0.01$) and more advanced stages ($P = 0.03$) when compared with those without cachexia, but no difference was observed about nodal status (see Table 1). Mean CRP levels were thrice higher in cachectic patients ($P = 0.01$), whereas hemoglobin was lower in those with cachexia ($P < 0.01$; see Table 1).

Exploratory multiplex analyses of serum cytokine levels indicated that IL-6 was higher in those with cachexia ($P = 0.04$), whereas IL-1β ($P = 0.15$) and granulocyte colony stimulating factor ($P = 0.11$) showed higher statistical trends (see Table 2). These results prompted the use of ELISA to further investigate the three cytokines. ELISA results were obtained for 22 of the 24 subjects; values were missing for 2 of the patients due to difficulty in obtaining sera samples. Serum ELISA results confirmed that IL-6 was higher in cachectic patients ($P = 0.02$), whereas IL-1β was too low to be detected and granulocyte colony-stimulating factor exhibited no difference (see Table 3).

Substantial proportions of those with (73%, 8 of 11) and without (54%, 7 of 13) cachexia had mild dysphagia at presentation. After excluding three patients with severe dysphagia from the study, adjusted MDADI scores were not statistically significantly different for those with and without cachexia ($P = 0.17$). Thus, an association of cachexia with mild dysphagia was not detected (see Table 4).

The FAACT results were lower for patients with cachexia ($P < 0.01$). All of the subscores had mean levels that were lower for the cachectic patients. The greatest differences between cachectic and noncachectic patients were observed in the Emotional, Functional, and Additional-Cachexia subscores. The difference between the two groups was not statistically significant for the Karnofsky performance scale (see Table 5).

Serum cytokine levels indicated that some proteins are being disproportionately produced in cachectic patients. The production could be occurring in the tumor, in lymphoid tissue as the host's response, or as some combination of both. The secondary study objective was to determine if cytokines were being produced in the tumor tissue. Previously collected tumor tissues from 19 retrospectively enrolled HNSCC patients with (n = 11) and without (n = 8) cachexia who met the study's inclusion/exclusion criteria were used in an attempt to detect tumor tissue expression of cytokine RNA and cachexia factor RNA. The clinical characteristics show statistical trends of increased N_2 and N_3 status as well as increased stage IV disease in cachectic compared with noncachectic patients (see Table 6). Real-time RT-PCR results showed that all tumor tissues

Table 4. Dysphagia in cachectic and noncachectic patients

	Cachectic (n = 11)	Noncachectic (n = 13)	P
Adjusted MDADI dysphagia score*	71.5 (16.7)	77.7 (10.3)	0.17
Dysphagia at presentation	73% (8/11)	54% (7/13)	0.24

NOTE: Values in table expressed in mean (SD).
*The value of the MDADI score was imputed to have the value of 80 for those with g-tubes (n = 4) or no complaint of dysphagia (n = 3).

Table 5. Quality of life and performance status

	Cachectic (n = 9)	Noncachectic (n = 11)	P
FAACT-total	88.2 (13.3)	120.7 (21.7)	<0.01
FAACT-physical	15.8 (7.4)	22.1 (5.9)	0.07
FAACT-social	21.7 (5.3)	23.7 (3.3)	0.49
FAACT-emotional	14.4 (3.4)	19.3 (4.4)	0.01
FAACT-functional	10.7 (5.2)	19.5 (7.2)	0.01
FAACT-additional/cachexia	25.6 (7.8)	36.1 (8.9)	<0.01
Karnofsky performance status	64.5 (20.7)	75.4 (19.4)	0.17

NOTE: Values in table expressed as mean (SD).

expressed the six cytokines tested as well as the cachexia factor LMF. However, the expression of PIF RNA was not detected in any of the 19 tumor tissue samples. The mean relative quotient measurements were compared for the cachectic and noncachectic groups. No statistically significant differences for any of the cytokines or factors were observed between these groups (see Table 7).

Discussion

Investigating the pathogenesis of cancer cachexia is challenging due to the multifactorial nature of weight loss. We formed strict inclusion/exclusion criteria to control for factors that may contribute to weight loss. We limited enrollment to patients who had not begun treatment for a recently diagnosed HNSCC because chemotherapy, radiation, and surgery could affect weight loss and cytokine expression.

In patients with HNSCC, dysphagia must also be considered as a potential contributor to the weight loss. The severity of physical dysphagia may be predictive of the cessation of alimentation, but some patients with severe dysphagia continue to eat sufficiently. Due to the higher potential that severe dysphagia could decrease food intake, we excluded patients with severe dysphagia, and we included patients with mild dysphagia. However, mild dysphagia could also limit food intake, thereby serving as an uncontrolled confounder in the study. The difference in MDADI scores for patients with mild dysphagia in the cachectic and noncachectic groups was not statistically significant. Larger comparison groups would allow for the development of a regression model, which could adjust for differences in dysphagia scores between groups. Among the patients enrolled, a substantial proportion of those with (73%) and without (54%) cachexia had mild dysphagia at presentation. This observation is pertinent to the design of the inclusion/exclusion criteria of future interventional clinical trials involving head and neck cancer cachexia. Because a high proportion of cachectic and noncachectic HNSCC patients will present with mild dysphagia, trials will need to be designed to cope with this putative confounder.

A higher proportion of cachectic patients had T_4 status, and a statistical trend was seen showing higher N status in cachectic patients. The presence of larger tumors could contribute to weight loss associated with dysphagia and upper digestive tract obstruction. However, larger tumors could also be associated with greater production of cachexia factors and inflammatory markers involved with cachexia pathogenesis.

Table 6. Clinical characteristics of cachectic and noncachectic HNSCC patients retrospectively enrolled for tumor cytokine and cachexia factor expression study

	Cachectic (n = 11)	Noncachectic (n = 8)	P
Age (y)	49 (9)	58 (13)	
Gender			
Male	82% (9/11)	75% (6/8)	
Female	18% (2/11)	25% (2/8)	
T_4 status	45% (5/11)	13% (1/8)	0.17
N_2-N_3 status	91% (10/11)	50% (4/8)	0.11
AJCC stage IV	100% (11/11)	63% (5/8)	0.06
Site			
Oral	27% (3/11)	50% (4/8)	
Oropharynx	27% (3/11)	13% (1/8)	
Hypopharynx	18% (2/11)	13% (1/8)	
Larynx	27% (3/11)	25% (2/8)	
Weight (kg)	70 (18)*	80 (7.6)	
Weight loss over 3-6 mo (kg)	8.9 (2.8) †	0 (0)	
Hemoglobin (g/dL)	13.1 (1.3)	14.2 (1.4)	0.15

NOTE: Values in table expressed as mean (SD).
*n = 8 nonmissing values.
†n = 10 nonmissing values (1 patient was diagnosed as cachectic without recorded weight loss).

Table 7. RT-PCR relative expression of cytokine and cachexia factor mRNA by tumor tissues of cachectic and noncachectic patients

Cytokine	Cachectic (n = 11), (pg/mL), mean RQ (SE)	Noncachectic (n = 8), (pg/mL), mean RQ (SE)	P
IL-1α	220.7 (204.2)*	36.2 (32.4)	0.74
IL-1β	265.8 (250.4)	49.8 (32.2)	0.56
IL-6	25.5 (18.7)	10.0 (5.6)	0.74
IL-8	555.3 (516.1)	108.6 (87.8)	0.87
TNF-α	21.9 (18.4)	3.8 (2.4)	0.72
IFN-γ	44.7 (36.0)	4.7 (1.2)	0.56
LMF	1,532.2 (1,402.7)	2,011.4 (1,536.1)	0.51
PIF	No expression	No expression	

*Relative quotient (RQ) is used in the relative quantitation method of RT-PCR to compare all measurements to one arbitrarily chosen sample.

Whereas we maintained stringent inclusion/exclusion criteria, and studied a selective group of patients with HNSCC cancer cachexia, our study was limited by the number of available subjects. As a result, we carried out multiple hypothesis tests for associations between measured variables and cachexia, but we were unable to develop a regression model with predictive power. The performance of multiple hypothesis tests without the benefit of a multiple-comparisons procedure increases the chance that some of the differences tabulated are not reproducible.

Another limitation of our study involves the retrospective acquisition of data for the RT-PCR tissue studies. In particular, we have slightly less confidence in the cachexia classification of the 19 retrospectively enrolled patients because direct interview data and first-hand weight measurements were not feasible. The effect of misclassifying the cachexia status of some of these patients would reduce the power of the group comparison tests in Table 6.

We have observed evidence that IL-6 and CRP are associated with cachexia in patients with HNSCC. These observed associations are evidence that cachexia in HNSCC patients is associated with the acute-phase response.

An accelerated acute-phase response is associated with weight loss in patients with melanoma, pancreatic cancer, or lung cancer (17). In patients with squamous cell carcinoma and adenocarcinoma of the esophagus, preoperative elevated CRP is associated with increased tumor extension, increased metastases to lymphatics, and worse prognosis (18).

The acute-phase response is modulated in part by IL-6. IL-6 controls CRP transcription by the liver as well as regulates plasmacytosis and T-cell responses. Increased serum levels of IL-6 have been seen in leukemia, lymphoma, melanoma, and cancers of the breast, lung, ovaries, and pancreas (19). Increased serum IL-6 has been linked with weight loss in lymphoma and lung and colorectal cancers (20). Anti–IL-6 monoclonal antibody therapy for cancer was first reported in 1991 (21). Subsequently, anti–IL-6 monoclonal antibody has been used to treat multiple myeloma, renal cell carcinoma, and B-cell lymphoproliferative disorders, with no serious adverse effects observed in the majority of studies (19). CNTO328 is an anti–IL-6 monoclonal antibody that was shown to be safe in a phase I trial for multiple myeloma patients (22). Zaki et al. (23) administered CNTO328 to two separate mouse models with human-induced tumor cachexia; in both models, weight loss was halted or reversed. Because CNTO328 specifically targets human IL-6, the results indicate that the tumor tissue was secreting human IL-6, and that this represents a site of action for the anti-cytokine treatment in the murine model. Our RT-PCR results indicate that for HNSCC patients, the tumor is a site of IL-6 cytokine production, suggesting that this could be a potential target for cancer cachexia therapy in future clinical trials.

As previously mentioned, LMF and PIF were first identified in murine models of cachexia; human homologues of LMF and PIF were then detected in the urine of cachectic patients. In a recent report, RT-PCR was used to successfully detect PIF mRNA in prostate cancer tumor tissues obtained from patients whose cachexia status was not reported (14). One study showed expression of PIF in tumors of cachectic patients (13), whereas another observed no difference in PIF expression for cachectic patients (16). In our study, PIF mRNA was not expressed by any tumor tissues. For all of our samples, the endogenous control (human β-glucuronidase) mRNA was expressed, showing that samples were present and capable of expressing mRNA. It is possible that PIF is being expressed by nontumor tissue at a different site, or that the human homologue of PIF does not exist in HNSCC.

Head and neck cancer has an extraordinary effect on quality of life (24). Our study reports that cachexia in this population substantially worsens quality of life, especially affecting the Emotional and Functional subscores.

Conclusion

Cancer cachexia in head and neck cancer is a common and understudied disease state with devastating consequences for patients. Our exploratory analyses help to define the clinical and laboratory characteristics, leading to a better understanding of the pathogenesis of cachexia in this population. In HNSCC patients, cachexia was observed to be associated with T_4 status, elevated CRP and IL-6, and anemia. Our results suggest that tumor expression of cytokine and cachexia factor mRNA may not differ substantially between those with and without cachexia, and that PIF mRNA is not expressed in HNSCC tumors. IL-6 may represent an important therapeutic target as the study of cachexia becomes translated to clinical medicine. A larger randomized controlled trial of anti–IL-6 treatment in cachectic patients with HNSCC is a future study design that could expand on our results.

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