Serum Cytokines and Cancer in Involuntary Weight Loss

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Background: Tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and IL-6 may be associated with involuntary weight loss in patients with and without cancer. However, results of previous studies have been conflicting. We evaluated patients who had involuntary weight loss to determine cytokine levels and the correlation of these cytokines with weight loss, the association with inflammation, and the potential for use in cancer diagnosis.

Materials and Methods: In 290 consecutive patients with involuntary weight loss (74 patients [26%] with cancer and 216 patients [74%] without cancer), erythrocyte sedimentation rate (ESR), and serum levels of C-reactive protein, TNF-α, IL-1β, and IL-6 were determined.

Results: Higher ESR and levels of C-reactive protein, TNF-α, IL-1β, and IL-6 were associated with cancer. The levels of TNF-α, IL-1β, and IL-6 did not correlate with the amount of weight loss. In multivariable analysis, only ESR was associated with cancer.

Conclusions: In patients with involuntary weight loss, TNF-α, IL-1β, and IL-6 were associated with cancer but were not weight loss mediators.

Key Words: tumor necrosis factor, interleukin, cachexia

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Cytokines are involved in cancer cachexia; among them, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IL-6 are plausible mediators of cachexia because they may induce anorexia.[1] However, the results of previous small studies have been conflicting.[2–6] Furthermore, when patients have been compared with control subjects in these studies, the control subjects have been healthy,[7–11] which could have resulted in overestimation of differences. A large study with 118 patients with cancer did not show a correlation between weight loss and these 3 cytokines.[12] Yet, in some studies, an association between weight loss and cytokines has been shown in patients without cancer.[13,14]

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The aim of this study was to determine whether TNF-α, IL-1β, and IL-6 are correlated with weight loss in cancer and other diseases that are associated with weight loss; whether this correlation, if present, is independent of inflammation; and whether these cytokines could have a role in the diagnosis of cancer in patients with involuntary weight loss.

MATERIALS AND METHODS

Setting and Patients

From January 2009 to September 2010, there were 292 consecutive adult patients (age ≥18 years) with involuntary weight loss without known cause (even after clinical assessment) who were admitted as inpatients or referred to the day hospital in the Department of Internal Medicine of a secondary care university hospital. There were 2 patients who declined to participate in the study. The remaining 290 patients were included in the present study because of (1) documented involuntary weight loss of 5% or greater body weight within the previous 6 months (228 patients), or (2) declaration of "very much" or "much" weight loss, on a Likert scale with 5 levels ("very much," "much," "average," "little," and "not at all"; 62 patients). The second criterion was applied only for the selection of patients for whom there was no baseline weight documentation and the amount of weight loss could not be computed to fulfill the first criterion; for these patients, the presence of weight loss was confirmed by a family member or by changes in size of clothing or belt. Patients with voluntary weight loss or known malignancy were not included in the study. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the institutional ethics committee. All patients agreed to participate in the study and signed the informed consent form before enrolling.

Study Design

The investigative evaluation was not standardized; it varied with each participating physician, depending on clinical and biological diagnostic clues in each patient. All patients had blood samples collected at admission. Levels of TNF-α, IL-1β, and IL-6 were measured for this study; the blood was centrifuged, the serum was frozen at −70°C, and the cytokine levels were determined later in another laboratory (Cantacuzino Institute, Bucharest). As the main objective of the research project was the validation of several simple clinical and biological parameters found as diagnostic for cancer in patients with involuntary weight loss in 3 previous studies,[15–17] tests in the hospital laboratory for all patients included complete blood cell count (including mean corpuscular volume [MCV] and red blood cell distribution width [RDW]; Sysmex XT 1800i counter, Sysmex Corporation, Kobe, Japan), Westergren erythrocyte sedimentation rate (ESR), and serum C-reactive protein (CRP), iron, albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) (Cobas 6000 Modular P 800 analyzer, Roche Diagnostics, Rotkreuz, Switzerland). Serum levels of TNF-α, IL-1β, and IL-6 were measured with a test kit (ELISA, DRG Instruments GmbH, Marburg, Germany) and analyzer (Chemwell

TABLE 1. Clinical Characteristics and Laboratory Studies in Patients with Involuntary Weight Loss*

Characteristic	All Patients	Patients With Cancer	Patients Without Cancer	*P*†
Number (%) of patients	290 (100)	74 (26)	216 (74)	
Age, yrs	67 (22, 94)	69 (44, 93)	67 (22, 94)	NS
Male sex	146/290 (50%)	42/74 (57%)	104/216 (48%)	NS
Weight loss, kg	10 (3, 36)	10 (4, 36)	10 (3, 32)	NS
Weight loss, %	13 (5, 40)	13 (5, 40)	13.4 (5.12, 35)	NS
ESR, mm/h	40 (2, 140)	50 (3, 140)	28.5 (2, 140)	<0.001
CRP, mg/L	8.76 (0.5, 550)	29.6 (1, 550)	5.1 (0.5, 328)	<0.001
TNF-α, pg/mL	13.6 (2.4, 455)	16.7 (4, 455)	12.05 (2.4, 197)	<0.02
IL-1β, pg/mL	7.4 (0, 863)	15 (0, 435)	6.5 (0, 863)	<0.05
IL-6, pg/mL	25.8 (0, 2364)	59.6 (0, 2085)	15.6 (0, 2364)	<0.001

*Data reported as number (percent) for categorical variables, and median (minimum, maximum) for continuous variables that were not normally distributed.

†NS, not significant ($P \geq 0.05$); sex tested with the Fisher exact test, and all other variables tested with the Mann-Whitney test.

2910 analyzer, Awareness Technology, Palm City, FL). The quality of results was validated during the study by regular internal quality control procedures and participation in an external quality assessment program.

All data were recorded by each physician in a questionnaire and then transferred by one of the authors into the database. To avoid misclassification concerning the final diagnosis (cancer or other), the patients were followed up for 6 months after discharge from the hospital and had verification of the final diagnosis, survival, state of health, and further weight change.

Statistical Analysis

Data analysis was done with statistical software (Stata 11, StataCorp, College Station, TX; SPSS 16.0, SPSS, Inc, Chicago, IL). Categorical variables were reported as frequency and analyzed by the Fisher exact test. Continuous variables that were not normally distributed were reported as median (minimum and maximum) and analyzed with the Mann-Whitney *U* test and Kendall τ (tau) rank correlation. Receiver operator characteristic curves were generated; areas under the curve and confidence intervals (CI) were determined. For logistic regression, the variables were selected by the "enter" method. Hypothesis testing was 2-tailed, and statistical significance was defined by $P < 0.05$.

RESULTS

For the 290 patients in the study, 259 patients (89%) were followed up for 6 months to diagnose or exclude the diagnosis of cancer that was not discovered during the initial hospitalization. Of the remaining 31 patients (11%) that were lost to follow-up, initial diagnosis was cancer (total, 10 patients: histopathology in 7 patients and a combination of computed tomography and elevated serum tumor markers in 3 patients), depression (4 patients), hyperthyroidism (2 patients), and Alzheimer disease, polyarteritis nodosa, polymyositis, and sarcoidosis (each in 1 patient); no known cause for weight loss was evident in 11 patients.

Most patients did not have cancer (Table 1). Patients with cancer had significantly greater ESR, CRP, TNF-α, IL-1β, and IL-6 than patients without cancer (Table 1). There was no correlation evident between serum cytokine levels and the amount of weight loss, either in the entire group of patients or in the patients with cancer. However, there was a weak correlation between TNF-α, IL-6, and inflammatory parameters (TNF-α and ESR: $r = 0.113$, $P = 0.007$; TNF-α and CRP: $r = 0.142$, $P = 0.003$; IL-6 and ESR: $r = 0.194$, $P < 0.001$; IL-6 and CRP: $r = 0.226$, $P < 0.001$).

When assessed for the diagnosis of cancer in patients with involuntary weight loss, the areas under the receiver operator characteristic curves for TNF-α (0.61; CI, 0.55–0.67), IL-1β (0.57; CI, 0.513–0.633), and IL-6 (0.69; CI, 0.63–0.75) were comparable to those of ESR (0.67; CI, 0.61-0.72) or CRP (0.69; CI, 0.62–0.75). In the logistic regression model using all these variables, only ESR was significantly associated with cancer ($P < 0.001$); TNF-α, IL-1β, and IL-6 were not significantly associated with cancer.

DISCUSSION

The data showed no correlation between the serum levels of TNF-α, IL-1β, or IL-6 and the amount of weight loss in all patients or in the patients with cancer with involuntary weight loss. Therefore, the relationship between the studied cytokines and involuntary weight loss is not a dose-response one, a necessary criterion for establishing a causal relation.[18] Tumor necrosis factor α, IL-1β, and IL-6 were comparable to ESR and CRP as diagnostic tests for cancer in patients with involuntary weight loss. However, after adjusting for ESR and CRP in logistic regression, cytokine levels were not associated with cancer, suggesting that the cytokines serve as mediators of inflammation and not cachexia. Cancer was diagnosed in 74 patients with involuntary weight loss (26%), a proportion similar to that in previous studies.[19]

Several studies have suggested a role of TNF-α, IL-1β, and IL-6 as mediators of cachexia. These studies showed that the level of these cytokines may be greater in patients with cancer and that weight loss may be greater in patients with cancer than in control subjects.[3,6–11] Involuntary weight loss may be associated with different diseases including cancer, inflammatory or noninflammatory somatic diseases, and psychiatric diseases. This heterogeneous spectrum of disease, and small sample size, may limit the demonstration of a correlation between cytokine levels and the amount of weight loss. However, other studies have shown an association between TNF-α, IL-1β, and IL-6 with weight loss in patients without cancer with Alzheimer disease and chronic obstructive pulmonary disease, and in community-dwelling elderly people without known disease.[13,14,20]

Limitations of the present study include the diversity of types of cancer in the studied cohort, which limited the assessment of the role of these cytokines in different types of cancer

because of limited statistical power. Furthermore, when we evaluated for a correlation between the amount of weight loss and cytokine levels, we could not include 62 patients who had been included in the study based on the second inclusion criterion because the amount of weight loss could not be determined in these patients.

The study of the correlation between TNF-α, IL-1β, and IL-6 and weight loss was only a secondary objective of the present study, which was primarily done to evaluate cancer as a cause of involuntary weight loss.[21] The study included only patients with weight loss greater than 5%, and patients with mild or no weight loss were not included for comparison; this may have limited the observation of a possible relation between cytokine levels and weight loss.

The present results confirm those of other studies, including a previous single large study of 118 patients with cancer that did not show a correlation between weight loss and cytokine levels.[12] Therapies directed against these cytokines have failed to prevent cachexia, further evidence against a causal relation.[22,23] Studies to date have been cross-sectional, including patients only after cachexia has developed, and a temporal relation between cytokine levels and development of cachexia had not been demonstrated. More pathogenic knowledge is needed before therapeutic recommendations can be made. Future research may include a prospective study, including longitudinal observations in patients with progressive weight loss.

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