Elevated Serum Cytokines Correlated with Altered Behavior, Serum Cortisol Rhythm, and Dampened 24-Hour Rest-Activity Patterns in Patients with Metastatic Colorectal Cancer

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ABSTRACT

Purpose: **Incapacitating symptom burden in cancer patients contributes to poor quality of life (QOL) and can influence treatment outcomes because of poor tolerance to therapy. In this study, the role of circulating cytokines in the production symptoms in cancer patients is evaluated.**

Experimental Design: **Eighty patients with metastatic colorectal cancer with either normal (group I, $n = 40$) or dampened (group II, $n = 40$) 24-hour rest/activity patterns measured by actigraphy were identified. Actigraphy patterns were correlated with QOL indices, serum cortisol obtained at 8:00 a.m. and 4:00 p.m. and with serum levels of transforming growth factor-α, tumor necrosis factor-α, and interleukin 6 (IL-6) obtained at 8:00 a.m. and analyzed in duplicate by ELISA. Cytokine levels and survival were also correlated.**

Results: **Group II patients had significantly higher pre treatment levels of all three cytokines, displayed significantly poorer emotional and social functioning, had higher fatigue, more appetite loss, and poorer performance status compared with group I patients. Transforming growth factor-α (TGF-α) and IL-6 were significantly increased in the patients with WHO performance status >1 and in those with appetite loss. Fatigue was significantly associated with elevated TGF-α only. IL-6 was increased in those patients with extensive liver involvement and multiple organ replacement, and it was significantly correlated with dampened cortisol rhythm. In a multivariate analysis, IL-6 was correlated with poor treatment outcome.**

Conclusions: **Significant correlations were found between serum levels of TGF-α and IL-6, circadian patterns in wrist activity and serum cortisol and tumor-related symptoms in patients with metastatic colorectal cancer. These data support the hypothesis that some cancer patient's symptoms of fatigue, poor QOL, and treatment outcome are related to tumor or host generated cytokines and could reflect cytokine effects on the circadian timing system. This interplay between cytokine signaling pathways, the hypothalamic-pituitary-adrenal axis, the autonomic nervous system, and efferent pathways of the suprachiasmatic nucleus that control circadian physiology, opens the way to new rational interventions for symptom management in cancer patients.**

INTRODUCTION

Cancer patients frequently have fatigue, loss of appetite, and other symptoms that are assessed by quality of life (QOL) indices and performance status (PS) scores. The importance of these data is illustrated by poor QOL and low PS predicting for treatment failure and poor survival in several types of cancer (1–3). QOL indices have been shown to be correlated with the novel clinical measurement of circadian rest/activity patterns measured by wrist actigraphy (4). Mormont et al. showed that actigraphy patterns are significantly associated with fatigue and other QOL indices and predict for poor survival in patients with metastatic colorectal cancer (5). Likewise, poor survival is found in metastatic breast cancer patients with dampened cortisol rhythms, a chemical measure of circadian rhythm (6). These data taken together suggest a relationship exists between disrupted circadian rhythm, symptoms, and poor prognosis (7).

Behavioral changes in cancer patients are similar to those observed in the "sickness behavior model" that implicates brain signaling by proinflammatory cytokines (8–15). The hypothesis examined in this report is whether there is an association between symptoms and circulating levels of interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and transforming growth factor-α (TGF-α) in patients with metastatic colorectal cancer. We report here an analysis of archived serum samples from cancer patients assessed with actigraphy, QOL indices, and treatment outcome.

PATIENTS AND METHODS

Study Design and Patient Selection. Our main hypothesis is that elevated circulating levels of tumor-associated cytokines could significantly alter the QOL of cancer patients through the production of circadian clock dysfunction.

A prospectively studied population of 200 consecutive metastatic colorectal cancer patients referred for systemic

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chronochemotherapy at Paul-Brousse Hospital, Villejuif, France served as the index population as previously reported (5). Before receiving chronochemotherapy, these patients had rest/activity monitoring with wrist actigraphy and serum cortisol rhythm assessment as part of their initial work up. The analysis of the rest/activity records allowed the ranking of the patients according to r24, a robust index of the circadian rhythmicity in activity (see below). From these 200 patients, 80 were selected based upon r24 ranking. Forty patients in the top quartile displayed near normal circadian rhythm (group I) and 40 other patients in the lowest quartile had markedly dampened circadian rhythm (group II; ref. 16). This sample size was determined to allow detection of a 40% difference in mean serum concentration of at least one cytokine between the two groups (each one with a SD of ±50%), with an α error of 0.05 and a β power of 90% (two-sided test, Power and Sample Size Calculation software, version 2.1.31). The ethical committees of the Kremlin-Bicêtre (France) and the University of Virginia Health System Institutional Review Board approved the study, and each patient signed a written informed consent.

Cytokine Assessment. The serum concentrations ofIL-6, TGF-α, and TNF-α were measured in duplicate with specific ELISA kits, according to the manufacturer's instructions (QIA61, Oncogene Research Products, Cambridge, MA; DTA50 and D6050, R&D Diagnostics, Minneapolis, MN, respectively). The assays were done at the University of Virginia General Clinical Research Center without knowledge of the patient's group or the results of actigraphy or QOL scores. The cytokine concentrations that were considered were the average of two determinations in samples drawn at 8:00 a.m. on the first day of pretreatment evaluation (5).

Evaluation of the Rest/Activity Cycle. Individual 24-hour rest activity patterns were measured noninvasively with a wrist-worn piezoelectric accelerometer for three consecutive days (Actigraph; Ambulatory Monitoring, Inc., Ardsley, NY; refs. 17, 18). The variables used to estimate circadian rhythm were previously reported (17, 18): autocorrelation coefficient at

Table 1 Clinical features of 80 patients grouped according to actigraphy (r_{24})

	Group I, "good rhythm" ($0.47 < r_{24} < 0.77$)	Group II, "dampened rhythm" ($0.03 < r_{24} < 0.35$)	$P < 0.0001$
Age (y)			
Median (range)	59.5 (42-76)	60 (36-74)	NS*
Sex			
Male: *n* (%)	23 (57.5)	29 (72.5)	
Female: *n* (%)	17 (42.5)	11 (27.5)	NS†
PS (WHO)			
0: *n* (%)	33 (82.5)	20 (50)	
1: *n* (%)	6 (15)	15 (37.5)	
2: *n* (%)	1 (2.5)	5 (12.5)	0.008†
Metastatic organs, *n*			
1 : *n* (%)	20 (50)	19 (47.5)	
≥2: *n* (%)	20 (50)	21 (52.5)	NS†
Liver metastases			
No : *n* (%)	5 (12.5)	11 (22.5)	
Yes : *n* (%)	34 (85)	29 (77.5)	
Unknown: *n* (%)	1 (2.5)	0 (0)	NS†
% Liver involvement by tumor			
≤25%: *n* (%)	28 (70)	27 (67.5)	
>25%: *n* (%)	10 (25)	12 (30)	
Unknown:	2 (5)	1 (2.5)	NS†
Localization of primary tumor			
Colon + sigma: *n* (%)	29 (72.5)	30 (75)	
Rectum : *n* (%)	11 (27.5)	10 (25)	NS†
CT line			
1st : *n* (%)	13 (32.5)	17 (42.5)	
2nd : *n* (%)	27 (67.5)	23 (57.5)	NS†
Serum CEA			
Normal : *n* (%)	5 (12.5)	8 (20)	
Elevated : *n* (%)	31 (77.5)	28 (70)	
Not assessed:	4 (10)	4 (10)	NS†
Serum CA 19-9			
Normal : *n* (%)	10 (25)	7 (17.5)	
Elevated : *n* (%)	25 (62.5)	28 (70)	
Not assessed:	5 (12.5)	5 (12.5)	NS†
QoL symptoms (from EORTC QLQ-C30)			
Global QoL			
Good : *n* (%)	22 (55)	14 (35)	
Poor : *n* (%)	16 (40)	21 (53)	NS*,†
Not assessed:	2 (5)	5 (12)	

(Continued)

Table 1 Continued

	Group I, "good rhythm" ($0.47 < r_{24} < 0.77$)	Group II, "dampened rhythm" ($0.03 < r_{24} < 0.35$)	$P < 0.0001$
Emotional Functioning			
Good : *n* (%)	18 (45)	10 (25)	0.025*
Poor : *n* (%)	19 (48)	25 (63)	
Not assessed:	3 (7)	5 (12)	
Social Functioning			
Good : *n* (%)	25 (63)	13 (32)	0.017*
Poor : *n* (%)	12 (30)	21 (53)	
Not assessed:	3 (7)	6 (15)	
Nausea/Vomiting symptom			
No : *n* (%)	34 (85)	22 (55)	0.007*,†
Yes : *n* (%)	4 (10)	13 (33)	
Not assessed:	2 (5)	5 (12)	
Fatigue symptom			
No or low : *n* (%)	22 (55)	11 (28)	0.003†
High : *n* (%)	16 (40)	24 (60)	
Not assessed:	2 (5)	5 (12)	
Appetite loss			
No : *n* (%)	28 (70)	15 (38)	0.004†
Yes : *n* (%)	11 (28)	20 (50)	
Not assessed:	1 (2)	5 (12)	
HADS questionnaire			
Anxiety			
No : *n* (%)	23 (58)	24 (60)	NS*,†
Borderline : *n* (%)	9 (22)	8 (20)	
Yes : *n* (%)	8 (20)	7 (18)	
Not assessed : *n*(%)	0	1 (2)	
Depression			
No : *n* (%)	36 (90)	31 (78)	NS*,†
Borderline : *n* (%)	1 (2)	3 (8)	
Yes : *n* (%)	3 (8)	5 (13)	
Not asssessed : *n*(%)	0	1 (2)	

*Kruskal-Wallis rank test.
† χ^2 test.

24 hours (r_{24}), a measure of the regularity of the activity pattern over a 24-hour period from one day to the next, and dichotomy indices (I < O and O > I), which respectively measure the difference in activity between the time spent in bed and that spent out of bed. Mean activity was also calculated, although this was not used as a primary variable for rest activity rhythm assessment (17, 18).

More precisely, the autocorrelation function is depicted by a graphic display of correlation coefficients between time series staggered by given time lags, according to the following procedure. In the case of a 3-day time series (72 hours), if X_i is the measurement at time i, the correlation coefficient r_k between X_i and $X_i + k$ is computed for lags k, with $k = 1$ to 4,320 minutes (72 hours). When circadian variation is present, the correlation coefficient increases to its highest value with lags at or near 24 hours. This coefficient can range from −1 to +1. In the case of a prominent circadian rhythm, $r_{24} = 1$.

The dichotomy index I < O is the percentage of the 1-minute activity values while in bed that are inferior to the median value when out of bed. The dichotomy index O > I is the percentage of the 1-minute activity values while out of bed that are superior to the median value when in bed. Both indices can vary between 0% and 100%. In the case of a prominent circadian rhythm with optimal rest at night and optimal activity during daytime, I < O and O > I reach 100%.

Evaluation of Cortisol Circadian Rhythm. The serum concentration of cortisol was determined in blood samples collected at 8:00 a.m. and 4:00 p.m. for two consecutive days (17). The ratio between the mean values obtained at 8:00 a.m. and 4:00 p.m. was shown to be a reliable estimate of the amplitude of the cortisol circadian rhythm in control subjects and in colorectal cancer patients (17, 18).

Quality of Life Assessment. Performance status was rated according to WHO as part of initial patient work up by the oncologist.

QOL questionnaires were filled in by the patients before putting on the actigraph. A study investigator was available for questions about the study and how to fill in the forms. QLQ-C30, from the European Organization for Research and Treatment of Cancer, is a 30-item questionnaire that incorporates five functional scales, eight symptom scales, and a global QOL scale. The questions are formatted with either yes/no answers or with four-answer categories, except for the two questions on general QOL, which are answered on a scale numbered from 1 to 7 (5). All calculations were done after linearly transforming the scores to a 0-100 scale, according to the European Organization for Research and Treatment of Cancer scoring manual guidelines. Higher scores for the global QOL and functional scales represent better functioning, whereas higher scores on the symptom scales indicate a higher level of disturbance. The

Hospital Anxiety and Depression Scale consists of seven items that evaluate anxiety and seven that aim at measuring depression (19); all questions are formatted with four-categories answers. Individual anxiety and depression scores range between 0 and 21, and two cutoff points (8 and 11) are commonly used to differentiate between depressed/anxious, borderline, and normal subjects.

Tumor response and treatment-related toxicity were assessed as previously described (5).

Statistical Analysis. Median, 25th and 75th percentile, range and frequencies were calculated for cytokine serum levels and for all demographic, clinical, rest/activity, or other rhythm-related as well as QOL variables. The normality of the various distributions was checked using a Kolmogorov-Smirnov test.

Kruskal-Wallis nonparametric ANOVA was done to validate differences in cytokine levels between the two groups or as a function of categorical demographic, clinical, and QOL characteristics. χ^2 test was used to compare two dichotomic variables. Possible associations between cytokine levels, rhythm variables, QOL scores, or continuous clinical variables were tested with Spearman rank correlations.

The survival time of each patient was measured from the date of serum sampling. The prognostic value of cytokines serum levels and all the clinical, rhythm-related, and main QOL variables was first examined with univariate analyses. Multiple regression analyses were then conducted with Cox proportional hazard mode to determine which factors were jointly influential on survival.

Finally, by arbitrarily choosing the median value as cutoff point between low and high IL-6, TNF-α or TGF-α, levels, the survival of patients according to each of these factors was estimated with the Kaplan-Meier method, with a comparison of the survival curves by the log-rank test. All statistical analyses were carried out with SPSS 11.5 for Windows (SPSS, Inc., Chicago, IL).

RESULTS

Patient Characteristics and Quality of Life Indices. The clinical and demographic features of group I and II patients did not differ except for performance status which was worse in group II patients. No difference was found between groups with regard to surgical removal of primary tumor, prior adjuvant chemotherapy, or prior radiotherapy (Table 1).

The analyses of the QOL questionnaires revealed that pretreatment emotional and social functioning scores were significantly higher in group I patients, whereas fatigue, vomiting, and appetite loss symptom scores were significantly higher in group II patients (Table 1).

Cytokines Levels: Relation with Clinical and Quality of Life Variables. Median serum levels of IL-6, TGF-α, and TNF-α were higher in group II than in group I by 1.5-fold (P from Kruskall-Wallis ANOVA = 0.006), 4.5-fold (P = 0.002), and 1.7-fold (P = 0.028), respectively (Fig. 1). Positive correlations were found between IL-6 and TGF-α concentrations (r = 0.30, P = 0.007) and between TGF-α and TNF-α concentrations (r = 0.27, P = 0.02) but not between IL-6 and TNF-α (r = 0.10, P = 0.41).

IL-6 levels were significantly higher in the patients with more than one organ involved by metastases and in those with >25% liver replacement by the tumor (Fig. 2*A*). In these latter patients, TNF-α concentrations were also increased (median: 16.6 versus 11.1 pg/mL; P = 0.032). Metastases from rectal tumors were associated with lower TGF-α levels as compared with those from colon primary (median: 0.50 versus 2.02 pg/mL; P = 0.019). No other clinical variable displayed any significant relation with any of the three cytokine levels that were measured (data not shown).

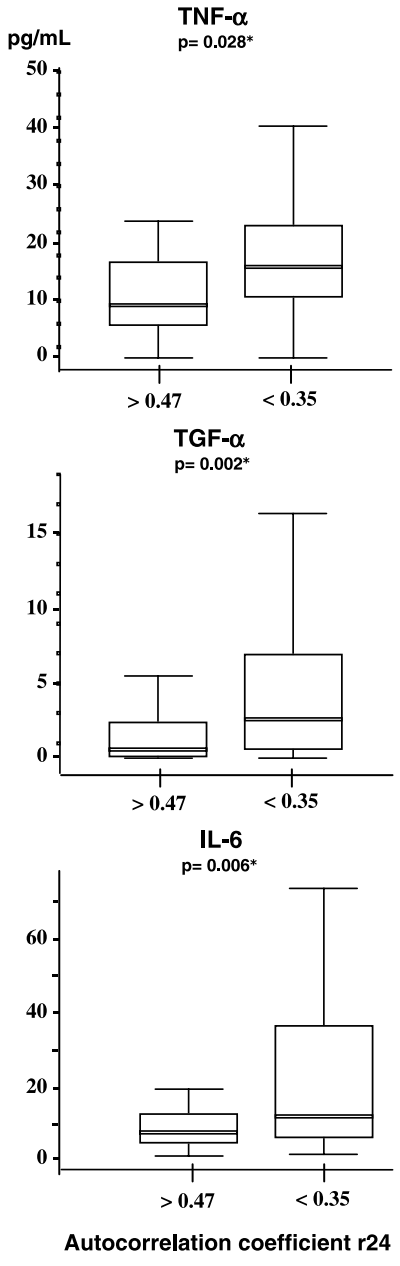

Fig. 1 Cytokine serum levels for TNF-α, TGF-α, and IL-6 in patients with "good" (>0.47) or "dampened" (<0.35) activity rhythm as measured by the autocorrelation coefficient r_{24}. Median (*dark horizontal line*), 25% to 75% quartiles (*open column*), and range (*light horizontal lines*) are shown for each cytokine in each group.

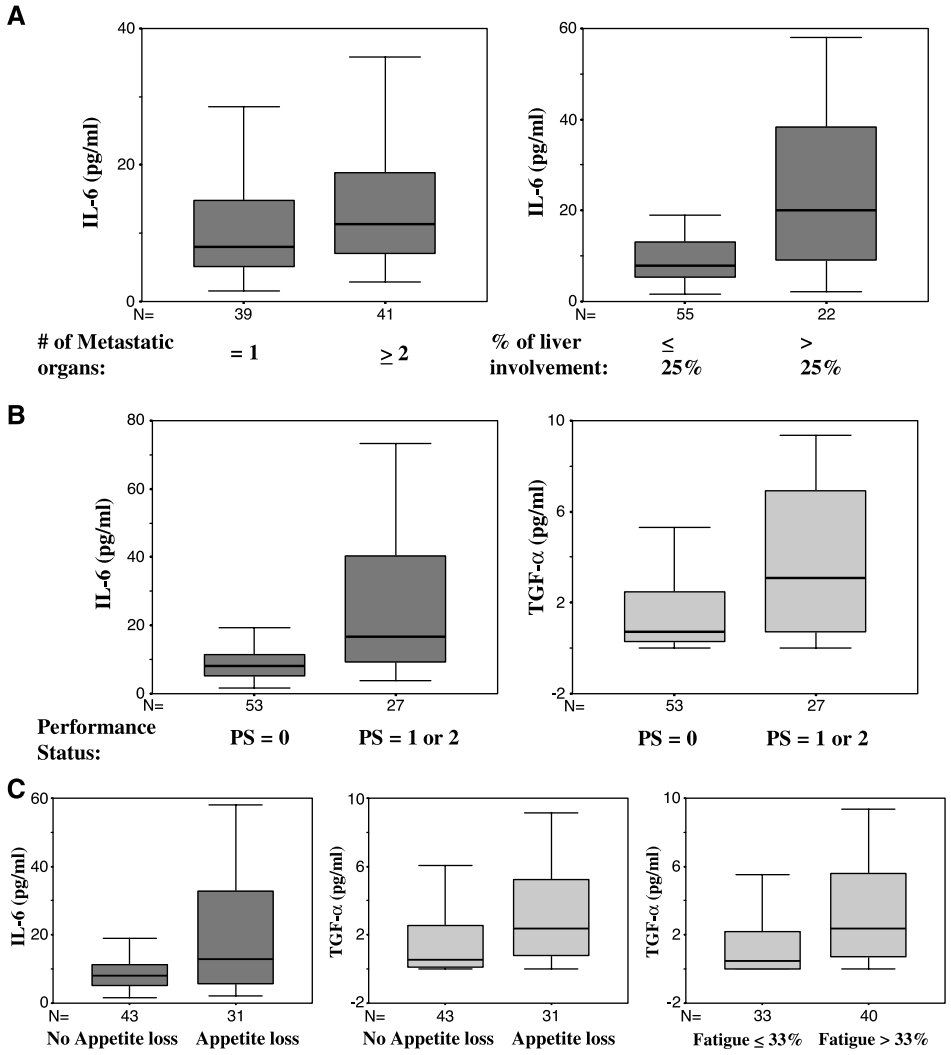

Fig. 2 Main relations between cytokine serum levels and extent of metastatic disease (*A*), WHO performance status (*B*), and QOL items (*C*). *A,* IL-6 is increased in the patients with metastases in more than one organ and in those with >25% involvement of the liver based on CT assessment ($P < 0.05$). *B,* IL-6 and TGF-α are significantly increased in those patients with poorer performance status ($P < 0.05$). *C,* IL-6 and TGF-α are increased in those patients with appetite loss; TGF-α is increased in those with higher fatigue scores ($P < 0.05$).

Patients with PS = 1 or 2 had higher serum levels of IL-6 ($P < 0.0001$) and TGF-α ($P = 0.02$) in comparison with patients with PS = 0 (Fig. 2*B*). No significant relation was found between PS and TNF-α serum concentration.

Patients with appetite loss displayed significantly higher serum levels of both IL-6 and TGF-α ($P = 0.012$ for either variable) as compared with patients with no such complaint. Patients with fatigue scores of >33% had significantly higher TGF-α serum levels as compared with nonfatigued patients ($P = 0.006$; Fig. 2*C*). Positive correlations were further found between IL-6 concentration and appetite loss score ($r = 0.30$, $P = 0.01$) or nausea/vomiting score ($r = 0.28$, $P = 0.016$) and between TGF-α concentration and appetite loss score ($r = 0.35$, $P = 0.002$) or fatigue score ($r = 0.28$, $P = 0.018$). No significant correlation was documented between TNF-α and any symptom score (data not shown).

Circadian Physiology. Group I patients with marked circadian rhythm (high r_{24} values) also had higher dichotomy indices (I < O and O > I), higher mean activity, and higher serum cortisol ratios between 8:00 a.m. and 4:00 p.m., in comparison to group II patients. Mean serum cortisol concentration did not differ significantly between both groups.

Although the patients in group I, with pronounced circadian rhythms, had significantly lower levels of all three cytokines as compared with the patients in group II, with dampened rhythms (Fig. 1), correlation studies indicated that the cytokine levels bore different relation with the few components of circadian physiology that were investigated here. Thus, all three cytokine levels were negatively correlated with both dichotomy indices, I < O and O > I. Both IL-6 and TGF-α concentrations were positively correlated with mean serum cortisol ($r = 0.342$, $P = 0.04$; $r = 0.48$, $P = 0.0001$). Only IL-6 level was further negatively correlated with circadian cortisol ratio ($r = 0.24$, $P = 0.042$; Table 2).

Cytokines and Treatment Outcomes. When comparing the study population according to disease response to chronochemotherapy, the serum levels of IL-6, TNF-α, and TGF-α were found to be higher in the subgroup of patients whose disease was refractory to the chronochemotherapy ($n = 21$), intermediate in patients whose best response was disease stabilization ($n = 25$) and lower in the patients with

Table 2 Rhythm-related parameters in the two groups

	Group I				Group II				
	Median	25th	75th	Range	Median	25th	75th	Range	*P*
r_{24}	0.58	0.51	0.62	(0.47-0.67)	0.22	0.13	0.30	(0.03-0.35)	<0.000l
I < 0 (%)	98.45	96.96	99.69	(95.02-100)	91.36	82.09	96.70	(48.70-99.53)	0.0001
0 > I (%)	96.57	93.40	97.83	(83.50-99.73)	83.89	74.52	89.16	(50.80-95.03)	0.0001
Mean activity (arbitrary units)	107.5	84.3	125.0	(26.0-161.0)	84.0	48.0	101.8	(4.0-127.0)	<0.0001
Cortisol ratio 08h/16h	1.72	1.48	1.98	(0.81-2.75)	1.60	1.14	1.77	(0.65-3.52)	0.031
Mean cortisol (nmol/l)	342.5	279.5	436.8	(210.3-600.5)	357.3	310.5	558.0	(37.3-942.3)	NS

objective response ($n = 34$). These differences were statistically significant (data not shown).

Median survival was 13.8 months (95% confidence interval, 9.7-17.9) in group I whereas it was 11.1 months (9.6-12.5) in group II (P from log-rank = 0.0176). Patients with serum IL-6 level below the overall median IL-6 value had a median survival of 15.7 months (13.0-18.3), whereas a median survival of 8.8 months (4.5-13.1) was found in those patients with serum IL-6 concentration above median value ($P < 0.0001$). Patients with high IL-6 had significantly poorer survival rate at 2 years regardless of their performance status, as estimated by the Kaplan-Meier survival life table. In the subgroup of patients with PS = 0, the 2-year survival of those with high IL-6 levels was 20% (±9%) compared with 36% (±8%) in those with low serum IL-6 ($P = 0.02$). Similarly, in the subgroup of patients with PS = 1 or 2, the survival rate was 0% and 43% (±19%) in patients with high and low IL-6 serum levels, respectively ($P = 0.0001$).

Univariate analyses showed a prognostic value on survival for serum IL-6 concentration, for four clinical factors (number of metastatic sites, number of previous chemotherapy lines for metastatic disease, Ca 19.9 level, and PS), for the scores of five QOL items (depression, appetite loss, fatigue, physical functioning, and global QOL), and for three circadian physiology-related variables (r_{24}, I < O and mean plasma cortisol). The multivariate Cox model identified the following factors to be jointly influential on survival: serum IL-6 concentration, number of metastatic sites, number of previous chemotherapy lines, CA 19.9, and appetite loss scores (Table 3).

Table 3 Statistical significance of prognostic factors for the survival in univariate and multivariate Cox analyses

	P	
Variable	Univariate	Multivariate
Cytokines		
IL-6	<0.0001	0.013
Clinical		
Previous treatment for metastases	0.036	0.018
Number of metastatic sites	0.004	0.002
CA 19.9	<0.0001	0.01
PS	<0.0001	NS
Liver involvement	0.047	NS
Rhythm-related		
r_{24}	0.001	NS
I < O	<0.0001	NS
Mean plasma cortisol	<0.0001	NS
QoL		
Appetite-loss	<0.0001	0.001
Fatigue	<0.0001	NS
Global QoL	0.018	NS
Physical functioning	0.007	NS
Depression	0.002	NS

DISCUSSION

Affective and constitutional symptoms in cancer patients show clustering patterns that suggest distinct and shared biological mechanisms in their production (8). Evidence from multiple disciplines (infectious disease, endocrinology, immunology, and neuroscience; ref. 9) supports the notion that symptoms in cancer patients could be caused by cytokines produced directly by tumors, or by host cells secondary to tumor invasion, that signal the central nervous system. The ''cytokine hypothesis'' is exemplified in the laboratory by sickness behavior observed in animals challenged with i.p. administration of lipopolysaccharide that display physiologic components of an acute phase response with fever, pain, social withdrawal, decreased food intake, and wasting (9). Systemic symptoms are mediated by circulating cytokines and/or by those produced centrally by afferent nerve fibers through a cascade of the proinflammatory cytokines IL-1, TNF-α, and IL-6 that signal the hypothalamic-pituitary-adrenal axis and the autonomic nervous system (9, 15, 20, 21). Further preclinical evidence for the cytokine hypothesis is supported by the finding of cytokine receptors for TGF-α, epidermal growth factor (EGF), TNF-α, and IL-6 in many regions of the brain (14, 22). They are thought to act in an autocrine and paracrine fashion as is evident in the regulation of neuroendocrine function, injury, inflammation, and physical and physiologic stress (14, 22). *In vitro* and *in vivo* studies suggest that signaling through these receptors occurs at picomolar concentrations of the ligand (22). Although the cytokine levels in our study using ELISA kits are low compared with those reported in the literature with RIA kits (23), this would not alter the correlative relationship with the outcomes examined in our study. Some degradation of the protein may have occurred over the 10 years of frozen storage and possibly equally for all samples before being analyzed.

Further support for the cytokine hypothesis comes from studies of the administration of the cytokines IFN-α and IL-2 in humans that are associated with the development of cognitive and depressive symptoms (24). Also relevant to our observations are data showing that administration of low doses of IL-6 in the evening to healthy men induces acute tiredness and feelings of being inactive and poor mental concentration (25). In cancer

patients, the cytokine hypothesis is supported by two reports showing a correlation between serum levels of IL-6 or vascular endothelial -growth factor (thought to signal via IL-6) and symptoms of depression or altered psychosocial functioning in pancreatic and ovarian cancer patients, respectively (26, 27). Likewise, we show that increased IL-6 in group II patients is associated with poorer performance status, impaired emotional and social functioning, worsened appetite loss, and disrupted circadian motor activity compared with group I patients. IL-6 has been shown to correlate to stage, disease extent, and survival in colon cancer and may reflect inherent disease aggressiveness (e.g., cell proliferation and metastatic proclivity; ref. 28). However, in the largest reviewed series of colon cancer patients, IL-6 was not independently predictive of survival in a multivariate analysis (29). Our data shows elevated IL-6 is correlated with high tumor burden consisting of extensive liver involvement and metastasis in multiple organs, a condition where compromised liver function and decreased clearance of IL-6 may take place. Another factor contributing to poor outcome in our patients may be the disruption of circadian physiology as evidenced by the significant correlation of increased IL-6 with dampened cortisol and rest/activity circadian rhythms. Laboratory studies show that administration of IL-6 increases serum glucocorticoid levels through hypothalamic pathways; in patients with chronic stress from cancer, altered patterns of cortisol rhythmicity are also associated with poor prognosis (6).

Another aspect of the cytokine hypothesis is guided by the emergence of an understanding of the role of TGF-α in cancer prognosis (30) and signaling within the central nervous system (31–35). TGF-α is a ligand for the EGF receptor (EGFR1) and colorectal cancer patients having both elevated tumor EGFR expression and increased circulating levels of TGF-α have relatively poor prognosis (23, 36). In our study, we show a significant correlation between symptoms, poor QOL, worsened appetite loss, and perhaps most significantly, increased fatigue in the patients with elevated serum levels of TGF-α. The latter finding is of particular interest in light of the recent report that TGF-α mediates hypothalamic signaling for the circadian regulation of motor activity, sleep, and body temperature (33). In this animal model, micromolar infusion of TGF-α into the hypothalamic subparaventricular zone blocks signaling from the suprachiasmatic nucleus which is the dominant circadian pacemaker controlling the rest/activity cycle as well as several physiologic and endocrine functions (37). Circadian rhythm can also now be understood in the context of a genetically controlled molecular clock (37–39) where 12 identified "clock" genes signal rhythmic output to multiple efferents including the EGFR-positive staining cells in the subparaventricular zone (33). Furthermore, behavioral abnormalities like inability to cope with stress and increased aggressivity have been reported in transgenic mice overexpressing TGF-α (40–42). We have also observed the loss of running wheel activity (mouse actigraphy) in tumor-bearing mice associated with elevated TGF-α tumor expression and increased serum TGF-α levels compared with nontumor-bearing controls.[6] TGF-α, a small polypeptide of 50 amino acids, has been found to slowly cross the blood brain barrier by a shared EGF/TGF-α receptor transport mechanism (43–45).

There is now evidence for a role of the circadian system and the molecular clock in cancer incidence and tumor progression (37, 46). A role for the molecular clock in tumor proliferation is suggested by experimental evidence that the clock gene *period 2* exerts tumor suppressor gene–like properties (47). These observations are consistent with the finding of accelerated tumor growth in animal models where the hypothalamic circadian pacemaker has been physically ablated or the molecular clock has been disrupted with chronic jet lag exposure (48, 49).

The implications of cytokines mediating an array of signals through the hypothalamic-pituitary-adrenal axis or the circadian axis may have practical consequences. First, behavioral effects on the host may compromise treatment outcome by decreasing the ability of the host to tolerate treatment. The development of therapeutic measures attempting to reset circadian time structure in patients with circadian disruption could bolster resistance and thus enhance tolerance to treatment. Second, the biological behavior of the tumor may be altered by SCN controls of peripheral clock regulation that could impact cell cycle check points, proliferation, and biochemical pathways in mammalian tissues (39, 50). New therapeutic strategies to assist in alleviating symptoms in cancer patients and treatment paradigms might result from a better understanding of the role of the interaction of cytokines, in particular the epidermal growth factor family, with circadian physiology and the biochemical behavior of the molecular clock.

[6] Unpublished data.

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