Diurnal Cortisol Rhythm as a Predictor of Breast Cancer Survival

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Background: **Abnormal circadian rhythms have been observed in patients with cancer, but the prognostic value of such alterations has not been confirmed. We examined the association between diurnal variation of salivary cortisol in patients with metastatic breast cancer and subsequent survival. We explored relationships between cortisol rhythms, circulating natural killer (NK) cell counts and activity, prognostic indicators, medical treatment, and psychosocial variables.** ***Methods:*** **Salivary cortisol levels of 104 patients with metastatic breast cancer were assessed at study entry at 0800, 1200, 1700, and 2100 hours on each of 3 consecutive days, and the slope of diurnal cortisol variation was calculated using a regression of log-transformed cortisol concentrations on sample collection time. NK cell numbers were measured by flow cytometry, and NK cell activity was measured by the chromium release assay. The survival analysis was conducted by the Cox proportional hazards regression model with two-sided statistical testing.** ***Results:*** **Cortisol slope predicted subsequent survival up to 7 years later. Earlier mortality occurred among patients with relatively "flat" rhythms, indicating a lack of normal diurnal variation (Cox proportional hazards, *P* = .0036). Patients with chest metastases, as opposed to those with visceral or bone metastases, had more rhythmic cortisol profiles. Flattened profiles were linked with low counts and suppressed activity of NK cells. After adjustment for each of these and other factors, the cortisol slope remained a statistically significant, independent predictor of survival time. NK cell count emerged as a secondary predictor of survival.** ***Conclusions:*** **Patients with metastatic breast cancer whose diurnal cortisol rhythms were flattened or abnormal had earlier mortality. Suppression of NK cell count and NK function may be a mediator or a marker of more rapid disease progression. [J Natl Cancer Inst 2000;92:994–1000]**

Cancer poses numerous physical and emotional stresses. While disease and treatment exert a heavy physiological toll, accompanying anxiety about diagnosis and prognosis, taxing medical treatments, and disruption of social, vocational, and family functioning constitute a series of psychological stressors. Cancer patients repeatedly endure physical and emotional events that activate stress-response mechanisms, including the hypothalamic–pituitary–adrenal (HPA) axis. Such repeated activation has been associated with HPA axis dysregulation and adverse health consequences *(1)*.

One sign of dysregulation in this endocrine stress response system is altered circadian cortisol rhythms *(2–4)*. In healthy individuals, cortisol levels are usually highest before awakening and decrease during the day *(5)*, but up to 70% of patients with advanced breast cancer show flattened circadian profiles, consistently high levels, or erratic fluctuations *(6,7)*.

Although the specific causes of circadian dysregulation in cancer are undetermined, cortisol dysregulation has been linked independently with the physical stress of cancer *(8)* and with psychological stressors *(4)*. Among patients with breast and ovarian cancers, severe endocrine disruption is seen in more advanced disease (e.g., patients with poor performance status and liver metastases have more markedly abnormal rhythms) *(7,9)*. However, psychological distress also accompanies aberration in cortisol levels among cancer patients *(10)*. Physically healthy individuals with altered diurnal cortisol profiles have been characterized as chronically stressed *(3,4)*, and aberrant rhythms are evident in subjects with de-

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pression *(11)*, unemployment *(12)*, and post-traumatic stress disorder *(2)*. Associations of cortisol profiles with psychological stress suggest that rhythm dysregulation may be a marker or mediator of psychosocial effects on cancer progression.

Dysregulation of the cortisol response may compromise tumor resistance. Glucocorticoids have been implicated in tumor growth, both in animal and in *in vitro* studies *(13,14)*. Cortisol may accelerate tumor growth via immunosuppressive actions *(15)* or effects on metabolic processes *(16)*. When cortisol profiles are affected, dysregulated patterns of immune activity and immune cell trafficking may also emerge *(17,18)*. Indeed, breast and ovarian cancer patients with altered cortisol rhythms show disruptions in patterns of circulating leukocytes, neutrophils, platelets, and serum proteins *(9)*.

The prognostic consequences of circadian disruption in cancer patients are as yet unknown *(8,9)*. In this study, we investigated HPA axis dysregulation as a predictor of metastatic breast cancer progression. The primary hypothesis was that aberrant diurnal salivary cortisol rhythms would predict earlier mortality from metastatic breast cancer. Second, we conducted exploratory analyses to determine whether associations of cortisol rhythm disturbance with traditional prognostic indicators, medical treatments, psychosocial variables, and immune function exerted statistically significant effects in the cortisol rhythm–survival relationship.

PATIENTS AND METHODS

Study Population

Women with metastatic breast carcinoma (n = 125) were recruited over a 5-year period from June 1991 through January 1996 for a new randomized, prospective study of Supportive/Expressive group psychotherapy and cancer survival designed to replicate and extend our earlier finding of longer survival with group support *(19)*. Subjects had breast cancer with systemic metastasis and a Karnofsky rating of 70% or more, were fluent in English, and lived in the San Francisco Bay area. Subjects were excluded if they had one of the following: 1) active cancers within the past 10 years other than breast cancer, basal cell or squamous cell carcinomas of the skin, or *in situ* cancer of the cervix; 2) positive supraclavicular lymph nodes as the only metastatic lesion at the time of diagnosis; or 3) a concurrent medical condition likely to influence short-term survival. The Stanford University Institutional Review Board approved the study procedures. Written informed consent was obtained from all participants.

A total of 104 patients contributed data to the current analyses, while 21 were excluded because they were taking hydrocortisone-based medications (n = 10), they did not provide saliva samples of adequate volume or number for analysis of diurnal cortisol profiles (n = 8), or they reported feeling too ill to collect any saliva samples (n = 3). Baseline endocrine data were collected after recruitment and before randomization (an average of 25 days passed from cortisol collection to randomization). Data concerning current medications, pain (Pain-Rating Scale) *(20)*, depression (Center for Epidemiologic Studies Depression Scale) *(21)*, and sleep quality *(22)* were also collected before randomization. Patients had been diagnosed a mean (standard deviation [SD]) of 1.8 (3.1) years before the study entry (Table 1).

Of the 104 patients in the current sample, 58 were randomly allocated to the psychotherapy group and 46 were randomly allocated to the educational control group. Baseline cortisol data were collected a mean (SD) time of 5.9 (1.4) years before the current analysis (range, 3.1–7.7 years). The mean survival time of the 71 deceased patients was 2.3 (1.5) years from the time of cortisol assessment (range, 0.3–7.1 years), and the remaining 33 had been followed for a mean of 5.4 (1.6) years (range, 3.1–7.7 years). No patients were lost to follow-up.

This study was designed to test two major hypotheses: 1) whether or not Supportive/Expressive group psychotherapy increased survival time, a replication of an earlier trial of the effect of this intervention on survival *(19);* and 2) whether endocrine dysfunction was associated with the rate of disease progression by examining the relationship between diurnal cortisol patterns and survival. In the current analysis, we tested only the second hypothesis rather than addressing treatment/control differences. At the time of analysis for this report, overall mortality was 68%, a rate at which one could reasonably expect to see any relationship between a physiologic measure previously associated with advancing disease and survival time. However, 68% mortality is too early to test the group psychotherapy hypothesis. In the previous trial *(19)*, the survival effect associated with group therapy did not begin to emerge until mortality had exceeded 50%. Were we to replicate

Table 1. Demographic characteristics and description of 104 women with metastatic breast cancer enrolled in the study (data taken at study entry)

Characteristic	Mean (standard deviation)
Age, y	53.2 (10.7)
Ethnicity, %	
Asian, 5.8	
Black, 1.0	
Hispanic, —	
Native American, 1.0	
White, 90.4	
Other, 1.9	
Educational level, y	16.2 (2.6)
Marital status, %	
Never married, 10.6	
Married, 52.9	
Separated, 1.9	
Divorced, 26.9	
Widowed, 6.7	
Other, 1.0	
Disease-free period, y	4.0 (3.0)
Years from cancer recurrence to study entry	1.8 (3.1)
Dominant cancer site at study entry, %	
Bone, 39.4	
Chest wall or regional lymph nodes, 30.8	
Viscera, 29.8	
Tumor estrogen receptor status, %	
Positive, 77.9	
Negative, 22.1	
Prior systemic treatment for metastatic disease, %	
Hormonal therapy, 77.9	
Chemotherapy, 46.2	
Currently on hormonal treatment at study entry, 69.2%	
Currently on megestrol at study entry, 12.5%	
Treatment within 2 mo of study entry, %	
Chemotherapy, 30.8	
Radiotherapy, 18.3	
Physicians' Karnofsky performance rating, %	
70, 3.8	
75, 2.9	
80, 20.2	
85, 2.9	
90, 30.8	
95, 2.9	
100, 36.5	

the previous findings, one would not expect the divergence to be statistically significant until well beyond 50% mortality. Indeed, in the previous study, we waited until the mortality was greater than 95% before testing the treatment versus control group hypothesis *(19)*. On the basis of our prior data, we are not yet able to conduct a valid test of the effect of Supportive/Expressive psychotherapy on cancer survival. In fact, recruitment of the current sample ended only in 1995, and many of the survivors are relatively healthy at this point. A definitive result of the intervention will be published when the sample reaches the predetermined threshold of 90% overall mortality.

Collection of Physiologic Data

The measurement of cortisol in saliva has been confirmed to reliably reflect free cortisol levels in blood *(23)*. Patients collected saliva at home with "Salivette" (Sarstedt, Inc., Newton, NC) devices, consisting of a cotton swab fitted into a plastic holder resting inside a centrifuge tube. Sampling was at 0800, 1200, 1700, and 2100 hours on 3 consecutive days. Medications, sleep, diet, exercise, and stressors during the sampling period were assessed by questionnaire. Samples were centrifuged for 4 minutes at 2500 rpm, and aliquots were frozen at −70 °C. From 95 patients who consented, blood samples for lymphocyte counts were drawn on 2 days approximately 1 week apart between 0700 and 1000 hours. Among healthy subjects, natural killer (NK) cell counts tend to peak during these hours.

Laboratory Methods

Cortisol levels were assessed by ^{125}I radioimmunoassay. Intra-assay coefficients of variation on three different saliva pools averaged 5.3%, and the inter-assay coefficient was 12%. Assay sensitivity was 0.008 μg/dL. Percents and absolute numbers of NK cells were measured by flow cytometry by use of monoclonal antibodies to identify cells that were positive for CD56 (NKH-1 clone) and negative for CD3 (CD3-T3 clone) cell surface antigens (both antibodies were received from Coulter Cytometry Inc., Hialeah, FL). Cytolytic activity was assessed by the chromium-release method. Lytic units reflecting 20% lysis (LU20) were calculated *(24)* and corrected for NK cell numbers. The mean absolute numbers of NK cells and the mean LU20 for the two specimens were used.

Statistical Methods

Because dysregulated cortisol rhythms have been characterized by flattened or aberrant daytime profiles and healthy cortisol rhythms by high morning and lower evening levels, the slope of diurnal change in the cortisol level was calculated to estimate how each patient fit the normal (i.e., descending) profile *(25)*. Since the distribution of raw cortisol values is typically skewed and the normal diurnal profile may be approximated by an exponential curve, raw values were log transformed. The regression of the 12 cortisol values on the hour of sample collection was calculated, with data pooled over the 3 days for each patient. Steeper slopes are represented by smaller β values for the slope of the regression, which indicate cortisol declining more rapidly. Flatter slopes (larger β values) indicate slower declines, abnormally timed peaks, or increasing levels during the day. The average area under the curve (AUC) was also calculated over 3 days of sample collection by trapezoidal estimation using log-transformed cortisol values.

The primary hypothesis was tested by analyzing the relationship between the baseline diurnal cortisol slope and the subsequent survival time. A regression was conducted on survival time, measured from the date of saliva collection, by use of the Cox proportional hazards model in a two-tailed test. The secondary hypotheses were exploratory in nature and were tested with conservative intent to rule out the possibility that any relationship between the cortisol slope and survival time was not explained by variance due to some other factor related to the disease or treatment process. These hypotheses evaluated the possible contribution of traditional prognostic indicators, including age, site of recurrence, estrogen receptor status, disease-free interval, time since diagnosis of cancer recurrence, and Physicians' Karnofsky Rating; medical treatments, including hormonal therapy (e.g., tamoxifen and megestrol), recent chemotherapy, and recent radiotherapy; psychosocial variables, including treatment versus control group assignment, marital status, sleep quality, pain, and depression; and two immune measures, NK cell counts (absolute numbers per cubic millimeter whole blood) and function (LU20). Spearman rank correlations with tie correction were used to test associations of the cortisol slope with each of these factors. One-tailed tests were used with the intent of most conservatively identifying factors associated with the cortisol slope that might influence the relationship of cortisol slope and survival. The contributions of these variables to the cortisol slope–survival relationship were tested by entering each variable as a covariate with the cortisol slope in a separate Cox regression. Here, the predictive value of the cortisol slope was considered to remain after adjustment for these other variables only when it met the more stringent criterion of significance in a two-tailed test. The relative risk of medical variables that are common prognostic indicators in breast cancer was evaluated by survival analyses performed by Cox regression. The Bonferroni correction was not applied to tests of the secondary hypotheses because they were exploratory in nature.

RESULTS

Flatter diurnal cortisol slopes predicted shorter subsequent survival times (Cox proportional hazards two-tailed P = .0036; hazard ratio = 464.9; 95% confidence interval [CI] = 7.5–28 953.0). Although the survival analysis was conducted using the entire sample and cortisol slope as a continuous variable, for descriptive (not statistical) purposes, subjects were split into two equal groups based on the cortisol slope. Fig. 1, A, shows the mean cortisol levels at morning, afternoon, and evening hours in these groups. Fig. 1, B, shows cortisol levels from four individual patients representing the various types of cortisol profiles that we observed. Kaplan–Meier survival plots of the two groups in Fig. 1, A, are shown in Fig. 2. The divergence in survival as a function of slope emerged approximately 1 year after cortisol assessment and extended at least 7 years after. Among patients split at the median cortisol slope, 77% of those with flat rhythms died (average survival, 3.2 years; 95% CI = 2.5–3.9 years), while only 60% of those with steep rhythms died (average survival, 4.5 years; 95% CI = 3.7–5.2 years); survival plots of these groups diverged significantly (log-rank, P = .016, two-tailed).

Fig. 1, B, shows data from four patients representing the various types of cortisol profiles observed. Only 37% of the patients had relatively normal diurnal rhythms, with cortisol concentrations that peaked at 0800 hours and levels that declined consistently thereafter. Peak concentrations occurred later in the day for 49% of the sample, while 14% had no apparent peak during the observed times of day. As suggested by the profiles shown in Fig. 1, B, in which all three patients with "aberrant" rhythms (open symbols) had lower morning and higher evening cortisol levels as compared with the "normal" diurnal rhythm (closed symbols), patients with flatter diurnal slopes had statistically significantly lower morning and higher evening cortisol levels. The minimum cortisol concentration over all times of day was greater for patients with flatter slopes (Spearman R = .22, P = .02).

Patients with flatter slopes had fewer absolute numbers of circulating NK cells (Spearman R = −.25; P = .007). Suppression of NK cell activity was also evident among patients with flatter slopes (R = .17; P = .05). Table 2 presents the mean and SD for NK cell number and NK cell lytic units reflecting a 20% lysis for groups dichotomized at the median cortisol slope for illustrative purposes as described in Figs. 1, A, and 2. Higher absolute NK cell counts predicted longer survival when used as a single independent variable in a Cox regression (P = .037; two-tailed; hazard ratio = 0.9977; 95% CI = 0.9956–0.9999) (n = 95). However, when both cortisol slope and absolute NK cell counts were included in the regression, the slope emerged as a stronger predictor of survival (cortisol slope—two-tailed P = .013, hazard ratio = 339.1, and 95% CI = 3.5–32 760.1 versus NK cell counts—P = .069, hazard ratio = 0.9980, and 95% CI = .9959–1.0002). NK cell activity was not associ-

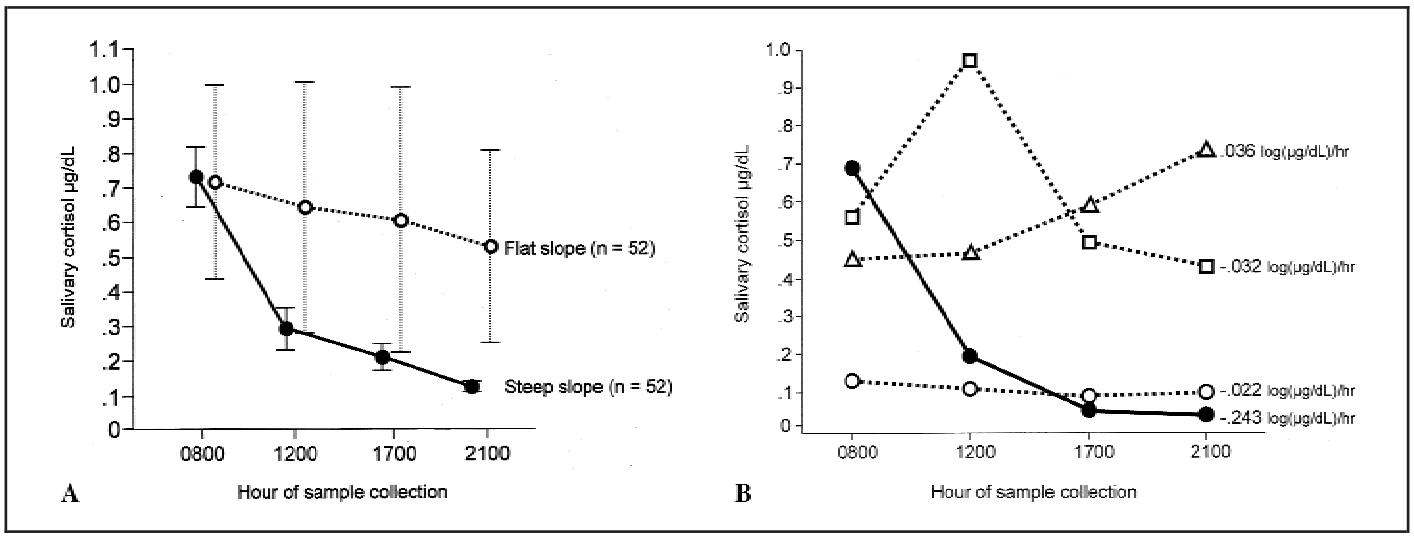

Fig. 1. Panel A: Mean (bars show 95% confidence intervals) diurnal salivary cortisol levels at four times of day for two equal groups of patients split at the median diurnal cortisol slope (−0.091 log [µg/dL] per hour). The average value of the diurnal cortisol slope for the flat slope was −.040 log(µg/dL)/hour and for the steep slope group it was −.128 log(µg/dL)/hour. **Panel B:** Mean salivary cortisol levels at four times of day, with the diurnal cortisol slope shown in the legend for each plot. Data from four study participants with cortisol profiles that represent the several types of profiles observed. The data from patients who were in the flat-slope group after the dichotomous split used in Figs. 1, A, and 2 are shown in the **open symbols.** The data from one patient in the steep-slope group are shown with **closed symbols.** Only 37% of the patients had cortisol concentrations that peaked at 0800 hours with levels that consistently declined thereafter. This pattern is represented by the patient whose data are shown with **closed circles.** Another 49% of patients had peaks occurring later in the day (□ and △), and 14% had no apparent peak during the observed times of day (○).

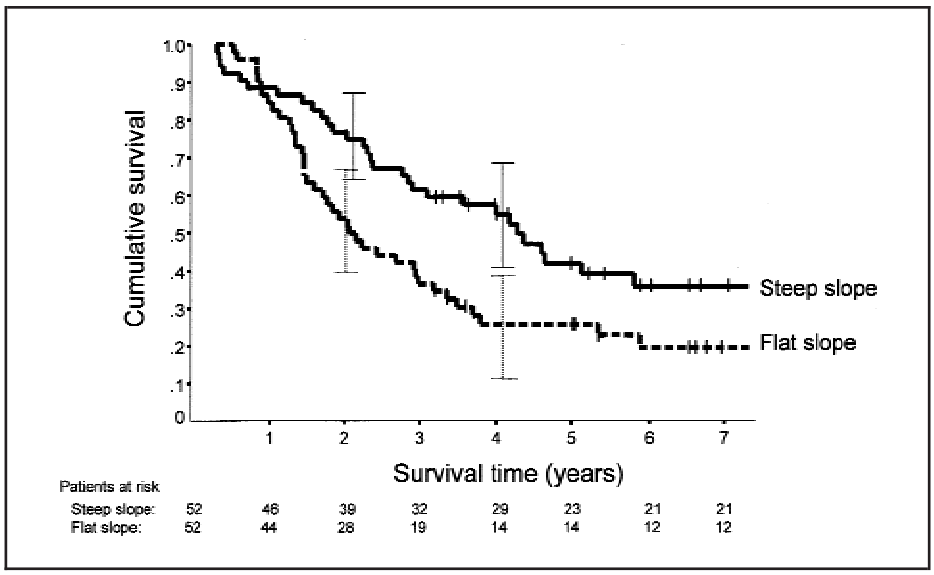

Fig. 2. Kaplan–Meier survival curves for patients split into two equal groups at the median diurnal cortisol slope (−.091 log [µg/dL] per hour). This grouping was performed only to illustrate survival curves representing patients with relatively steep versus flat cortisol slopes. The definitive survival analysis was conducted on the entire sample using the continuous variables of cortisol slope and survival time in a Cox regression. Patients with relatively flat cortisol slopes experienced shorter subsequent survival (Cox proportional hazards, two-tailed $P = .0036$). Among the patients split at the median cortisol slope, 77% of those with flat rhythms died, after surviving an average of 3.2 years (**broken line**). In contrast, 60% of the patients with relatively steep rhythms died, but they survived more than 1 year longer on average, with an average survival of 4.5 years (**solid line**). Survival plots of these groups diverged significantly (log-rank, two-tailed $P = .016$). Patients still living at the time of analysis are indicated by **black vertical slash marks.** The mumbers of living patients at each year mark are listed for the "flat-slope" and "steep-slope" groups whose survival curves are shown in the figure.

ated with subsequent survival. Overall levels of cortisol (AUC) were not associated with NK cell numbers or with their functional activity.

As a check against possible confounding variables, we examined associations of the cortisol slope with the markers of disease status, medical treatments, and psychosocial variables listed previously. There was a small but statistically significant association ($P = .023$) between metastasis in the chest wall or adjacent lymph nodes as opposed to visceral or bone metastases and steeper slopes (steeper slopes—i.e., their cortisol profiles look more "healthy"). Patients who were taking megestrol had flatter slopes ($P = .000$). Patients who reported more nocturnal awakenings had flatter slopes ($P = .003$), and marital disruption was associated with flatter slopes ($P = .040$). Slope remained a statistically significant predictor of survival, even after adjustment for the effects of each of these variables. No other variable related to disease or treatment was statistically significantly associated with the cortisol slope.

As yet an additional check, we evaluated the predictive value of the common prognostic indicators in breast cancer (e.g., age at initial diagnosis, disease-free interval, and estrogen receptor status) in this sample by use of survival calculated in the more traditional manner, from the time of initial diagnosis. As expected, estrogen receptor-negative tumor status and shorter disease-free interval predicted shorter survival measured from the date of initial diagnosis. However, once again, cortisol slope remained a statistically significant predictor of survival after adjustment for each of these variables.

There were no differences in baseline

Table 2. Mean (standard deviation) numbers and functional activity* of natural killer (NK) cells in groups of patients with metastatic breast cancer dichotomized at the median diurnal cortisol slope

Cortisol slope log, μg/dL per h	NK cells per mm^3 whole blood	Lytic units,* 20% lysis	% lysis by effector-to-target cell ratio				
			100 : 1	50 : 1	25 : 1	12 : 1	6 : 1
Steep slope, ≤−.091	212 (131)	800 (473)	49 (22)	45 (21)	39 (19)	28 (14)	18 (9)
Flat slope, >−.091	164 (134)	851 (520)	44 (19)	39 (19)	33 (17)	25 (14)	16 (9)

*Lytic units are corrected for NK cell numbers and are expressed as the number of NK cells required to kill 20% of the targets in a suspension of 10^7 cells per mm^3.

cortisol slopes between patients subsequently randomly allocated to the psychosocial treatment group versus the control group. Moreover, when the association between the cortisol slope and survival was controlled for the effects of the randomized group assignment, the slope remained a statistically significant predictor of subsequent survival ($P = .004$, hazard ratio = 459.6; 95% CI = 7.1–29 799.7). As an additional check of the robustness of our finding, we examined the relationship of the cortisol slope to subsequent survival specifically among control group patients. The predictive effect of cortisol slope on survival held when analyzed in the control group only (Cox proportional hazards, $P = .006$, hazard ratio = 1376.9; 95% CI = 7.8–242 263.5), despite the restricted statistical power that comes with a reduced sample size (n = 46).

Discussion

For the first time, we document that variability in the diurnal cortisol rhythm is a statistically significant predictor of survival time with metastatic breast cancer. Prior work *(26)* has shown that circadian abnormalities have prognostic value in predicting the initial occurrence of breast cancer, in that those at high risk showed a markedly different circadian pattern for an array of hormones than did women at low risk. Moreover, other studies *(8,9)* have associated circadian abnormalities with later stages of cancer development or with prognostic indicators. We did not observe an association between the cortisol slope and several standard prognostic disease variables. Furthermore, the cortisol slope predicted mortality more than 12 months after assessment. This suggests that flattening of the cortisol slope does not simply reflect preterminal changes; rather, it is a relatively long-term prognostic indicator.

Both mathematically and empirically, overall levels of cortisol measured by the AUC between 0800 and 2100 hours are functionally determined by a linear combination of the morning (0800) cortisol and the diurnal cortisol slope. Some readers will question whether high cortisol levels overall (AUC) would have been a better predictor of subsequent survival. A Cox regression was performed to test the predictive value of AUC on survival time. However, there was no association between AUC and subsequent survival, highlighting cortisol slope, and not the morning cortisol level, as a unique indicator of survival in metastatic breast cancer.

Lower NK cell numbers and NK cell activity were associated with flattened slopes. Effects of glucocorticoids on activity and trafficking of immune cells have been demonstrated *(27)*, and NK cell counts may vary with cortisol levels *(18)*. Patients with flatter diurnal patterns did not generally experience low levels of cortisol in the evening. It is possible that the absence of the typical evening cortisol nadir affected NK profiles and that the normal morning peak of NK cell count was disrupted in these patients. Thus, rather than reflecting a deficit of NK cells, our association of flat cortisol slope with lower NK cell counts may simply reflect an abnormality of NK cell trafficking secondary to the disrupted cortisol rhythm. Nevertheless, lower numbers of NK cells were implicated in survival, and patients with flatter cortisol slopes had poorer NK cell activity. NK cells do kill several types of experimental tumors and may substantially defend against breast cancer progression *(28)*. Thus, immunosuppression related to flattened cortisol slopes may partly explain the survival effect. Reductions in NK cell numbers have not been observed in disorders characterized by hypercortisolism (e.g., Cushing's disease and depression) *(29,30)*. Thus, it is not surprising that no association was found between overall cortisol levels (AUC) and NK cell numbers.

The chest wall as the dominant metastatic site was associated with the cortisol slope but did not weaken the relationship between the slope and survival. In contrast with chest metastases, more distant invasion such as visceral metastasis is a relatively end-stage event. Taken together with the lack of association between the slope and age, disease-free interval, estrogen receptor status, time since recurrence, and Karnofsky rating, this finding suggests that the diurnal cortisol slope reflects a different dimension of physiologic dysregulation related to earlier disease spread rather than to more severe changes associated with preterminal disease states.

There was no association between recent chemotherapy (i.e., treatment administered within 2 months of data collection) and the diurnal cortisol slope, indicating that endocrine dysregulation is not simply a result of chemotherapy. The association between megestrol treatment and flatter slopes is worthy of further consideration, since loss of the cortisol rhythm may have consequences relevant to disease-resistance processes. Other hormonal therapies (e.g., tamoxifen therapy) were not associated with aberrations of the diurnal cortisol rhythm.

We explored the possibility that links between flattened cortisol rhythms and shorter survival were an epiphenomenon of other factors related both to cortisol variability and to survival. Results give tentative evidence that disruption of marital ties through separation, divorce, or widowhood may be associated with the loss of normal diurnal cortisol variation. Flattened diurnal profiles may reflect the severe and chronic stress of losing marital support, which itself has been associated with poorer cancer outcomes *(31)*. Indeed, other work *(1,3)* suggests that the physiologic burden of accumulated stressors on endocrine response systems may result in flattened cortisol rhythms and may have long-term health consequences.

Cortisol slope was not related to pain or depression, and neither of these factors predicted survival. Nevertheless, persistent hypercortisolism could have triggered psychophysiologic responses that perpetuated the abnormality. For example, nocturnal hypercortisolism is associated with sleep disturbance *(32)*. In the current sample, flatter slopes were associated with more nocturnal awakenings. The failure of a normal nadir at bedtime may have disrupted sleep, a likely cause of further circadian disruption.

These data were collected, not only to test the relationship of the diurnal cortisol slope to survival but also to examine the hypothesis that psychosocial treatment will increase survival time. Although participants were assigned to either the treatment or the control group only after cortisol data were collected, group assignment constitutes a "nuisance" parameter in this analysis. Indeed, in the context of this randomized trial, any variability in survival time introduced by the intervention would tend to cloud relationships between pre-existing physiologic markers, such as the baseline cortisol slope, and subsequent survival. Our finding that the cortisol slope remained a statistically significant predictor of survival, both after adjustment for assignment to the treatment or control group and when examined among control patients only, suggests that it is a robust prognostic indicator of survival time.

Altered cortisol rhythmicity in cancer has been associated with other circadian abnormalities as well as poor prognosis *(8)*, making it possible that our results reflect circadian abnormalities extending beyond the adrenocortical axis. Aberrant cortisol rhythms may be associated with hypersensitivity to stress *(3)* or may disrupt other endocrine and immune rhythms *(18)*. These effects in combination with the psychologic stress of cancer may cause deterioration of disease-resistance capabilities *(1)*. We are examining this possibility by studying stress reactivity as well as the rhythms of other physiological end points in these patients.

Relevance of These Findings to Cancer Prognosis and Treatment

Abnormal circadian rhythms among cancer patients have previously been documented and have been linked to poor prognosis. To our knowledge, this is the first prospective study to demonstrate the relevance of diurnal rhythm alterations to survival. Flattened cortisol rhythms may reflect processes of disease and/or the cumulative effects of both physiologic and psychosocial stress on the body *(1)*. Assessment of altered HPA axis rhythms in relation to other endocrine, immunologic, and psychosocial functions among women with metastatic breast cancer may provide information about risk identification and reduction. Rhythm alterations among cancer patients may prove to be useful in determining treatment schedules as well as prognosis *(8)*: Circadian variation of cortisol has been associated with the variation of cell proliferation in bone marrow *(33)*. Thus, salivary cortisol rhythms may provide a marker for timing chemotherapy to minimize cytotoxicity in bone marrow *(33)*. Although chronomodulated chemotherapy administration is now common, cancer patients with altered rhythms, who may have the poorest prognosis, may be less likely to benefit from such practices *(8)*. To improve the accuracy of risk identification and treatment efficacy, further research is needed on the relationship between glucocorticoid-mediated stress response systems and cancer progression.

These data do not prove a direct causal connection between cortisol rhythms and cancer survival, since other mediating variables might influence both cortisol rhythm and survival. Nonetheless, the prospective nature of the data and the fact that the relationship holds even when other standard prognostic variables are controlled suggest that dysregulation of cortisol is associated with more rapid breast cancer progression.

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NOTES

All contributors were involved in the study design. S. E. Sephton generated the specific hypotheses, supervised the collection of salivary cortisol data, carried out the data analyses and primary interpretation of the data, and completed the majority of writing and revision of the manuscript. R. M. Saplosky provided laboratory equipment and technical assistance for cortisol measurement, consulted on the data interpretation, and contributed to writing the manuscript. H. C. Kraemer initiated the use of the cortisol slope to measure diurnal rhythm dysregulation, provided consultation on statistical methods and data interpretation, and edited the manuscript. D. Spiegel originated and is principal investigator of the study, provided the research subjects, directed the collection of all medical and psychosocial data, and contributed to the manuscript.

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