

Immune and Endocrine Mechanisms of Advanced Cancer-related Hypercortisolemia

P. LISSONI¹, F. BRIVIO², L. FUMAGALLI², G. MESSINA¹, G. SECRETO³, B. ROMELLI⁴,
G. FUMAGALLI⁵, F. ROVELLI⁵, M. COLCIAGO⁶ and G. BRERA⁷

*Divisions of ¹Radiation Oncology, ²Emergency Surgery and
⁵Laboratory of Analyses, San Gerardo Hospital, Monza, Milan;*

³National Cancer Institute, Milan;

⁴Bouty Italy Group, Sesto S.G., Milan;

⁶Laboratory of Analyses, Hospital of Carate, Carate, Milan;

⁷Ambrosian University, Milan, Italy

Abstract. *Background:* Cancer progression depend on the immune and endocrine status of the patients. In particular, it has been observed that abnormally high levels of cortisol and/or an altered circadian secretion are associated with a poor prognosis in advanced cancer patients. The present study was performed to establish whether cancer-induced hypercortisolemia depends on an activation of the hypothalamic-pituitary axis or on a direct adrenal stimulation by inflammatory cytokines, such as IL-6, which have been proven to induce cortisol secretion. *Patients and Methods:* The study included 50 metastatic solid tumor patients, who were evaluated before the onset of chemotherapy. Venous blood samples were collected in the morning to measure IL-10, IL-6, ACTH and cortisol serum levels. Moreover, to analyze its circadian secretion, cortisol levels were also evaluated on venous blood samples collected at 4.00 p.m. *Results:* Abnormally high morning levels of cortisol were observed in 19/50 (38%) patients. Moreover, a lack of a normal circadian rhythm of cortisol was seen in 8/50 (16%) patients. None of the patients showed high levels of ACTH. Abnormally high concentrations of IL-6 and IL-10 were present in 21/50 (42%) and in 14/50 (28%) patients, respectively. Mean serum levels of both IL-6 and IL-10 were significantly higher in patients with hypercortisolemia than in those with normal cortisol values ($p<0.005$ and $p<0.001$, respectively). According to previous clinical studies, these results confirm that the advanced neoplastic disease may be associated with enhanced cortisol levels and alterations of its circadian secretion. The lack of enhanced ACTH secretion excludes the possibility that the abnormal cortisol production is due to the

activation of the hypothalamic-pituitary axis. On the contrary, the evidence of significantly higher concentrations of IL-6 in hypercortisolemic patients would suggest that cancer-related enhanced cortisol production may depend on a direct adrenal stimulation by IL-6 itself. The well-demonstrated stimulatory role of cortisol on IL-10 production would explain the enhanced IL-10 secretion in hypercortisolemic patients. *Conclusion:* Cancer-related hypercortisolemia would seem to depend on alterations of the feedback mechanisms between endocrine and cytokine secretions, occurring in the neoplastic disease.

Several clinical investigations have demonstrated the possible occurrence of an abnormally enhanced secretion of cortisol with cancer progression, independently of the histotype of tumor (1-5). Cancer-related hypercortisolemia would not be only a simple epiphénoménon, since it has been associated with a poor prognosis and with a reduced response to anticancer treatments in several tumor histotypes, including breast cancer, ovarian carcinoma and lung cancer (1-5). Therefore, the evidence of cancer-related hypercortisolemia has to be considered as a biological marker, depending on the endocrine status of patients, in neoplastic disease (1-5), because of its potential prognostic significance. The evaluation of cortisol secretion would have to be included within the routine examinations of advanced cancer patients before the onset of conventional anticancer therapies. In fact, because of its immunosuppressive effects (6), the abnormal secretion of cortisol could contribute to the generation of cancer-related immunosuppression, as already suggested by clinical investigations, which have shown the association between high levels of cortisol and low number of total lymphocytes and NK cells (7) in cancer patients. The mechanisms responsible for cancer-related hypercortisolemia have still to be defined, and in particular it remains to be established

Correspondence to: Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale San Gerardo, 20052 Monza, Milan, Italy.
Fax: +39 2332284, e-mail: p.lissoni@hsgerardo.org

Key Words: Cancer, cortisol rhythm, interleukin-6, interleukin-10.

Table I. Clinical characteristics of 50 metastatic solid tumor patients.

Characteristics	n
M/F	28/22
Median age (years)	59 (43-66)
Median performance status (Karnofsky's score)	90 (80-100)
Tumor Histotype	
Non-small cell lung cancer	24
Colorectal cancer	14
Breast cancer	12
Dominant metastasis sites	
Bone	8
Lung	15
Liver	16
Lung + Liver	11

□ Normal
■ High

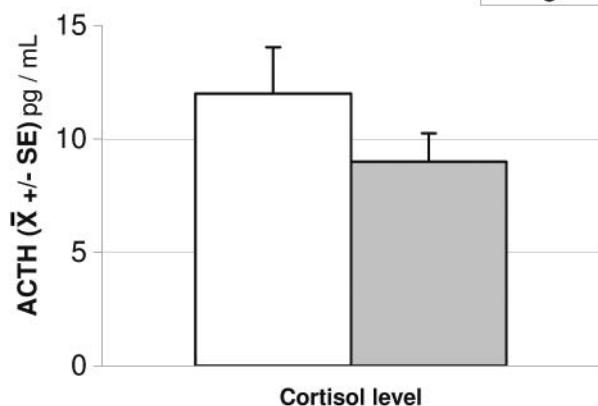


Figure 1. Mean ACTH serum morning levels in metastatic cancer patients with normal or high cortisol secretion.

whether the abnormal secretion of cortisol may depend on an increased stimulation of the adrenal gland following a hyperactivation of the hypothalamic-pituitary axis, with a consequent concomitant presence of abnormally high levels of the pituitary hormone ACTH, or whether it may be due to a primary dysfunction of the adrenal gland induced by cancer-associated abnormally elevated production of inflammatory cytokines, such as IL-6, which may stimulate cortisol secretion (8), and whose levels are often abnormally high in patients with advanced cancer (9). On the other hand, cortisol may stimulate the release of IL-10 from TH2-lymphocytes (10), which represents, as well as IL-6 (8), one of the most potent immunosuppressive cytokines in the anticancer immunity (11).

Preliminary results would seem to exclude cancer-related high cortisol levels being dependent on hypothalamic-pituitary mechanisms (1). Another potential mechanism responsible for the enhanced cortisol secretion with cancer progression could consist of a direct stimulatory action of IL-6 (8), which is produced through the cortisol-stimulation. The present study was carried out to establish whether advanced cancer-related hypercortisolemia depends on abnormal ACTH production from the pituitary gland, or whether it is due to the action of inflammatory cytokines, by concomitantly measuring ACTH and IL-6 blood concentrations, as well as to investigate a possible relation between the increased cortisol secretion and the endogenous production of IL-10, as expected on the basis of the well documented stimulatory role of corticosteroids on IL-10 secretion (11), which represents the main cytokine produced by T regulator (T-reg) lymphocytes.

Patients and Methods

The study included 50 consecutive metastatic solid tumor patients. Eligibility criteria were as follows: histologically proven metastatic solid neoplasm, measurable lesions, no double tumor, no brain metastasis, no previous chemothe-rapy for the metastatic disease and no concomitant chronic treatment with corticosteroids or with other drugs influencing cortisol secretion. The clinical characteristics of patients are reported in Table I. For the immune and endocrine evaluations, venous blood samples were collected in the morning after an overnight fast and before the administration of cancer chemotherapy. Moreover, to evaluate the circadian secretion of cortisol, venous blood samples were also collected at 4.00 P.M. Serum levels of IL-6 were measured by an IRMA assay (Technogenetics) and IL-10 by an ELISA immunoassay (Bender MediSystems). Cortisol serum concentrations were determined by an automated analyser with an ECLIA method (Elecys Systems Immunoassay; Roche Diagnostics, Mannheim, Germany); ACTH serum concentrations were measured by the automated chemiluminescent Nichols Advantage ACTH assay (Nichols Institute Diagnostics).

Normal values obtained in our laboratory (95% confidence limits) were below 31 pg/ml for IL-6, below 1.5 pg/ml for IL-10, below 220 ng/ml for cortisol morning values, with afternoon levels lower by at least 50% with respect to morning concentrations, and below 28 pg/ml for ACTH. Data were reported as mean $\bar{X} \pm$ SE, and statistically analyzed using the Student's *t*-test, the Chi-square test and the coefficient of correlation, as appropriate.

Results

Abnormally high morning levels of cortisol were seen in 19/50 (38%) patients. Moreover, within the group of hypercortisolemic patients, a normal cortisol rhythm, with a decline of at least 50% in the afternoon with respect to the values seen during the morning, was present in 11/19 patients,

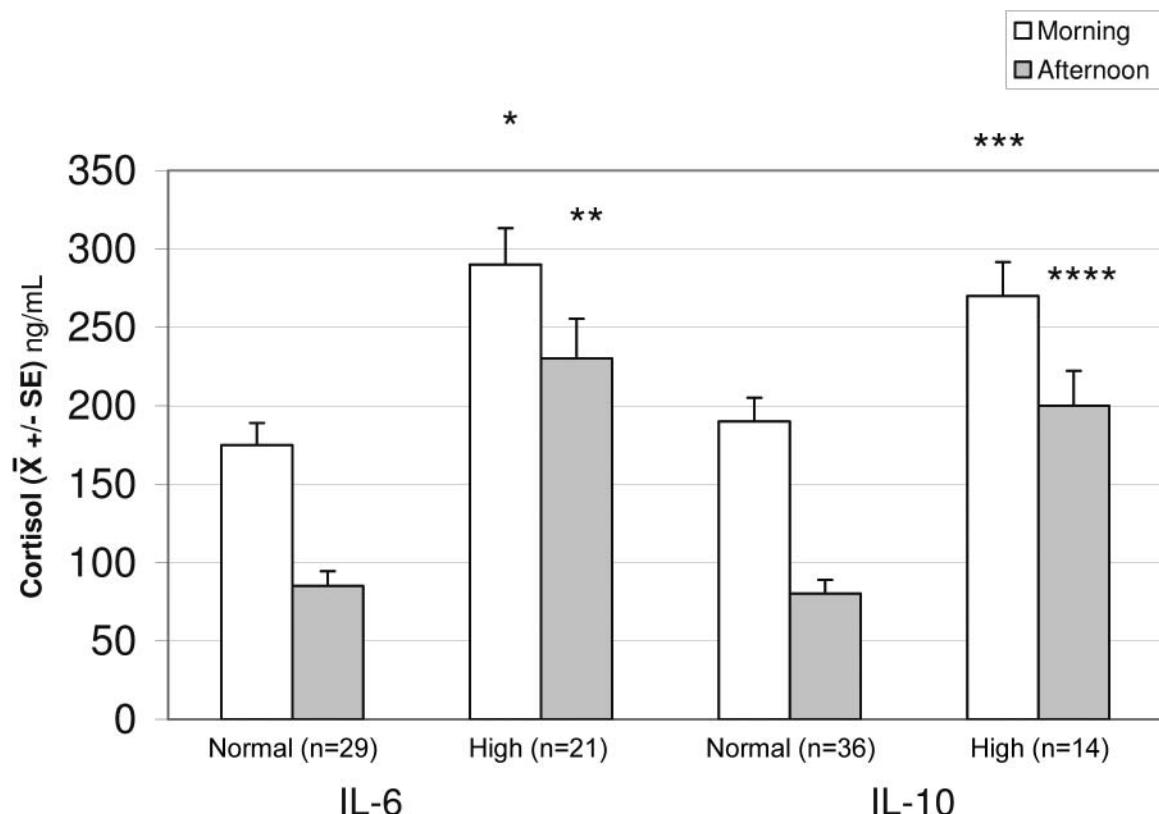


Figure 2. Mean serum morning and afternoon levels of cortisol in metastatic cancer patients with normal or abnormally enhanced serum concentrations of IL-6 and IL-10. * $p<0.01$ vs. normal IL-6; ** $p<0.005$ vs. normal IL-6; *** $p<0.05$ vs. normal IL-10; **** $p<0.01$ vs. normal IL-10.

whereas the remaining 8 patients (16%) showed no circadian secretion. Serum levels of ACTH were normal or low in all patients with abnormally high cortisol concentrations. As illustrated in Figure 1, mean ACTH levels were lower in hypercortisolemic patients than in those with normal morning cortisol values, without, however, statistically significant differences. IL-6 concentrations were high in 21/50 (42%) patients and the mean values of IL-6 observed in hypercortisolemic patients were significantly higher than in those with normal cortisol levels ($p<0.005$). On the same way, IL-10 concentrations were abnormally high in 14/50 (28%) cancer patients, and IL-10 mean values were significantly higher in hypercortisolemic patients than in those with normal cortisol concentrations ($p<0.001$). Moreover, within the hypercortisolemic group, patients who failed to present a circadian secretion of cortisol showed significantly higher levels of both IL-6 ($p<0.025$) and IL-10 ($p<0.05$) with respect to the hypercortisolemic patients with a maintenance of a normal circadiarhythm of cortisol secretion. A positive correlation was seen between cortisol levels and those of IL-6 and IL-10, even though none of these correlations reached statistical significance (IL-6: $r=0.32$; IL-10: $r=0.37$). IL-6 and IL-10 mean concentrations in relation to cortisol secretion are

Table II. IL-6 and IL-10 in relation to cortisol secretion in metastatic cancer patients.

Cortisol secretion	n	IL-6 ($\bar{X} \pm SE$) (pg/ml)	IL-10 ($\bar{X} \pm SE$) (pg/ml)
Normal cortisol levels	31	21±5	1.1±0.3
High cortisol levels	19	58±6*	4.3±0.5**
Presence of rhythmicity	11	46±4	3.8±0.4
Lack of rhythmicity	8	72±6***	5.9±0.5****

* $p<0.005$ vs. normal cortisol levels; ** $p<0.001$ vs. normal cortisol levels; *** $p<0.025$ vs. high cortisol levels with rhythm; **** $p<0.05$ vs. high cortisol levels with rhythm.

reported in Table II. Mean serum levels of cortisol observed in the morning and in the afternoon in relation to IL-6 and IL-10 production are illustrated in Figure 2. Both morning and afternoon values of cortisol were significantly higher in patients with abnormally elevated levels of IL-6 than in those with normal IL-6 concentrations ($p<0.01$ and $p<0.005$, respectively), and in patients with high IL-10 values than in those with normal values ($p<0.05$ and $p<0.01$, respectively).

Discussion

The present study, by showing that cancer-related hypercortisolemia is associated with normal levels of ACTH and abnormally high concentrations of IL-6, excludes the possibility that the abnormally increased cortisol secretion may depend on pituitary stimulation. We suggest that this may be due to mechanisms not depending on the endocrine system but on the immune system through the release of inflammatory cytokines, capable of modulating the endocrine secretions, as confirmed by the significantly higher levels of IL-6 in hypercortisolemic cancer patients. Therefore, because of the presence of normal ACTH values, the abnormal cortisol secretion could simply represent a direct consequence of the stimulatory action of IL-6 (8). Moreover, the occurrence of the highest concentrations of IL-6 in hypercortisolemic cancer patients, who had no cortisol rhythmicity, would suggest that cancer growth-related progressive increase in IL-6 blood levels may be the mechanism responsible for the aggressive disruption of cortisol circadian rhythm. In addition, the evidence of a concomitant hyperproduction of IL-10 in patients with high levels of cortisol and IL-6 is not surprising, since IL-10 has been proven to be under a stimulatory control played by cortisol (11). Abnormal production of cortisol in response to a chronic stimulation by IL-6 could determine an enhanced IL-10 production. Therefore, the abnormally high levels of IL-10 would simply represent, at least in part, an immunoendocrine consequence of the high cortisol concentrations, obviously in addition to the possible direct autocrine production of IL-10 by cancer cells themselves (11). In contrast, cancer-related hypersecretion of IL-6 would represent a primary event induced by the chronic response of macrophages to cancer growth (12), not determined by the hypercortisolemic status, since cortisol tends to reduce IL-6 production (6, 8), and no stimulatory effect of cortisol on IL-6 production has been documented.

Moreover, further studies will be required to better define the mechanism responsible for cortisol-induced immuno-suppression. In addition to the well documented immunosuppressive role of macrophages (12), recent studies have documented the immunosuppressive activity of T-reg lymphocytes (13-17), whose number has appeared to be abnormally high in advanced cancer patients. Cortisol could suppress the anti-cancer immunity by stimulating T-reg generation and activity.

Conclusion

This study shows that cancer-related enhanced secretion of cortisol is associated with normal levels of ACTH and enhanced concentrations of IL-6 and IL-10, suggesting that IL-6 hypersecretion may constitute the main mechanism responsible for the abnormally increased cortisol production, whereas the high levels of IL-10 are not the cause but the effect of hypercortisolemia.

References

- Van der Pompe G, Antoni MH and Heijnen CJ: Elevated basal cortisol levels and attenuated ACTH and cortisol response to a behavioural challenge in women with metastatic breast cancer. *Psychoneuroendocrinology* 21: 361-374, 1996.
- Touitou Y, Bogdan A, Levi F, Benavides M and Auzeby A: Disruption of the circadian patterns of serum cortisol in breast and ovarian cancer patients: relationships with tumour marker antigens. *Br J Cancer* 74: 1248-1252, 1996.
- McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spencer RL and Weiss JM: The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Rev* 23(1-2): 79-133, 1997.
- Sephton SE, Sapolsky RM, Kraemer HC and Spiegel D: Diurnal cortisol rhythm as a predictor of breast cancer survival. *J Natl Cancer Inst* 92: 994-1000, 2000.
- Mormont MC and Levi F: Circadian system alterations during cancer processes: a review. *Int J Cancer* 70: 241-247, 1997.
- Claman HN: Corticosteroids and the immune system. *Adv Exp Med Biol* 245: 203-210, 1998.
- Gatti G, Masera RG, Pallavicini L, Sartori ML, Staurenghi A, Orlandi F and Angeli A: Interplay *in vitro* between ACTH, beta endorphin and glucocorticoids in the modulation of spontaneous and lymphokine-inducible human natural killer(NK) cell activity. *Brain Behav Immun* 7: 16-28, 1993.
- Kishimoto T: The biology of interleukin-6. *Blood* 74: 1-10, 1989.
- Katsumada N, Eguchi K, Fukuda M, Yamamoto N, Ohe N and Oshita F: Serum levels of cytokines in patients with untreated primary lung cancer. *Clin Cancer Res* 2: 553-559, 1996.
- Tabardel Y, Duchateau J, Schmartz D, Marecaux G, Mohammad S, Barvais L, Leclerc JL and Vincent JL: Corticosteroids increase blood inter-leukin-10 levels during cardiopulmonary bypass in men. *Surgery* 119: 76-81, 1996.
- Moore KW, O'Garra A, de Waal-Malefyt R, Vieira P and Mosmann TR: Interleukin-10. *Ann Rev Immunol* 11: 165-172, 1993.
- Bruder S, Muul L and Waidmann TA: Suppressor cells in neoplastic disease. *J Natl Cancer Inst* 61: 5-11, 1978.
- Karakhanova S, Munder M, Schneider M, Bonyhadi M, Ho AD and Goerner M: Highly efficient expansion of human CD4+CD25+ regulatory T cells for cellular immunotherapy in patients with graft-versus-host disease. *J Immunother* 29(3): 336-349, 2006.
- Thornton AM and Shevach EM: CD4+ CD25+ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 188: 287-296, 1998.
- Pasare C and Medzhitov R: Toll pathway-dependent blockade of CD4+ CD25+ T cell-mediated suppression by dendritic cells. *Science* 299: 1033-1036, 2003.
- Elenkov IJ: Glucocorticoids and the Th1/Th2 balance. *Ann NY Acad Sci* 1024: 138-146, 2004.
- Franchimont D: Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. *Ann NY Acad Sci* 1024: 124-137, 2004.

Received November 13, 2006

Revised February 26, 2007

Accepted March 21, 2007