Urol Res (1991) 19:39–44

Urological Research

Plasma osteocalcin values and related hormonal parameters in patients subjected to a variety of prostate anticancer agents

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Accepted: September 1, 1990

Summary. Circulating osteocalcin (OC) and cortisol levels were measured in blood samples from 93 patients with dissaminated prostate cancer. Among these subjects 79 had not responsed to therapy, while 14 had responded to a variety of anticancer treatment strategies (orchiectomy, cyproterone acetate (CPA), flutamide, Buserelin, diethylstilbestrol (DES), Estracyt, and polyestradiol phosphate). The control group consisted of 19 patients with benign prostatic hypertrophy. In the majority of these patients blood adrenocorticotropic hormone (ACTH), estradiol human growth hormone (hGH), and thyroid stimulating hormone (TSH) levels were also assessed. In nonresponders to therapy with DES and Estracyt subnormal circulating OC levels were measured, while normal OC values were found in nonresponders to other treatment strategies. In patient given Estracyt highly elevated estradiol levels were recorded. Subnormal and/or low-normal estradiol concentrations were found in patients subjected to CPA and DES. Elevated blood cortisol levels were assessed in subjects treated with DES and Estracyt while at the same time either subnormal and low-normal plasma ACTH concentrations were measured in these same patients. Accordingly, the decline observed in OC concentration seems to be a consequence of the well-established inhibitory effect of glucorticoids on osteoblast activity. The decline in blood cortisol levels obtained after administration of dexamethasone in patients given DES and Estracyt may be attributed both to possible changes in catabolic pathways and to the contribution of the negative neuroendocrinological feedback.

Key words: Serum osteocalcin – Hormonal control – Prostate cancer therapy

The results of radioisotopic bone scans in untreated patients with metastastic cancer of the prostate are prognostic factors of major importance [7, 11]. However, sequential bone scans to monitor these same patients are, according to many studies, clinically efficacious and cost-effective only in symptomatic patients [11]. With the age of the male population constantly increasing, a sharp increase in a number of clinically manifest prostatic malignancies can easily be foreseen, thus giving rise to the substantial increase in the overal cost of the treatment. Biochemical tests such as circulating alkaline phosphatase value, serum osteocalcin (OC) concentration, and urinary hydroxyproline excretion data are therefore considered as alternatives to bone scintiscanning in follow-up procedures. A high pretreatment level of bone and total alkaline phosphatase may suggest an increased tumor burden and, hence, a poor prognosis. In 90% of prostate cancer patients with both X-ray and scintigraphic evidence of bone metastases, elevated levels of alkaline phosphatase were measured [24]. An increased level to total alkaline phosphatase was found in approximatelly 60% of patients with untreated prostate cancer and in only 6% of patients free of prostatic malignancies [13]. We have recently reported the possible application of serum osteocalcin (OC) concentration as a nonspecific marker of bone lesions in patients with prostatic carcinoma [31]. Elevated OC values reflect bone healing as early as 30 days. Tumor remission in bone could easily be distinguished from either metastatic progression (normal and subnormal concentrations) or tumor stabilization, regardless of the kind of therapy. Hence, the combined application of bone alkaline phosphatase and OC serotest is proposed as a means of detecting both progression and remission of the osseous tumor spread [31].

In nonresponding patients treated with Estracyt (including progression and stabile disease category, according to WHO standards) either subnormal or low-normal OC concentrations have been preliminarily recorded [31]. In nonresponders to various nonestrogenic androgen withdrawal procedures, such as orchiectomy and the administration of cyproterone acetate (CPA) and flutamide, normal OC concentrations were measured. The observed OC levels in subjects treated with Estracyt might possibly reflect a more rapid metastatic progression rate attributed to less differentiated, aggressive and thus chemohormonally treated tumors. If this hold true, blood

OC might represent a potential predictor of tumor metastatic potential. Our very preliminary studies also revealed subnormal or low-normal OC concentrations in patients treated with diethylstilbestrol (DES) [31]. The replacement of DES during tumor progression with either orchiecomy or antiandrogen therapy (CPA) led to an instant rise in osteocalcin concentrations from 1.0–2.5 ng/ml to 6–9 ng/ml. However, the images from bone scans taken therafter were unchanged [31]. Glucocorticoids exert an inhibitory effect on osteoblast cells function [5] while the reversal in postmenopausal bone resorptive processes is induced by the administration of estradiol and its esters [12]. Accordingly, intriguing results of our previous studies [30, 31] prompted continuous investigations of hormonal parameters related to osteoblast activity in patients subjected to a wide spectrum of hormonal anticancer agents.

Materials and methods

Subjects

A total of 79 nonresponders with disseminated cancer of the prostate and 14 responders to the same disease were followed during therapy. Their age ranged from 41 to 82 years. Tumor stages of bone metastases were determined only as had been done in the previous study [31]. The following modes of treatment and anticancer drugs were used: DES, 3-1 mg daily, 14/93 patients (15%); orchiectomy, 11/93 patients (11.8%); orchiectomy + CPA, 50 mg daily, 17/93 patients (18.3%); orchiectomy + flutamide, 3 × 250 mg daily, 9/93 patients (9.7%); CPA, 4–6 × 50 mg daily, 16/93 patients (17.2%); LHRH analog (Buserelin) + flutamide, nasal spray + 3 × 250 mg daily, 3/93 patients (3.2%); polyestradiol phosphate depot, 80–160 mg i.m. monthly, 2/93 patients (2.1%); DES + CPA, 0.5 mg + 4 × 50 mg daily, respectively, 2/93 patients (2.1%); Estracyt, 420–840 mg daily, 19/93 patients (20.4%). Tumor response to therapy, both negative (progression of "no change" status) and positive (partial osseous remission) was recorded on the bases of bone scan results and findings related to local tumor spread. Total tumor remission has not been recorded. If necessary, radiological evidence was included in the monitoring protocol. Circulating OC levels were measured in all reported patients, as were serum cortisol concentrations. In 55 of these same patients both human growth hormone (hGH) and estradiol concentrations were measured, in 45 patients thyroid secreting hormone (TSH) serotest level was measured, and in 71 of these subjects plasma adrenocorticotropic hormone (ACTH) concentration was assessed. The control group included 19 men with proven benign prostatic hypertrophy (BPH) and with no symptoms of malignancy. Their age ranged from 53 to 77 years. In all patients serum prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and testosterone levels were routinelly assessed. The data obtained have been supplementally utilized in determining the outcome of the treatment but their values are not presented herein.

In 3 patients presented herein the initial administration of DES was terminated due to cardiovascular difficulties. In 2 those subjects DES was replaced by CPA (200 mg daily) and 1 patient was subjected to orchiectomy. Corresponding serotest values have been included in the data of the respective groups of subjects.

Methods

Blood samples for the assessment of OC, cortisol, estradiol, hGH, and TSH serotest levels as well as for plasma (with EDTA) ACTH concentrations were drawn at least 5 days after any minor prostatic manipulation and/or 15 days after transurethral resection of the prostate (TURP). Plasma was kept in an ice bath for a short period of time until processed further. Serum or plasma had been separated and were frozen in aliquots thereafter at −20°C until assessed. Serum OC and cortisol levels were measured by with commercial radioimmunoassay kits from CIS, France (osteocalcin and TSH-IRMA), Biodata, Milan, Italy (estradiol), Institute for Immunology, Zagreb, Yugoslavia (cortisol), Pharmacia, Uppsala, Sweden (human growth hormone, hGH). ACTH concentrations in plasma specimens were measured with a radioimmunoassay kit reagent purchased from Vinča Inc., Belgrade, Yugoslavia. The normal ranges of the above parameters, as detected by these protocols, were 3.5–11.5 ng/ml (OC), 8–25 μg/100 ml (cortisol), 20–90 pg/ml (estradiol) 0–10 mU/l (hGH), 0.3–4.5 mU/l (TSH), and 20–80 py/ml (ACTH).

Scintigraphic bone imaging was performed according to the previously published procedure [31] and was repeated at 4- to 5-month intervals. Tumor response was evaluated according to the NPCP response protocol applied to scintigraphic data in the United States [25] and not to the overall response criteria.

The results obtained were analyzed using a Macintosh software. Statistical analyses were performed using a *t*-test for unpaired samples. All variances in this study are standard deviations.

Results

Nonresponders to therapy were selected on the bases of two or more sequential bone scans which showed no improvement (see "Materials and methods"). The disappearance of a least 50% of hot areas registered in previous bone images or a definite loss in the intensity of at least 50% of the uptake spots (without new appearances) was taken as evidence of a partial remission. PSA and PAP serotest values served as a control to bone scan data.

Blood cortisol concentration is at a peak in most individuals at 8 a.m., and at that time the blood samples were drawn. Plasma ACTH is of low stability, and so the corresponding aliquots which had been frozen at −30°C were processed as soon as possible.

A total of 217 sets of findings (including OC and corticol concentrations, 2–4 sets per patient) were recorded on blood samples coming from 93 patients. Plasma ACTH concentrations related to 42 patients (61 findings) were assessed. The results of these measurements are given in Table 1.

Patients given Estracyt were either former nonresponders to estrogen or antiandrogen treatment (7/19, 36.8%) or untreated patients (12/19, 63.2%) with poorly differentiated (GS) prostate cancer.

In responders to therapy, regardless of the strategy applied, circulating OC concentrations exhibited an initial and sharp rise [31]. However, a gradual decline in OC level was recorded (despite apparent and continuous improvement in scanning data) during the next 10–12 weeks in patients administered DES and Estracyt. In contrast, in patients treated with CPA elevated OC values were found to be steady for as long as bone tumor remission was scintigraphically detectable (Fig. 1).

Circulating hGH, TSH, and estradiol concentrations were measured randomly in 61 of the patients (Table 1). All hGH values, with only one exception, were found to be within the normal range (Table 2). Variations in numerical values of hGH, TSH, and estradiol the patients treated

Table 1. Circulating osteocalcin, cortisol, and ACTH values in monitoring patients with both scintigraphically proven disseminated prostate cancer and benign prostatic hypertrophy

Therapy and tumor stage	findings (*n*)	patients (*n*)	Meanage (years)	Osteocalcin (range) (ng/ml)	Cortisol (range) (μg/100 ml)	ACTH (range) (pg/ml)
Estracyt						
1. Stabilization	17	8	76	2.1 ± 2.6 (0.4– 3.8)	51 ± 27 (26–60)	14 ± 5 (12–21)
2. Progression	21	8	70	3.0 ± 1.3 (0.7– 4.8)	64 ± 19 (57–72)	17 ± 5 (14–21)
3. Remission	9	3	69	18.1 ± 6.5 (12.4–31.6)	57 ± 11 (35–69)	21 ± 8 (7–30)
P values[a]				$P < 0.01$	$P > 0.05$	$P > 0.05$
DES						
4. Stabilization	20	10	70	2.7 ± 1.5 (0.8– 3.6)	49 ± 14 (20–97)	18 ± 8 (11–25)
5. Progression	5	2	69	2.0 ± 0.4 (1.1– 2.7)	44 ± 20 (31–61)	13 ± 11 (5–22)
6. Remission	4	2	65	23.9 ± 10.1 (15.9–40.1)	53 ± 21 (30–92)	24 ± 21 (11–38)
P values[a]				$P < 0.01$	$P > 0.05$	$P > 0.05$
DES + CPA						
7. Stabilization	4	2	67	1.9 ± 1.1 (0.5– 3.2)	49 ± 8 (45–59)	20 ± 7 (8–23)
Orchiectomy						
8. Stabilization	11	5	75	7.9 ± 2.5 (4.1–11.6)	16 ± 5 (9–25)	33 ± 14 (21–42)
9. Progression	8	4	74	7.7 ± 2.0 (3.9–10)	18 ± 4 (9–21)	–
10. Remission	5	2	66	15.8 ± 1.3 (12.9–18.7)	19 ± 9 (8–29)	37 ± 16 (11–49)
P values[a]				$P < 0.01$	$P > 0.05$	$P > 0.05$[b]
Orchiectomy + CPA						
11. Stabilization	19	1	70	6.4 ± 1.1 (4.9– 9.1)	13 ± 4 (8–17)	36 ± 11 (18–57)
12. Progression	8	3	72	8.5 ± 3.7 (6.3–10.6)	23 ± 9 (2–30)	29 ± 7 (20–44)
13. Remission	12	3	66	13.9 ± 5.2 (13.9–24.4)	25 ± 9 (9–31)	39–21 (24–64)
P values[a]				$P < 0.01$	$P > 0.05$	$P > 0.05$
Orchiectomy + Flutamide						
14. Stabilization	11	5	70	8.8 ± 1.7 (6.3–11.1)	20 ± 8 (7–28)	41 ± 14 (18–66)
15. Progression	8	3	69	7.4 ± 2.2 (4.4– 9.6)	15 ± 5 (8–22)	–
16. Remission	2	1	73	19.1 ± 1.2 (12.1–20.9)	14 ± 5 (8–19)	–
P values[a]				$P < 0.01$	$P > 0.05$	$P > 0.05$
Buserelin + Flutamide						
17. Stabilization	3	2	74	5.9 ± 1.1 (5.0– 7.1)	18 ± 7 (8–25)	–
18. Remission	4	1	70	14.8 ± 2.3 (11.1–22.9)	23 ± 4 (15–24)	31 ± 19 (24–41)
P values[b]				$P < 0.01$	$P > 0.05$	
CPA						
19. Stabilization	23	10	68	8.1 ± 3.3 (3.7–11.4)	21 ± 19 (6–31)	–
20. Progression	9	4	75	7.7 ± 2.4 (4.6–10.2)	13 ± 4 (8–18)	49[c]
21. Remission	8	2	67	14.9 ± 3.3 (10.2–17.7)	14 ± 8 (5–27)	57 ± 22 (29–79)
P values[a]				$P < 0.01$	$P > 0.05$	
Polyestradiol phosphate						
22. Stabilization	5	2	74	8.9 ± 1.2 (8.2– 9.1)	15 ± 3 (12–17)	–
Benign prostatic hypertrophy	45	19	65	7.6 ± 2.1 (5.9– 9.9)	14 ± 6 (6–25)	37 ± 14 (19–59)

Normal ranges or osteocalcin, cortisol, and ACTH are 3.5–11.5 ng/ml, 8–28 μg/100 ml, and 20–80 pg/ml, respectively. Statistical correlation between osteocalcin values tabulated in times 3, 6, 10, 13, 16, 18, and 21 vs all other osteocalcin levels yielded $P < 0.01$. Statistical analysis of all mean cortisol and ACTH values presented in this table gave *P* value > 0.05.
DES, diethylstilbestrol; CPA, cyproterone acetate
[a] Stabilization and progression were correlated with remission
[b] Here only stabilization and remission were correlated
[c] One ACTH concentration was measured

with Estracyt and CPA were found to be dependent upon the dose of the drug and not on tumor stage and spread. In patients given Estracyt higher hGH concentrations were assessed than in those treated with DES ($P < 0.01$). Both of them differ significantly from all hGH concentrations measured in other groups of subjects ($P < 0.01$). These data are tabulated in Table 2.

Serum estradiol concentrations were found to be highly elevated in patients treated with Estracyt, revealing a pronounced dependence upon the dose of the agent

Table 2. Circulating estradiol, human growth hormone, and thyroid stimulating hormone levels in both treated patients with metastatic prostate cancer and men with benign prostatic hypertrophy

Treatment	Dose	Patients (*n*)	Estradiol (pg/ml)	hGH (mU/l)	TSH (mU/l)
CPA (+ orchiectomy)	50 mg	8	25.5	0.28	0.8
CPA	100 mg	2	21.6	–	0.6
	200 mg	3	15.8	–	0.9
	400 mg	7	13.7	–	0.9
DES	1 mg	7	2.6	1.11	1.2
	2 mg	3	3.7	1.26	1.4
Estracyt	280 mg	4	2,266[b]	2.6[a,b]	3.1[b]
	560 mg	6	3,080[b]	3.2[a,b]	4.3[b]
Orchiectomy	–	8	22.4	0.21	0.4
Orchiectomy + flutamide		7	22.2	0.22	0.6
BPH controls		7	47.1	0.18	0.4

One set of serotest measurements was performed per patient. Subjects were randomly chosen from both responders and nonresponders to therapy presented in Table 1. Normal ranges are estradiol 20–90 pg/ml, hGH 0–10 mU/l, TSH 0.3–4.5 mU/l
hGH, human growth hormone; TSH, thyroid stimulating hormone; BPH, benign prostatic hypertrophy; CPA, cyproterone acetate; DES, diethylstilbestrol
[a] Mean hGH value in patients treated with Estracyt is 2.98 mU/l
[b] $P < 0.01$ was calculated in statistical analysis of blood estradiol, hGH, and TSH concentrations measured in patients given Estracyt when compared to those treated with DES, CPA, orchiectomy, and flutamide

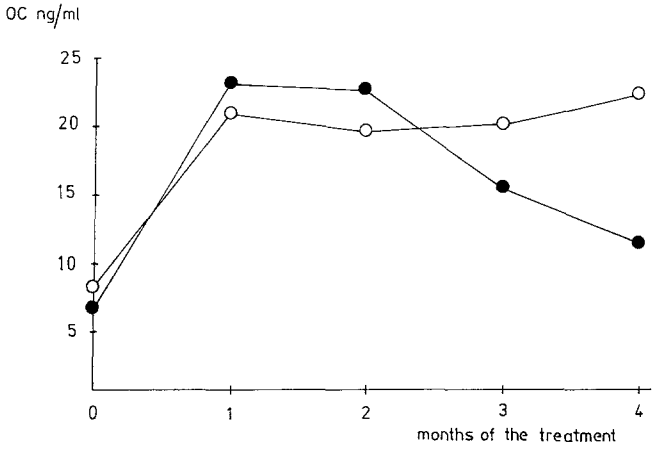

Fig. 1. Serial measurements of mean serum osteocalcin (*OC*) during scintigraphically documented bone tumor remission. Serotest concentrations were assessed in two patients treated with DES (1 mg/day) and in one patient treated with Estracyt (420–560 mg/day) (●) versus mean OC levels assessed in two patients treated by orchiectomy + CPA (○)

(Table 2). In contrast, in patients treated with CPA a dose-dependent decline in serum estradiol values were recorded (Table 2). The termination of treatment with CPA led to a sudden increase in circulating estradiol level (unpublished results)

Dexamethasone was given in a single dose (1 mg) at 11 p.m. to subjects whose cortisol concentration had been previously assessed. At 8 a.m. the next day blood cortisol level were measured and a 14-fold decline in the mean value was seen (from 17.1 to 1.2 µg/100 ml). In 3 patients given 1 mg DES/day and in 2 patients treated with 420 mg of Estracyt per day a corresponding 3-fold decline in mean cortisol concentration was assessed (from 51.0 to 16.3 µg/100 ml and from 57.6 to 16.7 µg/100 ml, respectively). A consecutive 2-day administration of dexamethasone did not significantly alter the aforementioned changes in blood corticol concentrations.

Discussion

Noncollagenous bone proteins which escape to a significant extent from bone to circulation are of potential use for clinical studies of bone metabolism processes. Among them only OC, the most abundant noncollagenous protein in bone, meets criteria fro a nonspecific marker related to bone healing. The precise function of OC in bone is yet unclear, but it certainly influences mineralization processes [22]. It is synthesized by osteoblasts and is cleared rapidly from serum by kidney filtration. Hence, a severe kidney failure might cause a rapid and extremely high rise in OC concentration. That fact should not be overlooked and might be of the utmost clinical importance when serum OC is applied as a marker in monitoring patients with bone metastases [6, 31].

Bone formation, resorption, healing, and pathologic destruction are complex processes which are regulated, facilitated, and/or impeded by systemic and local growth factors. Hormones are, however, likely to modulate the synthesis and the effects of some of such growth factors [15].

Osteoblasts lay down now bone matrix and are very active during bone healing processes. They contain glucocorticoid receptors [17]. Not only prolonged glucocorticoid therapy, but even small doses of these hormones seem

to be responsible for (a) a decrease in blood OC level [17], (b) the inhibition of OC biosynthesis in osteoblasts [3], and consequently (c) the reduced rate of bone formation [19]. The observed effects might results from a direct inhibitory action of glucocorticoids on osteoblast cells [5] by either (a) depressing protein synthesis, (b) reducing recruitment of osteoblast from osteoblast progenitors, or (c) interfering with the stimulative effect of 1,25-dihydroxy vitamin D_3 on OC production [22]. Positive cross-correlation between 1,25-dihydroxy vitamin D_3 action in bone and glucocorticoids has been already reported [26].

The recognition of estrogen receptors in cultured osteoblasts [9] has already been related to the direct action of estrogens on bone cells. Natural estrogens that inhibit bone resorption [3] and improve calcium balance are therefore already being used to prevent bone loss [2]. The activity of natural estrogens in bone might originate from estrogen receptor-steroid complex formation in osteoblasts and the accompanying molecular events leading to the biosynthesis of OC. Estrogens are administered to menopausal women as agents against osteoporoses, thus leading to the osteoblast reactivation. According to both recent findings [16, 20] and the results reported herein (Table 2), in patients given Estracyt normal endogenous estradiol plasma levels were increased up to several hundred fold. Accordingly, osteoblasts as target cells for estrogens have available enormous quantities of circulating estradiol in patients treated with Estracyt but not in those subjected to DES or to the other prostate cancer treatment modalities.

However, a decline in OC concentration is registered in both nonresponders to Estracyt [31] and DES (Table 1). In patients given Estracyt circulating estradiol, as one of the major metabolities of Estracyt, is dramatically elevated in a dose-dependent manner (Table 2). In contrast, subnormal estradiol concentrations were measured in subjects treated with DES and CPA. Accordingly, the estradiol-related promoting effect on the osteoblast activity is reversed during treatment with Estracyt. It appaears therefore that a well-recognized bone resorptive effect, connected with an excess of circulating glucocorticoids, may account for the observed diminution in blood OC concentration (Table 1). A close relationship between a high plasma glucocorticoid concentration and a lower osteoblast activity has been recently reported [23]. Such a conclusion is not only based on data related to nonresponders to therapy (Table 1) but is additionally supported by a short-term increase in OC concentration in responders to anticancer agents that raise circulating cortisol (Estracyt and DES). Such an effect has not been observed during partial bone tumor remission in responders to CPA (Fig. 1).

Serum ACTH and corticol levels were normal in most of the patients administered CPA (Table 1). This result is in harmony with the recent report that plasma cortisol levels in patients treated with orchiectomy plus CPA were unchanged during the first 6 months of treatment [32].

The administration of flutamide after orchiectomy has no influence on plasma ACTH and cortisol levels (Table 1), as had been already reported for plasma corticol concentrations [1]. More than a decade ago Fukushima and coworkers reported no changes in plasma cortisol concentration during treatment with flutamide despite a decrease in the cortisol production rate of more than 50% on average. The half-life of circulating cortisol is found to be prolonged under these conditions, probably due to changes in either hepatic or extrahepatic cortisol catabolism (or both). A recently published reinvestigation of these data [4] indicates the effects of flutamide on the adrenal level as being mediated *via* liver interactions.

A dexamethasone test is routinely used to determine the possible ACTH dependence of the increase in adrenal cortisol production [18]. Changes in plasma corticol level, if unrelated to ACTH concentrations, may be taken as evidence for the absence of the negative neuroendocrinological feedback relationship. The reported results, especially those related to trials with dexamethasone, trace back to the origin of the recorded raise in cortisol level (Table 1). Accordingly, it depends both on the adrenal steroidogenesis regulated by plasma ACTH concentrations and on a prolonged half-life of circulating cortisol provoked by anticancer agents that alter the rate of cortisol catabolism.

An increase in plasma hGH and TSH concentration has been attributed to therapy of prostatic carcinoma with estrogens and DES [22]. The assistance of hGH in juvenile bone formation is quite obvious. Glucocorticoids seem to play a role in regulating hGH gene transcription and expression [25]. Normal hGH concentrations were measured in all responders and nonresponders to therapy (Table 2), thus, revealing their full insensitivity regarding (a) bone healing processes and (b) the extent of the metastatic tumor spread. However, among these normal hGH values significantly higher concentrations were assessed in subjects treated with Estracyt ($P < 0.01$), thus being in line with the previously published data [22].

The observed rise in the circulating cortisol level may also occur due to the reported dramatic, but reversible, increase in blood cortisol binding globuline (CBG) during administration of estrogens [14]. The rate of CBG synthesis is reported to be controlled by the pituitary hormone TSH [14]. In patients with elevated circulating estradiol level blood TSH concentrations were found to be close to the upper limit of the normal range and thus statistically different from other TSH values tabulated in Table 2 ($P < 0.01$). Hence, the results of this study might be taken as supplemental evidence in favor of estrogen-related regulation of CBG synthesis modulated by serum TSH concentrations [8].

A slight decline in blood OC concentration was assessed (10%–15%) during the first 3 months after castration followed by a steady OC level thereafter [27]. During our preliminary and yet unpublished studies we have not detected any significant postcastrational change in OC level when compared to testosterone serotest values measured in BPH control group.

In conclusion, OC as a bone-derived protein related to mineralization processes during bone healing may serve as a marker of remission in patients with advanced prostate cancer. OC serotest values are found to be dependent on various hormonal parameters, in particular of circulating glucocorticoids. The future interplay between the clinical

evaluation of bone growth factors [15] and numerous noncollagenous proteins related to osteoblasts, osteoclasts, and macrophages promises to revolutionize our unterstanding of bone resorptive and bone healing processes that occur during prostate cancer metastatic spread.

Acknowledgement. The author is indebted to Dr. K. Kovačić for revealing bone scantiscanning data, to Dr. I. Kraljić for some of the results related to the routine urological findings, to Dr. A. Strelkov-Alfirević for the help in performing preliminary statistical analyses, to Dr. B. Mildner for assistance in the assessment of hGH and TSH serotest values, and to Dr. Bjørn Forsgren from Pharmacia LEO Therapeutics AB, Helsingborg, Sweden, for the helpful discussion. The technical assistance of Ms. Bešlić and Ms. Caput is gratefully acknowledged. This study was supported by Research Grant 1.08.04.00.31 administrated by Research SIZ, Zagreb, Croatia.

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