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Low-serum testosterone and high-cromogranin A rare case associated with high-grade prostate cancer and higher pathological stages of the disease

INTRODUCTION: *CgA and testosterone are two serum markers that may be involved in prostate cancer. The objective of this study was to evaluate the relationship of testosterone and CgA to grades and stages of prostate cancer, particularly whether low-serum testosterone and high-serum CgA are associated with more aggressive grades, and higher pathological stages of the disease.*
METHODS: *This perspective study included 121 men (Caucasian only) presenting with -newly-diagnosed, untreated prostate cancer. All the patients underwent radical prostatectomy.*
RESULTS: *We subdivided the sample into two homogeneous groups, Group A with Gleason score ≤7 (3+4), and Group B with Gleason score ≥7 (4+3). Low testosterone (< 3 ng/ml) was most common among the members of Group B 80 % versus 12.6 % of Group A (p = 0.001). At the same time, elevated CgA (> 80 ng/ml) was present for a rate of 72 % in Group B, 28.1% in the Group A (p = 0.001). The multivariate analysis we used revealed that low-serum testosterone and high-serum CgA are associated with higher pathological stages of the disease (p = 0.001).*
CONCLUSION: *The principal findings of this investigation were that low testosterone is correlated with elevated CgA levels, and these two parameters are associated with more aggressive grades and higher pathological stages of prostatic adenocarcinoma.*

KEY WORDS: Chromogranin A, Prostate cancer, Risk factor for prostate cancer, Testosterone

Introduction

TESTOSTERONE AND PROSTATE CANCER

Prostate cancer is the result of a complex and yet unclear interaction between ageing, genetic factors, hormones, growth factors, and the environment [1]. Although the relevance of the role testosterone plays in the development of prostate cancer is challenged by the fact that testosterone serum levels decrease with age (0.25-0.4% per year), while the incidence of prostate cancer increases in 2. The percentage of men with partial androgen deficiency as defined by total serum testosterone levels of less than 3,0 ng/ml, increases with age and is estimated to be in the range of 20% in elderly men [2]. Recent studies have suggested that low-serum testosterone may be associated with clinical prostate cancer [3,4]. Morgentaler et al. reported a prevalence of 14% of prostate cancer in a group of men with partial androgen deficiency a

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datum emerging following normal digital rectal examination (DRE) and PSA. The Authors' hypothesis was that low testosterone can reduce PSA levels thus making it an unreliable marker for this kind of patient [3]. This group of scientists and that of Marberger have also found that low testosterone levels were associated with more extensive prostate cancer and with a higher percentage of Gleason 7 score or a higher prostate cancer stage [4].

Chromogranin a (CGA) and prostate cancer

Neuroendocrine (NE) cells, in addition to basal and secretory cells, represent the third epithelial cell type of normal prostatic tissue, all three cell types originate from a pluripotent stem cell [5]. The function of NE cells in the prostate is unknown, but it is hypothesised that they may be involved in the regulation of the growth and differentiation of the developing prostate as well as in the regulation of secretory process in the mature gland[6]. The most predominant product of prostatic NE cells is CgA. Increased serum levels of CgA were more consistently found in patients with androgen-insensitive prostate cancer, and a significant correlation was found between NE serum markers and distant metastases .7 Immunohistochemical studies have shown that focal NE differentiation occurs in virtually all common prostatic adenocarcinomas [8].
Prompted by these data we performed a prospective evaluation of serum testosterone (total) and serum CgA of men with untreated, newly diagnosed prostate cancer. We were interested in the reverse correlation between serum levels of CgA and testosterone, and their correlation with pathological TNM and with the Gleason score in men with prostate cancer, that can have diagnostic and prognostic applicability.

Material and Methods

Patients

Between February 2008 and February 2009, 372 men (Caucasian only) aged 60 to 69 with suspect of prostate cancer seen at the Department of Urology, Rieti Hospital, were included in this study.
Inclusion criteria were: age 60-69, suspect of prostate cancer (or by DRE, or by PSA or both), no previous medicaments that can interfere with testosterone (e.g. Finasteride) or with CgA (e.g. analogues of somatostatin), clinically localized prostate cancer.
Serum samples for evaluation of testosterone, and CgA were obtained by cubital vein puncture of fasting patients between 7:30 and 10:00 am, in all the patients that have a prostate biopsy indication. Of this 372, 164 were positive at biopsy, and 43 were not fitting the inclusion criteria (22 for previous assumption of Finasteride and 21 for not clinically localized prostate cancer), and they were excluded from the study. The 121 patients remaining were all between 60 and 69 years old. The patients were included in the study consecutively. The patients with negative biopsies were enrolled in the control group (208 pts).
The diagnosis of prostate cancer was made by transrectal ultrasound guided biopsies. From 8 to 12 specimens were obtained from each patients and at least 2 of this specimens were obtained from the transitional zone, the other from the peripheral zone. Prostate biopsy indication was either a suspicious finding on DRE or elevated serum PSA (equimolar AxSYM PSA assay, Abbott Laboratories, USA) concentrations using age specific reference values: 60-69 yrs: < 4.5 and evaluation of PSA free/PSA total < 0.16. 23 of this patients underwent biopsy for elevated PSA (normal DRE), 33 for positive DRE (normal PSA), the other 65 had high PSA and positive DRE. The study was done in accordance with Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved by the hospital ethics committee and an informed consent was obtained from all patients. Further diagnostic included an assessment of prostate volume with transrectal ultrasound, nuclear bone scan, CT of the abdomen and pelvis.
The Gleason score was determined by a single pathologist specialised in Urology diseases. All the patient included in the study had a clinically localized prostate cancer and underwent radical prostatectomy, we collect the Gleason score at the biopsy and on the surgical specimen, but we performed the statistical analysis on the definitive Gleason score. The pathologist was blinded to the results of the serum testosterone and CgA.
The TNM system used was the 1997 TNM.

Serum Values of Testosterone and CGA

Serum samples for evaluation of testosterone, and CgA were obtained by cubital vein puncture of fasting patients between 7:30 and 10:00 am.
Testosterone was determined by a coat-a-count radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA). The inter- and intra-assay coefficient of variation are 8.9% and 5.2%. Normal range 2.7-10.7 ng/ml.
CgA was determined by RIA using a commercial kit (CIS bio International, Cedex, France), the detection limit of this kit was 1.7 ng/ml. The inter and intra-assay coefficient of variation of CgA assay was 5.8% and 3.8%, respectively. Normal value for CgA was < 80 ng/ml.

Statistical Analysis

Descriptive statistics were used to characterize the different parameters (mean, median, and range). Differences

between descriptive statistics were assessed using Mann Whitney U.
Comparison of clinical and endocrine parameters in patient with Gleason score ≤7 (3+4) [Group A] and in patient with Gleason score ≥7 (4+3) [Group B] were performed with the Pearson Chi Square test. The correlation between CgA and Testosterone was performed with the Sperman Rho test.
A multivariate analysis was performed evaluating the Fischer Exact test. We used logistic regression to determine the effect of testosterone and CgA as risk factors for more aggressive grade prostate cancer. Differences were considered significant for $p<0.05$. NCSS statistical computer software (NCSS 2007, Kaysville, Utah, USA) was used to perform all statistical calculations

Results

121 men (64 ± 3.6 yrs; mean ± standard deviation) were included in this study. Patients and control group characteristics are given in Table I. Differences between patients with positive biopsies and control group was statistically significant only for PSA value (p = 0.001). Median Total PSA pre-surgical operation was 6 ng/ml (range 3-13 ng/ml). The mean Gleason Score was 6.5 ± 1.5. 82 men (67 %) had Gleason Score ≤7 (3+4) at the pre surgical biopsy, and 11 of these men at the definitive histological exam presented a Gleason ≥7 (4+3). This 11 patients were enrolled in the Group B (Gleason ≥7 (4+3)). None patients presented with primary metastatic disease. Mean prostate volume as determined by TRUS was 53.7 grams ± 27.8. All the patients after diagnosis of clinically localized prostate cancer underwent radical retropubic prostatectomy with iliac obturatory limph node dissection. Clinical and pathological features in patients according to cutoff low and normal testosterone (3 ng/ml) and high and normal CgA (80 ng/ml) are showed in Table II.
We subdivided into two homogeneous groups, Group A with Gleason score ≤7 (3+4), and Group B with Gleason score ≥7 (4+3). Baseline characteristics and statistical analysis between Group A and Group B are showed in Table III and Fig. 1. At the end of the study, in group A was enrolled 71 men, and in group B 50. We evaluated the following variables for each patient: age, biopsy Gleason score, PSA pre-biopsy, prostate grams, testosterone and CgA pre-surgery, TNM, Gleason score after surgery, presence of association with PIN HG and or perineural infiltration, surgical margins. The statistical analysis showed differences statistically significant between the two groups for pre biopsy testosterone (p= 0.003) with a median value for Group A: 4.2 ng/ml versus Group B: 2.5 ng/ml, CgA (p=0.002) with a mean value for Group A: 69 ng/ml Group B: 97 ng/ml. Prostate grams evaluated with TRUS resulted smaller in the B Group (mean 52.1 grams) than in the A Group (55.5 grams) with a P value not statistically significant (p=0.41). The differences between the two groups in total PSA pre biopsy, positive lymph node and metastases was not statistically significant (p respectively 0.77; 0.48 and 0.75). Differences statistically significant was present also in presentation of pathological stage T, the Group A had 85.9 % (61 pts) in stage pT2 and 14.1 % (10 pts) in stage pT3, while Group B presented 26 % (13 pts) in stage pT2 and 74 % (37 pts) in stage pT3. The value of Fischer Exact Test was p=0.002. No stage pT4 was obtained. At the definitive histological exam there were differences statistically significant (Pearson Chi Square test p=0.010) in the incidence of positive surgical margins Group A 7.04 % (6 pts) vs Group B 22 % (11 pts) and association with PINHG Group A: 2.8 % (2 pts) Group B 16 % (8 pts). There was not difference for perineural infiltration.
Low testosterone (< 3 ng/ml) was present in the Group B 80 % (40 pts) versus 12.6 % (9 pts) of Group A (p = 0.001). At the same time elevated CgA (> 80 ng/ml) was 87.2 % (36 pts) in the Group B and 28.1 % (20 pts) in the Group A (p = 0.001).
We have also done a correlation analysis between CgA, and testosterone. The study showed that there were a strong correlation in the Group B between high serum levels of CgA and low levels of serum testosterone with a positive Correlation Coefficient = 0.25 (p < 0.001). There was a strong correlation with the value of Spearman's rho significant at the 0.01 level. This means that in the B Group, Gleason ≥7 (4+3), patients had low testosterone associated with higher CgA levels. In

TABLE I - *Baseline characteristics in 121 patients with PC. Median value (range)*

Baseline CH	PTS with positive biopsy	Control Group	P value
Age	64 ± 3.6(60-68)	65 ± 2.4 (61-68)	0,423
Total PSA pre-op. ng/ml	6 (3-13)	1.7 (0.4 – 5.3)	0,002
Gleason Score	6,5 (5-8)	–	
Prostate volume, grams	53,7 (28-73)	56.0 (23-83)	0.212
Testosterone ng/ml	3,35 (1,34-5,76)	3.5 (2.9-3.9)	0.124
CgA ng/ml	83 (54 –127)	71 (45-91)	0.114

PTS: patients; pre-op.: pre operative

TABLE II - *Clinical and pathological features in patients according to cutoff low and normal testosterone (3 ng/ml) and high and normal CgA (80 ng/ml)*

	TESTOSTERONE			CgA		
	Low	**Normal**	**p value**	**High**	**Normal**	**p value**
No. Patients (121 pts)	49	72		56	65	
Median PSA(ng/ml)	5,7	6,5	0,69	6,1	6,9	0,8
Prostate volume (gr)	54	57	0,39	51	60	0,43
			Subdivided by Gleason Score			
Group A [71 pts]	9	62	< 0,001	20	51	< 0,001
Group B [50 pts]	40	10	< 0,001	36	14	< 0,001
Subdivided by Stage						
pT2 [74 pts]	15	59	= 0,001	22	52	< 0,001
pT3 [47 pts]	31	16	< 0,001	29	18	< 0,001

TABLE III - *Baseline charateristics and statistical analysis in Group A (71 patients) and Group B (50 patients)*

	Group A [71 pts]	**Group B [50 pts]**	**p value**
Age	65 (61-67)	66 (62-68)	0.4 n.s.
Total PSA pre-op. ng/ml	7,8 (6-13)	7,1 (4-12)	0.77 n.s.
Gleason Score	6 (5-7)	8 (7-9)	0.04
Prostate volume, grams	55,5 (29-73)	52,1 (28-67)	0.41 n.s.
Testosterone ng/ml	4,2 (1,45-5,76)	2,5 (1,34 – 4,65)	0.003
CgA ng/ml	69 (54 –78)	97 (69 –127)	0.001
T2	85.9 % (61 pts)	26 % (13 pts)	0.002
T3	14.1 % (10 pts)	74 % (37 pts)	0.002
Positive surgical margins	7.04 % (5 pts)	22 % (11 pts)	0.004
PIN HG	2.8 % (2 pts)	16 % (8 pts)	0.003
Low testosterone (< 3 ng/ml)	12.6 % (9 pts)	80 % (40 pts)	0.001
High CgA (> 80 ng/ml)	28.1 % (20 pts)	72 % (36 pts)	0.001

Pts: patients; Pre-op: pre operative

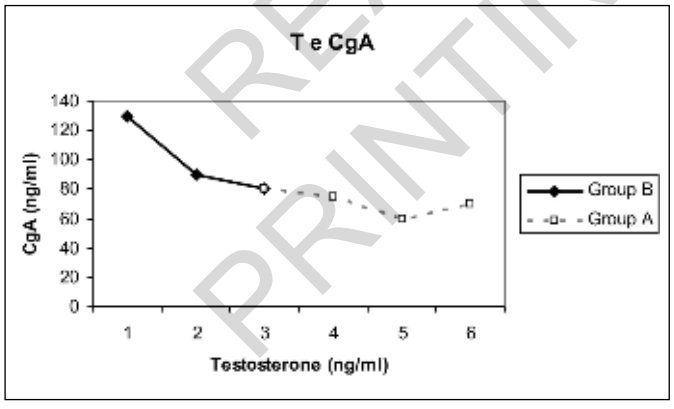

Fig. 1: Distribution of the patients according to testosterone and CgA serum values.

the Group A there was a significant correlation between testosterone > 3 ng/ml, with CgA < 80 ng/ml (p = 0.006). This data are showed in Fig. 1. The two Groups showed opposite coefficient of correlation.

On the multivariate analysis performed by logistic regression we obtained that testosterone < 3 ng/ml was a more present in aggressive prostate cancer (Odds Ratio : 0.41, 95% CI 0.29- 0.83, p < 0.01) as high CgA (odds Ratio: 0.39, 95% CI 0.35- 0.74, p < 0.01), while normal values of serum testosterone associated with normal values of CgA a was associated with the develop of less aggressive grade prostate cancer.

Discussion

The principal findings of this investigation were that low testosterone is correlated with high CgA levels, and this two parameters are correlated with the Gleason score and the pathologic findings, and have to be considered important factors for the more aggressive grade and for the higher pathological stage of the clinically localized prostatic adenocarcinoma. On the other side normal levels of testosterone and of CgA are correlated with lower Gleason Score and TNM staging lower.

It is important to note that our study population consisted of men with age range restricted to a fairly narrow range (60-69).
Approximately 20% of old men have a partial androgen deficiency, this percentage increases constantly with age: 16.2% (40-49 years), 20% (50-59 years), 22.6% (60-69 years), and 26% (70 years or older). In patients with prostate cancer the respective percentage were higher in all age groups: 50-59 years: 29,5%, 60-69 years: 30,3%, 70 years or older 40,3% 2.
Raynaud JP in 2006 [9] confirmed, in a study on a large prospective cohort of 10,049 men that high grade prostate cancer has been associated with low plasma level of testosterone. Furthermore, pre-treatment total testosterone was an independent predictor of extraprostatic disease in patients with localized prostate cancer; as testosterone decreases, patients have an increased likelihood of non-organ confined disease and low testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy. Meanwhile, although Teloken et al. [10] reported that low testosterone is associated with higher risk of positive surgical margins in RRP specimens, low testosterone levels were not observed to be significantly associated with higher Gleason scores or pathological stage.
In a previous report of prostate biopsies in hypogonadal men with PSA less than 4,0 ng/ml Rhoden et al. found that cancer rate was increased 2.15-fold in men in the lowest testosterone tertile compared with that in men in the highest tertile and also the cancer rate was 30% in hypogonadal men with PSA between 2 and 4 ng/ml [11].
It remains to be determined whether the risk of prostate cancer follows same continuous measure of testosterone or whether there exist a critical threshold point below which the risk is increased.
Recently it was reported by Massengill et al. that low total testosterone predict extraprostatic disease in patients with clinically localized prostate cancer who underwent radical prostatectomy [12].
NE differentiation in prostate cancer has received increasing attention in recent years because of the prognostic and therapeutic implications. NE differentiation in prostate cancer generally refers to the presence of NE cells focally in otherwise typical conventional adenocrcinomas Secretory products from NE cells have growth-promoting properties, and a number of oncogenes have been shown to activate or be activated by same of this hormone products .13
Fixemer et al. noted that irrespective of grade, stage and degree of NE differentiation, apoptotic activity was restricted to esocrine tumor cells and was undetectable in most of the NE tumor cells expressing CgA 14. NE tumour cells are found at all stages of prostate cancer and are freely dispersed throughout the tumour. Independent groups of researcher have shown that NE cells lack or do not express the androgen receptor.
NE differentiation increases in high grade/high stage tumours (Bohrer and Schmoll 1993 15) and particularly, in androgen deprived (Sciarra et al. 2003 [16]) and androgen independent tumours (Hirano 2004 [17]).
Significantly greater neovascularization has been seen in high-grade prostate cancer with many, compared with high-grade with few, NE tumor cells [18]. Vascular endothelial growth factor features among the most potent angiogenic factor identified. NE cells are a significant source of vascular endothelial growth factor in prostate tumors [19].
High values of CgA have a significant correlation with distant metastases [20].

Conclusion

The major finding of our study is that patients with clinically localized prostate cancer treated with radical prostatectomy have a statistically significant correlation between pre-treatment total testosterone and CgA levels with Gleason score and pathological stage. This correlation held up in multivariate analysis
This lead to the clinical expression that, when low testosterone and high CgA is presented together, the aggressivity of the prostate adenocarcinoma is higher than prostate cancer with the values of the two parameters normal. The pathological findings confirm this trend.
Pretreatment total testosterone serum levels and CgA, might help clinicians in the future assessment and management of localized prostate cancer.

Riassunto

La cromogranina A (CgA) e il testosterone sono due marker sierici che possono essere coinvolti nel cancro alla prostata. L'obiettivo di questo studio è stato quello di valutare la relazione tra testosterone e CgA in gradi e stadi del cancro alla prostata, in particolare se il testosterone a basso livello sierico e il CgA ad alto siero sono associati a condizioni più aggressive e a stadi patologici più elevati della malattia.
Lo studio prospettico ha riguardato 121 uomini (solo caucasico) che presentavano un cancro alla prostata appena diagnosticato e non trattato. Tutti i pazienti sono stati sottoposti a prostatectomia radicale.
Il campione è stato suddiviso in due gruppi omogenei: il gruppo A con il punteggio di Gleason ≤7 (3 + 4) e il gruppo B con il punteggio di Gleason ≥7 (4 + 3). Il basso livello di testosterone (<3 ng / ml) era più comune tra i membri del gruppo B 80% rispetto al 12,6% del gruppo A (p = 0,001). Allo stesso tempo, il CgA elevato (> 80 ng / ml) era presente per un tasso del 72% nel gruppo B, del 28,1% nel gruppo A (p = 0,001). L'analisi multivariata che abbiamo utilizzato ha rivelato che il testosterone a basso livello sierico e il CgA ad alto siero sono associati a stadi patologici più elevati della malattia (p = 0,001).

In conclusione i principali risultati di questa indagine indicano che il basso livello di testosterone è correlato con elevati livelli di CgA e questi due parametri sono associati a gradi più aggressivi ea stadi patologici più elevati dell'adenocarcinoma prostatico.

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