The prognostic value of serum chromogranin A and prostate specific antigen in prostate cancer patients for progression to the hormone resistance state

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Abstract

Prostate adenocarcinomas (PAC) consist mainly of tumour cells of luminal immunophenotype and scattered neuroendocrine (NE) cells. NE cells are defined by chromogranin A (CgA) immunoreactivity. *The aim* of this study is the evaluation of CgA serum levels in monitoring prostate cancer (PC) patients under complete androgen deprivation (CAD) in comparison with the prostate specific antigen (PSA) as a prognostic marker of androgen resistance and bone metastases. *Ninety-two patients* with newly diagnosed PAC and 30 healthy blood donors serving as the control group were enrolled in the study. Serum CgA and PSA values were measured. All patients had locally advanced or metastatic disease and received CAD treatment. In the group of PAC patients bone scanning with 925MBq ^{99m}Tc-MDP revealed the presence of bone metastatic lesions in 50 patients (29 with more than 3 lesions and 21 with less than 3 lesions). The other 42 patients had no bone metastases. The patients and the control group were re-evaluated after 1 year. *Our results showed* that serum CgA positively correlated with multiple bone metastases and higher Gleason score, serum levels of CgA and PSA. Levels of PSA were significantly higher in patients with PAC and bone metastases compared with those with no bone metastases (P<0.001). In patients with multiple bone metastases and Gleason Score >7 elevated serum levels of CgA higher than those of PSA were found. *In conclusion,* serum CgA levels is a valuable marker for predicting the presence of multiple bone metastases in PAC patients. Combined with PSA, CgA can predict disease progression in patients with advanced PAC under CAND treatment and is correlated with poor prognosis.

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Introduction

Prostate adenocarcinoma (PAC) is common in the aging male population and constitutes the sixth most common cancer worldwide with 543,000 new cases diagnosed every year [1].

Nowadays, with the widespread adoption of prostate serum antigen (PSA) screening many prostate cancers are detected in earlier stages and therefore are amenable to potentially curative treatments [2].

In cases of advanced disease, androgen suppression by either orchiectomy or administration of luteinizing hormone-releasing hormone analogues (LHRH-As) is the mainstay of treatment for those patients. Although this treatment frequently results in tumor shrinkage and improvement of symptoms, it is not curative and the majority of patients eventually develop hormone-refractory disease [3, 4].

Bones are the most common site of metastases for advanced PAC. The accurate evaluation of the presence of bone metastases in patients with PAC is of great importance both for tumor staging and for the follow up of patients during treatment [5]. Bone scintigraphy is considered to be quite accurate in depicting bone metastases, due to its high sensitivity; however it has a low specificity, since several "hot spots" in the skull or the cervical spine represent on bone scans false-positive areas [6].

It has recently been pointed out that neurondocrine cells (NE) differentiation is not a static phenomenon. The NE compartment in fact, increases after androgen deprivation and in refractory disease. The direct stimulation of NE differentiation by androgen-deprivation treatment was demonstrated in a preclinical study by Jongsma et al (2002) [7].

Regarding the role of PSA in the detection and follow-up of PC, there is general agreement that PSA estimation according to age, prostate size, the free-to-total PSA ratio and PSA velocity offer the highest prognostic value for PAC detection [7, 8].

The most significant application of PSA is in the follow-up of patients with PAC. It has

been estimated that 10%-27% of men aged 62-91 years with a PSA of 4.0ng/ml or slightly less, harbor prostate cancer [9]. Young individuals with a PSA in the range between 2.6-6 ng/ml have higher possibilities than the elderly to have curable PAC, while elders have a higher risk of harboring more aggressive PAC. Also, given that PSA is under androgen regulation it is worth mentioning that under hormonal treatment 34% of patients with clinical stage D2 disease had normal PSA despite the progression of bone metastases [10]. These findings indicate or showoff the potential usefulness of Chromogranin A (CgA) for monitoring androgen-independency in men with PAC.

The biological and clinical significance of NE differentiation in PAC patients is still to be elucidated. Chromogranin A appears to be a sensitive marker for detecting NE differentiation either at the tissue level or in serum [11]. The availability of such a specific circulating marker for the NE component could allow us to detect and monitor NE differentiation in PAC patients as in the early detection of resistance during complete androgen deprivation (CAD) is important for the prognosis of these patients.

The aim of our study was the evaluation of CgA serum levels in monitoring patients with PAC under CAD in comparison with the PSA as a prognostic marker of resistance to hormonal treatment and detect the early NE differentiation.

Patients and methods

Ninety two patients, median age 58 (range 42-81 years) with non-organ confined PAC diagnosed after prostate biopsy were enrolled in the study. All patients were T_3-T_4 stage according to the TNM system (The American Joint Committee on Cancer) [8]. Forty two patients had no bone metastases (negative bone scans) (Group A). Fifty patients had bone metastases assessed by bone scans (Group B) with twenty nine having more than three hot spots and twenty one having less than three hot spots on bone scan. (Fig. 1).

Thirty healthy blood donors median age 68.5 (range 53-84 years) served as the control group (Group C). None of the patients in Groups A and B nor any healthy donor suffered from decreased renal function or atrophic gastritis or were on medication with proton-pump inhibitory drugs which increase the plasma levels of CgA. All patients with PAC were subjected to whole body bone scans after the intravenous infection of 925MBq technetium-99m methylene diphosphonate (^{99m}Tc-MDP) using a tomographic gamma - camera (GE-Millennium MPR, U.S.A) and also plasma CgA and serum PSA were measured. In Groups A and B, serum CgA and PSA values as well as bone scans were reevaluated after one year.

The diagnosis of bone metastatic lesions was established with whole body scintigraphy and confirmed with plain X-rays. Computerized tomography (CT), was used in order to delineate lesions that appeared positive on scintigraphy and negative on X-rays. The extent of metastases in bone scintigraphy was evaluated according to the criteria proposed by Soloway (1988) [12].

In Groups A and B, CAD was accomplished by surgical castration in 22 patients and by hormonal treatment using LHRH agonists combined with antiandrogens, in 70 patients.

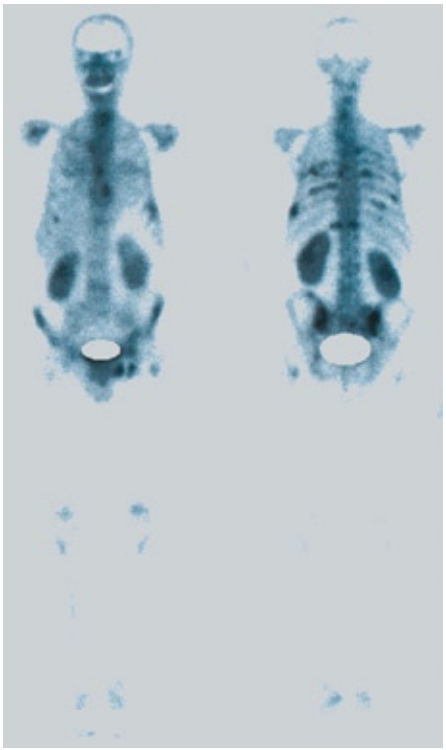

Figure 1. Whole body bone scan with 925MBq ^{99m}Tc-MDP in patient with skeletal events of prostate cancer with more than 3 hot spots at bone scan.

Blood samples were drawn in the morning after overnight fasting and plasma and serum were separated and frozen at -70^{o}C until assayed. Commercially available kits for measuring plasma CgA values by a solid- phase, two- site immunoradiometric assay (IRMA) for the measurement of CgA provided by CIS-Bio International (IRMA, CIS-bio international-Shering, Gif-sur-Yvette, France) were used. Serum PSA was measured by a solid phase two site IRMA the Tandem- R PSA assay kit (Hybritec Inc.). The reference upper values for CgA and PSA were 70nmol/L and 4ng/ml respectively.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS V.11. Statistical analyses of CgA and PSA concentrations related to Groups A, B and C were performed using the chi-square test. The dependence of CgA on the others variables (PSA, bone scan and Gleason score) was assessed by one way analysis of variance (ANOVA) followed by tests of multiple comparisons. A P value of less than 0.05 was considered to be statistically significant.

Results

Table 1 displays the baseline characteristics of the 92 patients with PAC and the control group. The reference normal levels of CgA were below 70 nmol/L according to our measurements in healthy blood donors, which is in contradiction with previous studies [10-11]. CgA concentrations of 4.0-10.0 nmol/L raise suspicion of a NE tumor and should be checked with new sample collection and assay. CgA concentrations above 10.0

Table 1. *Baseline characteristics of all groups studied*

Variables / Group	A /%	B /%	Control /%
No	42/35	50/41	30/24
Age / Mean	65-82 /73.5	61-85/73	53-84/68.5
Initial gleason gcore			
2-4	14/33	8/16	-
5-6	10/24	18/36	-
≥7	18/43	24/48	-
Initial Stage TNM*			
$T_3N_0M_0$	12/28	-	-
$T_3N_{1-3}M_{1b}$	30/72	-	-
$T_4N_0M_0$	-	14/28	-
$T_4N_{1-3}M_{1b}$	-	36/72	-
Bone scan ($T_{3-4}N_{1-3}M_{1b}$)			
<3	-	21/42	-
>3	-	29/58	-
PC treatment			
Surgical castration	10/24	12/24	-
Hormonal treatment	32/76	38/76	-
Initial serum PSA ng/ml			
<50	22/52	11/22	30/100
>50	20/48	39/78	-
Chromogranin A nmol/L			
<70	29/45	09/18	30/100
>70	13/55	41/82	-

Table 2. *Assessed by one way analysis of variance (ANOVA) (Dependent variable: Chromogranin A)*

		Sum of squares	df	Mean square	F	Sig.
PSA	Between Groups	2.319	1	2.319	14.455	0.001
	Within Groups	19.254	90	0.160		
	Total	21.574	91			
Bone scan	Between Groups	3.545	1	3.545	6.295	0.013
	Within Groups	67.570	90	0.563		
	Total	71.115	91			
Gleason score	Between Groups	6.402	1	6.402	18.974	0.001
	Within Groups	40.491	90	0.337		
	Total	46.893	91			

nmol/L strongly suggest the presence of a NE tumor [13]. Fifthy of them (Group B) had bone metastases without visceral involvement. Plasma CgA values of less than 70nmol/L were observed in 38 patients (41%) and supranormal CgA in 54 patients (59%). Elevated serum CgA levels, higher than those of PSA, were found in patients with multiple bone metastases and Gleason score >7 (P<0.001) (Table 2).

At one-year follow-up we examined plasma CgA, serum PSA and bone scan in all groups studied (Table 3). Eight new patients were moved to Group B from Group A because they had new bone metastases. Five of them had more than 3 hot spots and 3 had less than 3 hot spots. All patients with new bone metastases had baseline PSA of more than 50ng/ml and CgA of more than 70 nmol/L. Furthermore, five patients with more than three hot spots on the bone scan, PSA >50 ng/ml and CgA >70 nmol/L died during this one year follow-up period. There is a significantly positive prognostic value of elevated CgA (>70nmol/L) for predicting the presence of bone metastases.

Table 3. *Characteristics of all groups studied after one year of follow-up*

Follow-up / Group	A /%	B /%
No	34/37	58/63
Staging TNM		
$T_{3-4}N_0M_0$	34	-
$T_{3-4}N_{1-3}M_{1b}$	-	58
Bone scan		
<3	-	24/41
>3	-	34/59
Serum PSA ng/ml		
< 50	34/100	13/22
>50	-	45/78
Chromogranin A nmol/L		
< 70	34/100	10/44.5
>70	-	48/55.5

Discussion

Likewise the resistance to endocrine treatment and the development of hormone-refractory prostate cancer (HRPC) has been largely attributed to NE cell differentiation of the primary tumour [14].

The measurement of serum NE markers constitutes a more representative indicator and a more objective quantification of significant NE tumour differentiation compared to IHC staining of needle biopsy specimens.

Neuroendocrine differentiation in PC is typically detected by immunohistochemistry (IHC) as single cells in conventional PAC. There is evidence linking the development of Large Cell Neuroendocrine Carcinoma (LCNEC) to long-term stimulation from hormonal treatment for PAC, resulting in clonal progression under the selection pressure of treatment [15].

According to our results patients with pretreatment serum PSA value of more than 50ng/ml and high CgA values (>70nmol/L) had poorer prognosis. Others suggest that serum CgA levels above 100nmol/L indicate PC [16]. We also found that CgA values were significantly correlated to PSA values (P<0.001). Furthermore, CgA was not suppressed by androgen ablation treatment, while on the contrary PSA levels were decreasing

Therefore early appearance of serum CgA would create a chance for early adjustment of treatment and the prevention of any further development of metastases. These findings support the androgen-independence of prostatic NE cells. In addition, CgA values consistently increase during androgen deprivation treatment regardless of the PSA response.

Other investigators reported that CgA values increased

during endocrine treatment, but found no correlation between serum PSA and CgA values. Furthermore CgA levels were not suppressed by androgen ablation while PSA values were markedly decreased. These findings supported the androgen- independency of prostatic NE cells [17].

Others reported elevated CgA levels in patients with pT3 prostate cancer under androgen deprivation treatment; in patients with biochemical recurrence following radical prostatectomy and in M1 stage patients. CgA values increased by 0.60ng/ml/month in patients who underwent castration, and by 0.29 in Pt3 patients under bicalutamide monotherapy. Their follow-up period was 24 months and their findings did not include hormone refractory PCa [18].

In our study the follow-up period was 12 months. During that time 22 new patients developed bone metastases with pretreatment PSA and CgA exceeding 50 ng/ml and 70nmol/L respectively. These new patients from group B represent cases of hormone refractory PCa. The same authors comparing the velocity of CgA, came to the conclusion that NE differentiation is more aggressive when androgens are more intensively suppressed (i.e., anti-androgen only < castration only < combined androgen blockade) [19]. Intermittent androgen deprivation is thought to delay or reduce acceleration of NE differentiation. On the other hand NE differentiation is accelerated more aggressively when androgen is suppressed more intensively [20].

To our knowledge, no study has evaluated the prognostic value of serum CgA and PCA for monitoring disease progression to the androgen-independent state. Initial reports on immunohistochemical (IHC) tissue staining [21, 22] and measurements of blood serum concentration [19], found CgA to be a potentially significant prognostic factor for final outcome of PCa patients on endocrine treatment.

On the other hand, results from studies evaluating the significance of serum and tissue values of CgA in predicting clinical response to octreotide acetate treatment [23] and final outcome [24] for patients with PCa were not encouraging.

There are results indicating that serum CgA elevation precedes elevation of PSA for patients with advanced CaP under androgen depression treatment, implying that elevation of CgA signals failure of hormonal treatment for these patients [25].

In conclusion, our results suggest that serum CgA levels is a valuable marker for predicting the presence of bone metastases in PAC patients. Combined with PSA, CgA can predict disease progression in patients with advanced PAC under CAND treatment and is correlated with poor prognosis.

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