Independent prognostic role of circulating chromogranin A in prostate cancer patients with hormone-refractory disease

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Abstract

The presence of neuroendocrine (NE) differentiation in the context of predominantly exocrine prostate cancer may play a key role in androgen-independent tumor growth. The prognostic significance of plasma chromogranin A (CgA) was assessed in a series of consecutive prostate cancer patients with hormone-refractory disease.

One hundred and eight patients with newly diagnosed hormone-refractory prostate cancer entered the study. Plasma CgA levels and other biochemical parameters, such as serum prostate specific antigen, serum alkaline phosphatase, serum lactate dehydrogenase, serum albumin and hemoglobin concentration, were measured at baseline (i.e. when hormone refractoriness occurred) and their prognostic role was evaluated together with patient performance status, Gleason score (at diagnosis of prostate cancer) and the presence of visceral metastases. Furthermore, plasma CgA was prospectively evaluated in 50 patients undergoing chemotherapy.

At baseline, 45 patients (43.3%) showed elevated CgA values. Plasma CgA negatively correlated with survival, either in univariate analysis ($P=0.008$) or in multivariate analysis, after adjusting for previously mentioned prognostic parameters ($P<0.05$). In the patient subset undergoing chemotherapy, median CgA (range) values were 13.3 (3.0–141.0) U/l at baseline, 19.1 (3.0–486.0) U/l after 3 months, 20.8 (3.0–702.0) U/l after 6 months and 39.4 (3.0–414.0) U/l after 9 months ($P<0.01$). The corresponding supranormal rates were 17/50 (34%), 23/50 (46%), 26/50 (52%) and 34/50 (68%) respectively ($P<0.005$).

Elevated plasma CgA levels are frequently observed in prostate cancer patients with hormone-refractory disease and correlate with poor prognosis. NE differentiation in hormone-refractory patients is a time-dependent phenomenon and is not influenced by conventional antineoplastic treatments.

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Introduction

Prostate cancer is frequent in the aging male population (De Angelis *et al*. 1997). Androgen suppression by either orchiectomy or administration of luteinizing hormone-releasing hormone analogs (LHRH-As) is the mainstay of treatment for patients with advanced disease. Although this therapy frequently results in tumor shrinkage and improvement of symptoms, it is not curative and the majority of patients eventually develop hormone-refractory disease. Once disease becomes refractory to hormonal manipulation, prognosis is dismal and the overall survival is about 15–16 months (Smaletz *et al*. 2002, Halabi *et al*. 2003). Treatment options for hormone-refractory disease include intensive supportive care, radiotherapy, bisphosphonates, second-line hormonal manipulations, cytotoxic chemotherapy and investigational agents (Sandler *et al*. 2003). Chemotherapeutic agents, such as docetaxel, have recently yielded improved response rates and survival in two randomized independent clinical trials (Petrylak *et al*. 2004, Tannock *et al*. 2004). Docetaxel represents therefore the new standard treatment. The survival benefit obtained with this drug in

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hormone-refractory patients, however, is modest. Understanding mechanisms underlying the development of hormone refractoriness is the basis for developing new treatment strategies for the disease at this stage.

The majority of conventional prostate cancers display at diagnosis focal neuroendocrine (NE) differentiation, usually revealed by immunohistochemistry as scattered individual cells or nests of cells in the context of predominantly exocrine tumors (Abrahamsson 1999*a*,*b*, di Sant'Agnese 2001). NE mechanisms are emerging as important factors in the evolution, progression and overall prognosis of prostate cancer (di Sant'Agnese 2001). They appear to be especially important in facilitating prostate cancer progression to androgen-deprivation therapy (Abrahamsson 1999*b*, di Sant'Agnese 2001). Several mechanisms have been identified: NE cells are androgen receptor negative, therefore they survive to androgen deprivation (Bonkhoff 1998); NE cells produce peptides, hormones and growth factors (Bonkhoff, Abrahamsson 1999*b*) that could stimulate the proliferation of exocrine prostate cancer cells and increase their aggressiveness through apoptosis inhibition (Xing *et al*. 2001) and neoangiogenesis stimulation (Mazzucchelli *et al*. 2000).

Some authors, including our group, have found that NE cell numbers in prostate cancer correlate with stage and Gleason score (Deftos *et al*. 1996, Ahlegren *et al*. 2000, Bollito *et al*. 2001). These findings notwithstanding, the relationship with survival is still controversial, since some studies demonstrated a negative prognostic role (Weinstein *et al*. 1996, McWilliam *et al*. 1997, Theodorescu *et al*. 1997), while others did not (Noordzij *et al*. 1995, Bubendorf *et al*. 1997, Abrahamsson *et al*. 1998, Ahlegren *et al*. 2000, Bostwick *et al*. 2002). All these studies involved hormone-naive patients.

It has recently pointed out that NE differentiation is not a static phenomenon. The NE compartment, in fact, increases after androgen deprivation and in refractory disease (Abrahamsson 1999*a*, di Sant'Agnese 2001). The direct stimulation of NE differentiation by androgen-deprivation therapy was first demonstrated in an elegant preclinical study by Jongsma *et al*. (2002). These authors demonstrated in xenograft models that castration results in a dramatic increase in the number of cells expressing NE markers. These data have been confirmed in humans by Sciarra *et al*. (2003), showing a significant increase in circulating chromogranin A (CgA) levels after LHRH-A administration. Interestingly, this increase was lower during intermittent therapy than after traditional continuous androgen ablation.

The biological and clinical significance of NE differentiation in prostate cancer patients who become hormone-refractory is still to be elucidated.

CgA appears to be the most sensitive marker and is most frequently used for detecting NE differentiation either at the tissue level or in the general circulation (Berruti *et al*. 2001). The availability of such a specific circulating marker for the NE component could allow us to easily detect and monitor NE differentiation in prostate cancer patients.

Plasma CgA levels were measured in a series of prostate cancer patients with hormone-refractory disease. The primary study aim was to determine the prognostic role of elevated levels. A secondary aim was to evaluate the changes of this marker over time in a subgroup of patients undergoing chemotherapy.

Patients and methods

Patients

From January 1998 to January 2003, 108 consecutive patients with newly diagnosed hormone-refractory prostate cancer were enrolled in the present study.

Eligible patients were required to have histologically proven adenocarcinoma of the prostate that progressed to LHRH-A administration, in the presence of castrate levels of testosterone. From 1998 to 2000, castrate testosterone levels were defined as less than 50 ng/dl; from 2000 onwards it was reassessed as less than 20 ng/ml. The time from the diagnosis of hormone refractoriness to the enrolment in the study should have been not greater than 2 months. Disease progression was defined by rising prostate specific antigen (PSA) levels on two consecutive measurements at least 2 weeks apart, in addition to a new lesion on a bone scan and/or an increase in the size of a measurable lesion on a computed tomographic scan of the abdomen/pelvis or chest. All patients previously treated with antiandrogen in addition to LHRH-A (total androgen ablation) were required to undergo antiandrogen withdrawal. Patients were required to be off all antiandrogens for at least 4 weeks with further evidence of disease progression after cessation of the antiandrogen. Further inclusion criteria included an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-3 and normal renal and hepatic function. Patients were excluded from the study for severe uncontrolled co-morbidity, second malignancies, brain metastases and concomitant treatment with proton pump inhibitors. All patients were required to give written informed consent before registration.

Biochemical measurements

Plasma levels of CgA and serum values of PSA were evaluated at baseline condition in all patients. Both markers were also prospectively measured after 3, 6 and 9 months in 50 patients submitted to chemotherapy.

Blood specimens were obtained in the early morning after an overnight fast. Blood for CgA assessment was collected in a frozen vial until plasma separation. All serum and plasma samples were immediately frozen and stored at -20°C until analysis. Commercially available kits were used to measure serum PSA levels (IRMA kit; Hybritech, Liege, Belgium), and plasma CgA values (ELISA kit; DAKO, Glostrup, Denmark). The reference upper value of serum PSA was defined as 4 ng/ml. The reference upper value for circulating CgA was defined as 20 U/l as previously reported (Berruti *et al*. 2000). A number of biochemical parameters, such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP), albumin (ALB) and hemoglobin (Hb), were also evaluated at baseline conditions using automated procedures (Architect; Abbott Laboratories).

Among patients who had plasma CgA prospectively measured, the following subsets were identified, according to the changes in marker concentrations: (i) patients with a marker level below the normal threshold at baseline that remained negative after treatment; (ii) patients with normal marker levels at baseline that became elevated during treatment; (iii) patients with elevated marker levels at baseline, showing a marker decline >50% during treatment; (iv) patients with normalization of elevated pretreatment marker levels; (v) patients with marker increase >25% of baseline value; and (vi) patients with no change in marker levels, defined as reduction <50% or increase <25%.

Statistical analysis

Differences between paired groups were tested using the Friedman ANOVA non-parametric test. Difference in proportion was done by the chi-square test. The main end point was survival duration, which was defined as the time between the diagnosis of hormone-refractory disease and death. Patients were censored if they were known to be alive or they were lost to follow-up. Survival curves were estimated using the Kaplan–Meier method. Unadjusted difference in these estimates was tested by the log-rank test. The Cox proportional hazard model was employed to evaluate the independent prognostic significance of baseline factors. Biochemical markers and Hb were considered as continuous variables. PSA, LDH, ALP and CgA levels had a marked right-skewed distribution and were modeled using the log transformation. Statistical computation was performed using the 'Statistics for Windows' software.

Table 1 Patient characteristics

No.	108
Median age (range)	74 (58–86)
Gleason score at diagnosis	
5	5 (4.6%)
6	10 (9.2%)
7	36 (33.3%)
8	41 (38.0%)
9	12 (11.1%)
10	1
Missing	2
Median (months) since start of hormone therapy	24.4
Range	1.13–211.0
ECOG PS	
0	26 (24.1%)
1	55 (50.9%)
2	19 (17.6%)
3	8 (7.4%)
Sites of disease	
Bone	105 (97.2%)
Visceral	11 (10.2%)
Soft tissue	24 (22.2%)
No. of patients with elevated PSA levels	91 (84.2%)
Median PSA (ng/ml) (range)	97.0 (0.1 – 3393.0)
No. of patients with elevated CgA levels	45 (41.7%)
Median CgA (U/L) (range)	17.3 (3.0 394.0)
Median Hb (g/dl) (range)	12.4 (7.3–15.6)
Median ALP (U/l) (range)	150 (47.0–2480.0)
Median LDH (U/l) (range)	398 (143.0–3011.0)
Median ALB (g/dl) (range)	3.73 (2.04–6.74)

Results

Patients

Table 1 displays the baseline characteristics of the 108 patients enrolled. Seventy-five of them had an ECOG PS of 0-1. Ninety-seven percent had bone metastases and 10% had visceral involvement. Supranormal CgA values were observed in 45 patients (41.7%), while 91 patients (84.2%) had elevated PSA levels. At the last follow-up on June 2004, 80 patients (74.1%) had died. Median survival was 16.7 months (range 1.0 to 79+ months).

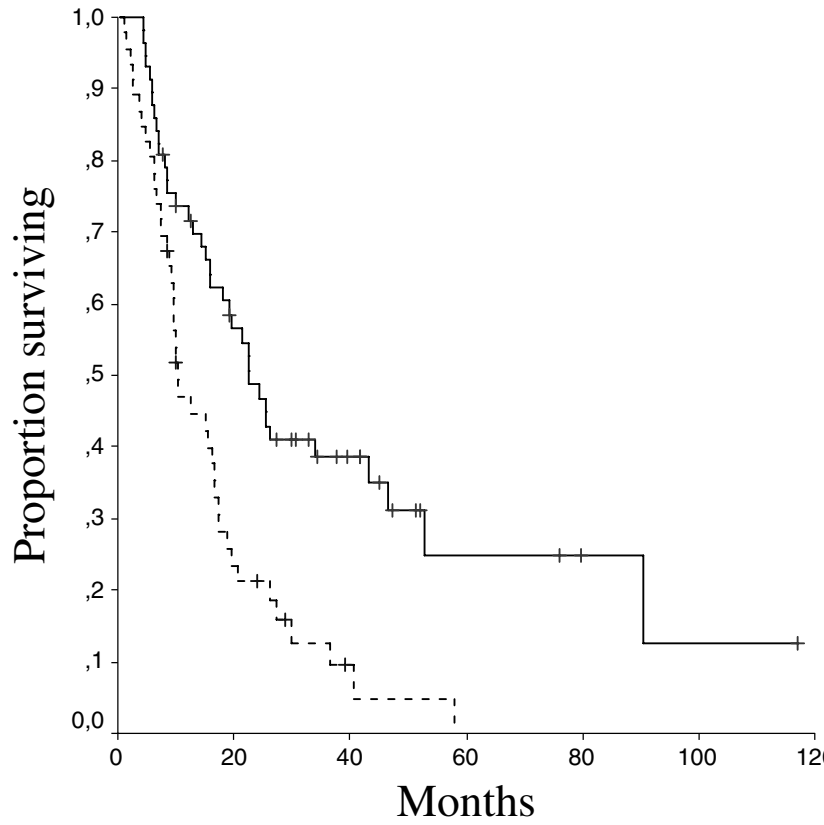

Figure 1 Survival curves of patients with elevated plasma CgA levels (broken line) vs patients with normal CgA values (solid line) at baseline conditions.

Univariate survival analysis

As reported in Fig. 1, survival of patients with elevated CgA levels (median 10.2 months (95% confidence interval (CI) 6.5–13.9)) was significantly lower than that of patients with normal CgA (median 22.6 months (95% CI 15.9–29.2)), $P=0.0002$.

Table 2 presents the univariate survival analysis of the baseline prognostic factors. Statistically significant factors of survival were: patient PS, Gleason score of the original prostatectomy or prostate biopsy specimen, plasma CgA, serum ALP, serum LDH, Hb, serum ALB and serum PSA. The presence of visceral metastases failed to significantly influence the prognosis. Stratifying patients according to baseline PSA values, the negative prognostic role of elevated CgA levels was maintained in the subset with PSA less than or equal to the median value (97.0 ng/ml) (median survival 9.9 months (95% CI 9.0–10.7) in CgA+ patients vs 26.2 months (95% CI 0.0–53.5) in CgA− ones, $P=0.001$), but not in the subset with PSA greater than 97.0 ng/ml (median survival 15.7 months (95% CI 10.2–21.2) in CgA+ patients vs 12.7 months (95% CI 4.0–21.4) in CgA− ones, $P=0.29$).

Multivariate survival analysis

Baseline factors that statistically correlated with overall survival in the univariate analysis, were considered in a multivariate analysis (Table 3). Plasma CgA confirmed its prognostic role in multivariate analysis, after adjusting for patient PS, serum ALP, Hb, Gleason score, serum ALB and serum LDH. Independent statistically significant prognostic factors of overall survival were also patient PS, serum ALP and Hb, while serum LDH just failed to enter the model.

Serial CgA evaluations

Plasma CgA was prospectively measured after 3, 6 and 9 months in 50 patients undergoing chemotherapy. Fifteen patients received estramustine alone, 15 patients epirubicin and 20 patients estramustine+docetaxel. Chemotherapy was administered while maintaining

Table 2 Prognostic role of baseline biochemical and clinical parameters in an univariate analysis

	Survival		
	Median	*95% CI*	*P*
Gleason score			
5–7	22.5	13.9–31.0	0.05
8–10	12.7	5.5–19.9	
Visceral metastases			
Yes	10.2	0.0–20.6	0.36
No	17.3	13.1–21.4	
ALP			
≤ 150.0 U/l	22.4	17.3–27.6	0.0002
>150.0 U/l	10.2	7.0–13.4	
PSA			
≤ 97.0 ng/ml	21.4	13.6–29.2	0.04
>97.0 ng/ml	15.4	9.4–21.4	
LDH			
≤ 398.0 U/l	24.4	17.0–31.7	0.01
>398.0 U/l	12.7	5.8–19.6	
CgA			
≤ 17.6 U/l	22.4	14.1 – 30.6	0.008
>17.6 U/l	15.1	8.2 – 22.1	
PS			
0	43.7	n.d.	0.0002
1	16.6	12.5–20.7	
2–3	9.4	7.4–11.4	
Hb			
≤ 12.4 g/dl	9.7	4.3–15.3	0.001
>12.4 g/dl	26.1	18.5–33.7	
ALB			
≤ 3.7 mg/dl	12.2	5.7–18.0	0.04
>3.7 mg/dl	22.4	14.3–30.6	

androgen-deprivation therapy (LHRH-A). Eighteen patients showed a PSA response (36.0%). Median CgA plasma levels (range) were: 13.3 (3.0–141.0) U/l at baseline, 19.1 (3.0–486.0) U/l after 3 months, 20.8 (3.0–702.0) U/l after 6 months and 39.4 (3.0–414.0) U/l after 9 months ($P<0.01$). The corresponding supranormal rates were 17/50 (34%), 23/50 (46%), 26/50 (52%) and 34/50 (68%) respectively ($P<0.005$). In the 33 patients with normal CgA values at baseline, only 13 maintained normal marker levels during all of the treatment period, while the remaining 20 showed a consistent marker increase above the normal range. In the 17 patients with elevated CgA at baseline conditions, the marker decreased (>50%) in 2 patients and increased (>25%) in 10 patients, while it did not change in 5 patients. In no patient did elevated pre-treatment CgA levels return to normal after treatment. As outlined in Fig. 2, the individual variation in plasma CgA did not differ after stratifying patients according to PSA response.

Table 3 Multivariate analysis of independent variables predicting for overall survival

	HR	95% CI	*P*
CgA	1.20	0.99–1.44	0.05
Hb	0.87	0.74–0.92	0.001
LDH	1.67	0.95–2.93	0.07
ALP	1.33	1.03–1.71	<0.03
PS	1.30	1.01–1.68	<0.05
Gleason score	1.17	0.94–1.45	0.15
ALB	0.91	0.64–1.28	0.58
PSA	1.01	0.90–1.12	0.81

HR, hazard ratio.

Discussion

Recent studies suggest that NE differentiation in prostate cancer appears to be especially important in promoting the androgen-independent growth phase of the disease (di Sant'Agnese 2001). This concept is supported by the results of this study. In our series of prostate cancer patients evaluated at diagnosis of hormone-refractory disease, plasma CgA was frequently elevated (41.7% of patients) and elevated marker values were significantly predictive of poor prognosis.

The prognostic role of NE differentiation in prostate cancer has been already explored by several investigators. In the majority of published studies, CgA immunostaining in tumor specimens obtained after radical prostatectomy failed to show a prognostic role (Noordzij *et al*. 1995, Bubendorf *et al*. 1997, Abrahamsson *et al*. 1998, Ahlgren *et al*. 2000, Bostwick *et al*. 2002). Similarly, Lilleby *et al*. (2001) did not show a significant correlation between elevated circulating CgA values and prognosis in a patient subset with hormone-naive disease submitted to radiotherapy. To our knowledge, the present is the first prospective study evaluating the prognostic role of circulating CgA in hormone-refractory patients.

Patients with hormone-refractory prostate cancer are destined to die of this disease (Smaletz *et al*. 2002, Halabi *et al*. 2003) and available antineoplastic therapies only have a modest impact on survival (Petrylak *et al*. 2004, Tannock *et al*. 2004). The median survival observed in the present study was 16 months, but survival was distributed in a wide range (from 1 to 79+ months), thus confirming previous observations (Smaletz *et al*. 2002, Halabi *et al*. 2003). Due to the heterogeneity of this population, the identification of prognostic factors is useful to physicians to guide treatment selection and to counsel patients about their long-term outlook. Along this line, two nomograms have been developed on patients included in large prospective clinical trials (Smaletz *et al*. 2002, Halabi

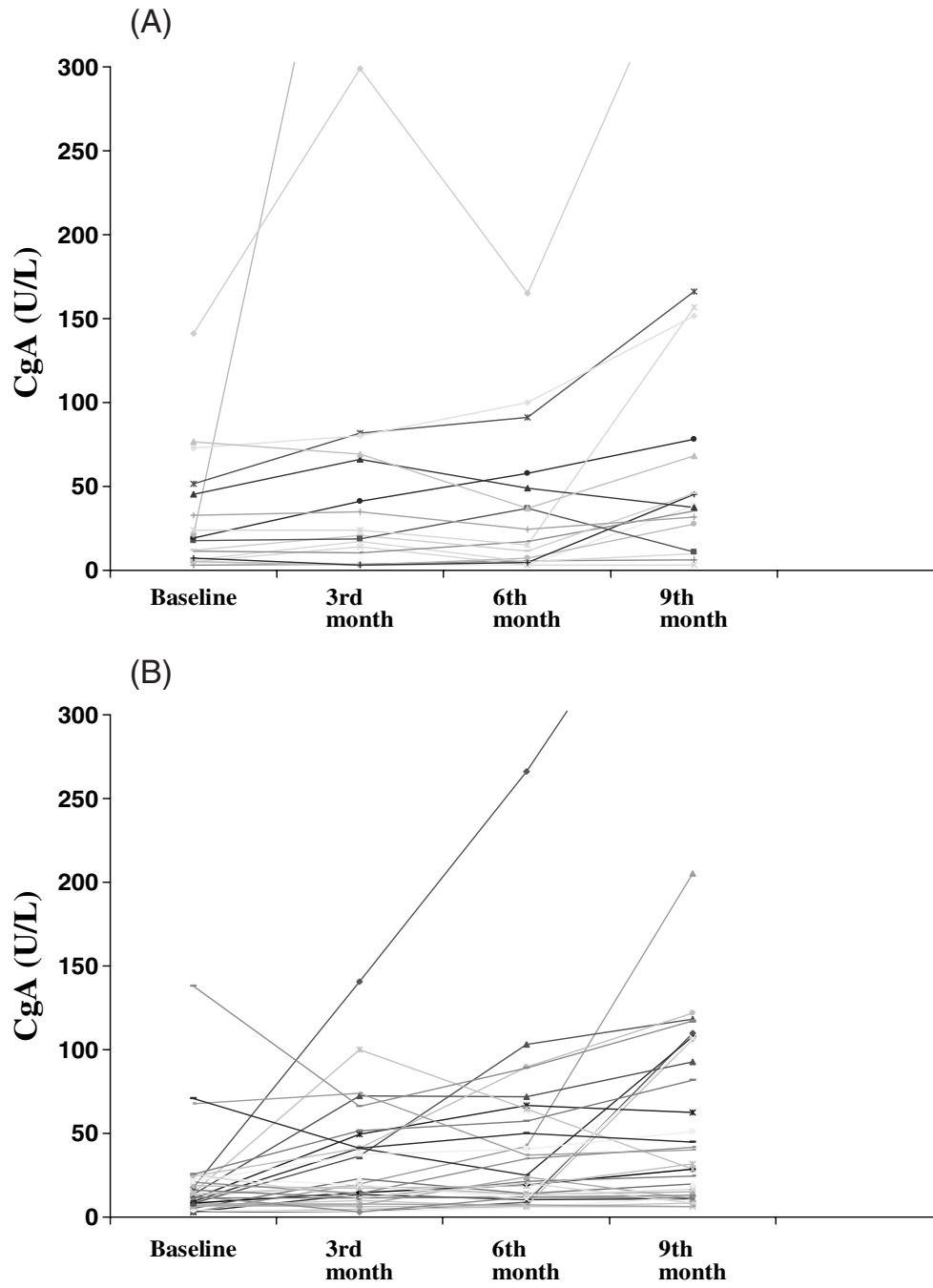

Figure 2 Individual variation of plasma CgA after chemotherapy in patients obtaining a PSA response (A) and in those failing to obtain a PSA response (B).

et al. 2003). These models are based on clinical and biochemical parameters commonly considered in predicting the outcome in many solid tumors (such as Hb, LDH, PS and the presence of visceral metastases) and on specific prognostic parameters for prostate cancer patients (such as Gleason score at diagnosis, PSA and ALP). With the exception of visceral metastases, all these prognostic parameters were confirmed in the present study to correlate with prognosis in univariate analysis. In multivariate analysis, however, only PS,

Hb and ALP showed an independent prognostic role; LDH just failed to attain the statistical significance, while ALB, PSA and Gleason score did not enter the model. Plasma CgA values maintained an inverse relationship with survival in multivariate analysis, after adjusting for PS, Hb, LDH, ALB, PSA and Gleason score. These data suggest that CgA could really provide independent prognostic information with respect to validated prognostic factors.

The prospective nature and the measurement of CgA and all biochemical parameters in a single laboratory are the strengths of this study. Limitations include the relatively low number of patients enrolled and the absence of an external validation data set. Our data anyway were recently confirmed by preliminary results of a Cancer and Leukemia Group B study (Taplin *et al*. 2004).

The absent relationship among PSA, ALB, Gleason score and survival in multivariate analysis seems to be in contrast with previously published results (Smaletz *et al*. 2002, Halabi *et al*. 2003). It should be noted, however, that the two mentioned prognostic studies reported discrepant results with respect to these three parameters. ALB was included as a prognostic parameter in the study by Smaletz *et al*. (2002), but not in the study by Halabi *et al*. (2003); vice versa, Gleason score was included in the second study but not in the first one. Finally, PSA was a strong independent prognostic variable in one study (Halabi *et al*. 2003), but not in the other (Smaletz *et al*. 2002).

In 50 stage D patients with hormone-naive disease, Issiki *et al*. (2002), have reported that the negative prognostic role of elevated circulating CgA was confined to the subset with relatively low serum PSA levels (<172 ng/ml). Our data confirmed this observation. In our series, elevated CgA was significantly associated with poor prognosis in patients with PSA levels less than or equal to the median value, but not in patient with higher PSA values. We have no explanation for this phenomenon. As mentioned in the Introduction, a mechanism of the relationship between neuroendocrine differentiation and prostate cancer aggressiveness is the capability of neuroendocrine cells to stimulate neoangiogenesis. PSA has recently shown to have an antiangiogenetic property (Fortier *et al*. 1999). We can therefore speculate that, in CgA+ patients, high PSA expression could counteract the neoangiogenesis stimulated by neuroendocrine differentiation, whereas low-level expression of PSA may not be sufficient to overcome the increased angiogenesis stimulation. This interesting hypothesis, however, deserves further study.

In the patient subset with CgA prospectively evaluated during chemotherapy, we clearly showed that antineoplastic drugs commonly employed against prostate cancer are unable to affect CgA levels. NE prostate cancer cells notoriously do not proliferate (Bonkhoff 1998) and this finding could explain the resistance of this cell compartment to cytotoxic drugs. Our study also points to the dynamic role of NE phenotype in prostate cancer. CgA increased a lot over time; plasma values at the end of study, in fact, were on average about twice those of values observed at baseline. The marker increase was observed either in patients with already elevated values at baseline or in patients with baseline marker values within normality. These data suggest that the NE phenotype is a time-dependent phenomenon and might be predominant in the late phase of the natural history of the disease. The mechanisms underlying this increasing trend are unknown. It has been shown that androgen-deprivation therapy strongly stimulates NE phenotype (Jongsma *et al*. 2002, Sciarra *et al*. 2003). Our patients did not interrupt androgen-deprivation therapy and this may have perpetuated the stimulation of the NE compartment. Of course we cannot exclude that NE stimulation may be the result of the disease progression.

To conclude, elevated plasma CgA levels are frequently observed in prostate cancer patients with hormone-refractory disease and correlate with poor prognosis. Plasma CgA should be considered among biological markers to be incorporated in future prognostic nomograms. Serial CgA evaluation showed that NE differentiation in hormone-refractory patients is a time-dependent phenomenon and is not influenced by conventional antineoplastic treatments.

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