ELSEVIER

# EFFECT OF NONSTEROIDAL ANTIANDROGEN MONOTHERAPY VERSUS CASTRATION THERAPY ON NEUROENDOCRINE DIFFERENTIATION IN PROSTATE CARCINOMA

ALESSANDRO SCIARRA AND FRANCO DI SILVERIO


## ABSTRACT

**Objectives.** To determine whether the administration of the nonsteroidal antiandrogen bicalutamide reduces the risk of an increase in chromogranin A (CgA) levels in patients with prostate cancer who experienced biochemical failure after radical retropubic prostatectomy (RRP) compared with pharmacologic castration therapy. It has been hypothesized that continuous androgen suppression for the treatment of prostate cancer results in hyperactivation of neuroendocrine cells and an increase in CgA levels.

**Methods.** Forty-eight patients with pT3pN0M0 prostate cancer and biochemical (prostate-specific antigen) progression after RRP were randomized to bicalutamide monotherapy or pharmacologic castration. The serum levels of CgA and prostate-specific antigen were measured at 1, 3, 6, 12, 18, and 24 months of therapy. The changes in serum CgA levels were compared for patients who successfully responded to the first 24 months of therapy.

**Results.** In both treatment groups, a statistically significant trend was noted for CgA levels to increase from baseline to 24 months. This trend was lower in the bicalutamide group (slope = 0.60, 95% confidence interval 0.28 to 0.92; $P = 0.004$) than in the castration group (slope = 0.29, 95% confidence interval 0.08 to 0.50; $P = 0.01$).

**Conclusions.** The results of this study provide the first evidence to show that in patients with prostate cancer undergoing hormonal therapy, nonsteroidal antiandrogen monotherapy produces a significantly lower increase in serum CgA compared with castration.

UROLOGY **63:** 523–527, 2004. 

Neuroendocrine (NE) cell differentiation, consisting of NE cells with a focal distribution,[1] in prostate cancer and its possible clinical significance has received increasing attention. NE cells may play a local regulatory role in the growth and differentiation of prostatic tissue through bioactive neuropeptide production.[2,3] The serum levels of NE cell products may reflect NE cell activity, with chromogranin A (CgA) appearing to be the best marker.[4,5] Patients with elevated CgA levels may have a poor prognosis,[6] and prostate cancer with NE cell differentiation tends to be more aggressive.[4,7] NE cells consistently lack androgen receptors and are resistant to hormonal therapy.[8] Their activity may represent an independent factor in tumor progression.

The NE tumor cell number[9] and CgA serum levels increase during androgen deprivation.[10,11] In radical retropubic prostatectomy (RRP) specimens from patients randomized to 3 months of neoadjuvant castration or surgery alone, the number of CgA-positive cells and the proportion of NE-positive tumors were greater in the neoadjuvant group than in the control group ($P < 0.003$).[12] Similarly, after RRP, the serum concentration and prostatic tissue gene expression of CgA were greater in patients who received 3 months or longer of neoadjuvant, complete androgen deprivation (CAD) compared with untreated patients ($P < 0.01$).[1] If CgA expression reflects NE activity, continuous androgen suppression could cause NE cell hyperactivation, a possible mechanism by which prostate cancer progresses to androgen independence.

*From the Department of Urology U. Bracci, University La Sapienza, Rome, Italy*

*Reprint requests: Franco Di Silverio, Department of Urology "U. Bracci," University La Sapienza, V. Policlinico, Rome 00161, Italy*

*Submitted: May 19, 2003, accepted (with revisions): October 14, 2003*



0090-4295/04/$30.00
doi:10.1016/j.urology.2003.10.043

Different hormonal therapies may influence CgA levels and NE differentiation to different extents. For example, intermittent CAD significantly reduced the increase in serum CgA levels compared with continuous CAD.[13] The present study, therefore, investigated the direct effects of nonsteroidal antiandrogen monotherapy and pharmacologic castration on CgA levels.

## MATERIAL AND METHODS

### *Subject Selection*

Patients with pT3pN0M0 prostate cancer and biochemical progression (defined as a PSA level greater than 0.4 ng/mL)[14] within 12 months of RRP were enrolled. The other inclusion criteria were histologically proven prostate cancer; no preoperative hormonal therapy or radiotherapy; RRP with regional lymphadenectomy performed at our institution; and negative surgical margins.[14]

### *Study Design*

Patients were treated according to the Declaration of Helsinki, and all patients provided signed, informed consent.

After RRP, when serum PSA levels exceeded 0.2 ng/mL, the PSA determinations were repeated every 2 weeks. Patients entered the study once their PSA level progressed, defined as three or more consecutive elevated levels (greater than 0.4 ng/mL).

Patients were randomized to bicalutamide 150 mg daily or triptorelin 3.75 mg monthly. Serum CgA levels were analyzed at baseline (PSA progression) and 1, 3, 6, 12, 18, and 24 months after randomization. Total PSA levels were measured every 4 weeks for the first 12 months of treatment and every 8 weeks thereafter. Treatment was considered to have failed when the PSA level increased to greater than 0.4 ng/mL or at clinical progression. Biopsy of the urethrovesical anastomosis, abdominal-pelvic magnetic resonance imaging, and a total body bone scan were performed 12 and 24 months after randomization or at PSA progression.

Blood samples collected in the early morning after overnight fasting were evaluated centrally. The serum CgA levels were measured by radioimmunoassay (CIS Bio International, Cedex, France; detection limit 1.5 ng/mL; interassay and intra-assay coefficient of variation 5.8% and 3.8%, respectively). The same sample was used to determine the PSA level (Hybritech, San Diego, Calif).

### *Statistical Analysis*

The study objectives required a design that would detect a statistically significant difference between measures (month 24 versus baseline) of 25% at $P < 0.05$, with a power of 90% (type II or beta error of 0.1). The effect size seemed to be clinically reasonable. Using standard power analysis methods, a sample size of 24 patients in each group was estimated. The statistical analyses were carried out using the Statistical Package for Social Sciences, version 10.0.7.

Descriptive statistics were used to characterize the parameters (mean ± SD, median, and range). Differences were assessed between mean values using Student's *t* test and between treatment groups using repeated measures analysis of variance. The Machuly sphericity test was performed to test the spherical form of the common covariance matrix and validate the F test and associated *P* values for within-patient factors. The degree of association among the variables was determined using Pearson's *r* correlation test, validated by Spearman's rank and Kandal-Tou correlation tests.

## RESULTS

Between December 1998 and January 2001, 186 patients with clinically localized prostate cancer underwent RRP and bilateral pelvic lymphadenectomy at our institution; 59 fulfilled the inclusion criteria and agreed to randomization. No patient had a history of other disorders, therapies, or conditions known to interfere with CgA levels.

Only the 48 patients who concluded and successfully responded to the first 24 months of treatment were analyzed. All had PSA progression within 12 months of RRP. In each patient, the total body bone scan, abdominal-pelvic magnetic resonance imaging, and biopsy of the urethrovesical anastomosis on PSA progression proved negative. No differences were found between the groups in the baseline characteristics or serum PSA and CgA levels (Table I). During the first 24 months of follow-up, no patient showed evidence of clinical progression and the PSA serum levels remained at 0.4 ng/mL or less.

Repeated measures of analysis of variance ($F = 12.190$; $P = 0.001$, power 0.925) supported the hypothesis that a statistically significant difference exists between groups on the overall CgA measurements and indicated that, in our population, a main effect of therapy was present. In particular, a statistically significant trend was noted for CgA levels to increase from baseline to 24 months (slope = 0.60, 95% confidence interval 0.28 to 0.92; $P = 0.004$) in the castration group (Fig. 1). A statistically significant, but lower, trend was found in the bicalutamide group (slope = 0.29, 95% confidence interval 0.08 to 0.50; $P = 0.01$; Fig. 2).

At 12 months of follow-up, the serum CgA levels were significantly lower in the bicalutamide group, than in the castration group ($P = 0.0433$; Table II), which was greater at 24 months' follow-up ($P = 0.0025$). The median increase in serum CgA levels from baseline to 12 and 24 months was lower in the bicalutamide group (25.4%, range 1.0% to 42.8%, and 30.0%, range 1.0% to 48.6%, respectively) than in the castration group (48.9%, range 27.2% to 82.4%, and 60.2%, range 16.6% to 78.2%, respectively).

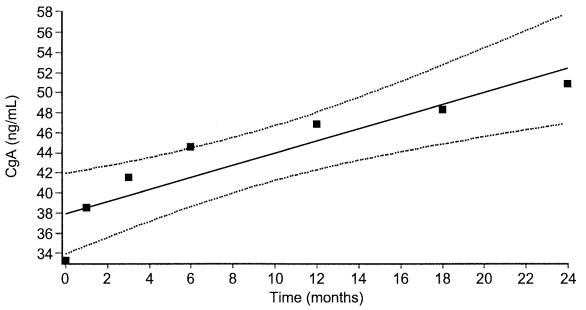
In both groups, no statistically significant association was found between CgA and PSA levels at 12 and 24 months ($r < 0.200$; $P > 0.05$). The increases in CgA levels were independent of the variations in PSA levels.

When stratified by Gleason score (less than 7 and 7 or greater) at baseline, the serum CgA levels were greater in patients with a Gleason score of 7 or more (bicalutamide 38.04 ± 6.34 ng/mL; triptorelin 35.22 ± 6.52 ng/mL) than in those with a Gleason score of less than 7 (bicalutamide 24.0 ± 2.41 ng/mL; triptorelin 23.77 ± 2.26 ng/mL) in both

**TABLE I.** *Clinical and pathologic characteristics*

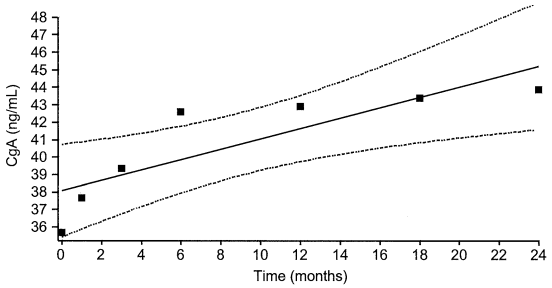
| Characteristic | Bicalutamide Group (150 mg) | Triptorelin Group (3.75 mg) | *P* Value |
|---|---|---|---|
| Patients (n) | 24 | 24 | |
| Age (yr) | 64.2 ± 3.7 (66); 54–70 | 64.8 ± 3.5 (66); 56–70 | 0.980 |
| Pathologic stage at RRP | | | |
| T3aN0 | 18 | 20 | |
| T3bN0 | 6 | 4 | |
| Gleason score at RRP | | | |
| 6 (3 + 3) | 4 | 4 | |
| 7 (3 + 4) | 8 | 11 | |
| 7 (4 + 3) | 10 | 7 | |
| 8 (5 + 3) | 2 | 2 | |
| Negative surgical margins | 24 | 24 | |
| Pre-RRP PSA (ng/mL) | 12.60 ± 2.85 (12.0); 7.0–17.40 | 12.32 ± 2.76 (11.7); 7.5–16.0 | 0.730 |
| PSA after RRP (ng/mL) (1 mo) | 0.11 ± 0.04 (0.10); 0.10–0.20 | 0.10 ± 0.03 (0.10); 0.10–0.15 | 0.250 |
| Time to PSA progression after RRP (mo) | 6.30 ± 1.40 (6); 3–9 | 5.70 ± 1.80 (6); 3–9 | 0.200 |
| PSA at baseline (ng/mL) (progression after RRP) | 1.12 ± 0.32 (1.0); 0.60–1.80 | 1.05 ± 0.30 (1.0); 0.60–1.60 | 0.530 |
| CgA at baseline (ng/mL) (progression after RRP) | 35.70 ± 7.90 (35.0); 20.70–50.70 | 33.31 ± 7.40 (33.20); 20.40–49.70 | 0.283 |

*KEY: RRP = radical retropubic prostatectomy; PSA = prostate-specific antigen; CgA = chromogranin A.*
*Statistical significance of differences between the bicalutamide and triptorelin groups is reported (*P *values).*
*Data given as "mean ± SD (median), range", unless otherwise indicated.*




Slope=0.60; CI 0.28-0.92.
The slope differs significantly from zero (*P*=0.004).
Y intercept is 37.93 (r=0.90).

FIGURE 1. *Linear regression model for castration therapy showing mean CgA variations during 24 months of therapy.*




Slope=0.29; CI 0.08-0.50.
The slope differs significantly from zero (*P*=0.01).
Y intercept is 38.09 (r=0.8501).

FIGURE 2. *Linear regression model for bicalutamide treatment showing mean CgA variations during 24 months of treatment.*

groups ($P < 0.0001$). At 12 months' follow-up, statistically significant differences were found between the bicalutamide and triptorelin groups in serum CgA levels in patients with a Gleason score of 7 or greater (46.0 ± 5.40 versus 50.83 ± 4.92 ng/mL; $P = 0.0094$) but not in patients with a Gleason score less than 7 (27.65 ± 0.95 versus 27.20 ± 4.77 ng/mL; $P = 0.6857$). This was also true after 24 months' follow-up (Gleason score of 7 or greater, 47.37 ± 5.18 versus 54.58 ± 6.14 ng/mL; $P = 0.0001$; Gleason score less than 7, 27.0 ± 1.79 versus 29.45 ± 1.10 ng/mL; $P = 0.1478$). The greatest increase in serum CgA levels was in the castration group with a Gleason score of 7 or greater (baseline to 24 months, median increase 54.8%, range 33.3% to 78.2%).

## COMMENT

It has been hypothesized that continuous androgen deprivation results in NE cell hyperactivation in the prostate, an increase in CgA levels, and, ultimately, a tumor unaffected by hormonal therapy.[1,9–12] In the present study, the effects of nonsteroidal antiandrogen monotherapy and pharmacologic castration on CgA levels and NE prostate cell hyperactivation were compared. This is important given the recent interest in the use of bicalutamide in nonmetastatic disease and as adjuvant to RRP or radiotherapy.[15–17] To our knowledge, this is the first report to indicate that nonsteroidal antiandrogen monotherapy has less effect on serum CgA levels in prostate cancer than does castration.

Patients who experienced biochemical progression after RRP were admitted once their PSA level

**TABLE II. *Serum PSA and CgA levels in bicalutamide and triptorelin groups during 24 months of follow-up***

| | Baseline | Time 1 | Time 3 | Time 6 | Time 12 | Time 18 | Time 24 |
|---|---|---|---|---|---|---|---|
| Bicalutamide (150 mg) | | | | | | | |
| Serum PSA (ng/mL) | 1.12 ± 0.32 (1.0) | 0.52 ± 0.22 (0.60) | 0.26 ± 0.12 (0.30) | 0.22 ± 0.12 (0.30) | 0.24 ± 0.11 (0.20) | 0.23 ± 0.10 (0.20) | 0.22 ± 0.08 (0.20) |
| Serum CgA (ng/mL) | 35.70 ± 7.90 (35.0) | 37.73 ± 8.98 (38.60) | 39.36 ± 8.81 (40.40) | 42.60 ± 9.07 (42.65) | 42.94 ± 8.54 (43.90) | 43.45 ± 8.55 (45.30) | 43.97 ± 9.09 (45.50) |
| Triptorelin (3.75 mg) | | | | | | | |
| Serum PSA (ng/mL) | 1.05 ± 0.30 (1.0) | 0.32 ± 0.14 (0.35) | 0.21 ± 0.10 (0.20) | 0.24 ± 0.09 (0.20) | 0.20 ± 0.08 (0.20) | 0.19 ± 0.09 (0.20) | 0.17 ± 0.10 (0.15) |
| Serum CgA (ng/mL) | 33.31 ± 7.40 (33.20) | 38.55 ± 8.68 (38.40) | 41.55 ± 8.98 (40.55) | 44.60 ± 9.18 (44.55) | 46.89 ± 10.19 (49.45) | 48.32 ± 9.87 (50.85) | 50.39 ± 11.08 (53.20) |
| Bicalutamide (150 mg) vs. triptorelin (3.75 mg) | | | | | | | |
| Serum CgA differences (*P* value) | 0.2837 | 0.8609 | 0.5430 | 0.4832 | 0.0433 | 0.0100 | 0.0025 |

*Abbreviations as in Table I.*
*Statistical significance of differences in CgA levels between bicalutamide and triptorelin groups is reported (P value).*
*Data presented as the mean ± SD, with the median in parentheses.*

was greater than 0.4 ng/mL, a level considered indicative of residual disease[18] and a patient type and PSA level at which significant variations in serum CgA levels during hormonal therapy can be effectively analyzed.[13] Only patients who responded successfully to the first 24 months of therapy without additional biochemical or clinical progression were considered. Therefore, the direct effect of the two hormonal therapies on the CgA serum levels was analyzed, regardless of the response to therapy. Variations in the CgA levels in relation to the clinical response to bicalutamide or castration were not analyzed.

Castration produced a significant and progressive increase in serum CgA levels. This trend and rate of increase was comparable to that reported in our previous analysis of patients with pT3pN0M0 disease and biochemical progression undergoing CAD.[13] The selection of therapy-responsive patients, with PSA-only progression and with very low PSA levels during therapy, increases the significance of our results. Therefore, even in patients with a favorable prognosis and regardless of the response to therapy, castration produced a statistically significant increase in CgA levels despite stable and low PSA levels. Importantly, the increase in CgA level was independent of any variations in the PSA level, as previously reported.[1,19] CgA may, therefore, represent a useful index of progression regardless of the PSA level.

After castration, a 60% median increase in serum CgA levels was observed after 24 months, which was both statistically significant and clinically relevant. In contrast, bicalutamide monotherapy produced a significantly lower increase. The difference between the two therapies, although lower than that described in a study comparing intermittent and continuous CAD,[13] was statistically significant even after 12 months. As previously reported,[1,12,13,20] the effect of androgen deprivation on CgA levels occurred rapidly and was sustained with time.

These different effects on the serum CgA levels may be related to the different mechanisms of action of the two compounds. Castration suppresses gonadotropin secretion, producing castrate testosterone levels. In contrast, bicalutamide blocks the androgen receptor peripherally and, by inhibiting negative feedback of the gonadal steroids, testosterone and estradiol levels are maintained. One may speculate that castrate testosterone levels induce NE cell hyperactivation and therefore significant increases in the CgA levels. In contrast, maintenance of testosterone levels during bicalutamide monotherapy may reduce this risk. Importantly, castrate testosterone levels may induce a reduction in androgen receptor expression. This mechanism may explain the observed differences in NE system

activity; however, the opposite effect of castration and bicalutamide on the hypothalamic-pituitary axis may also result in different effects on the NE system and serum CgA levels.

A possible limitation of our study was that we only measured the serum CgA levels. We have previously reported a statistically significant correlation between serum CgA levels and tissue mRNA CgA expression in prostate cancer.[1] However, serum levels of NE markers may not be specific to prostate gland activity and an analysis of a large, stratified, patient population may determine whether a prostate cancer-specific serum marker exists. In the present study, prostatic tissue samples were obtained at RRP in 15 patients and analyzed by reverse transcriptase-polymerase chain reaction for CgA and PSA mRNA expression. This subgroup was not well distributed between groups (samples obtained before randomization); therefore, these data were not presented. However, CgA mRNA was expressed in all tissue samples and was associated with the serum CgA levels ($r = 0.428$; $P = 0.036$). Modifications in CgA tissue expression during therapy were not analyzed.

In previous studies, NE differentiation and CgA levels represented a statistically significant independent prognostic factor in patients with poorly differentiated tumors.[1,2,13] In the present study, most patients presented with a Gleason score of 7 or greater, reducing the significance of these results. However, patients with baseline Gleason score of 7 or greater had higher CgA levels compared with patients with a Gleason score of less than 7, and, during treatment, a statistically significant difference in CgA levels between treatments was found only in those with a Gleason score of 7 or greater.

## CONCLUSIONS

Our results affirm that different methods of androgen deprivation can influence the serum CgA levels to different extents in prostate cancer. In particular, bicalutamide produces a significantly lower increase in serum CgA compared with castration. In light of other evidence that supports a significant relationship between serum CgA levels, tissue CgA expression, and NE activity, we hypothesize that bicalutamide may reduce the risk of NE cell hyperactivation in prostate cancer.

It is important to determine whether increases in CgA levels and NE cell activation are associated with progression toward hormone-independent prostate cancer. Additional investigation with longer periods of observation and analysis of patients with progression during therapy is warranted, in particular to determine whether a significant increase in CgA levels during therapy is associated with earlier clinical progression compared with stable CgA levels.

## REFERENCES


1. Monti S, Sciarra A, and Falasca P, *et al:* Serum concentrations and prostatic gene expression of chromogranin A and PSA in patients affected by prostate cancer and benign prostatic hyperplasia. J Endocrin Invest **23**(suppl 8): 53–61, 2000.
2. Cohen RJ, Glezerson G, and Haffejee Z: Neuro-endocrine cells: a new prognostic parameter in prostate cancer. Br J Urol **68:** 258–262, 1991.
3. Cohen MK, Arber DA, Coffield KS, *et al:* Neuroendocrine differentiation in prostatic adenocarcinoma and its relationship to tumor progression. Cancer **74:** 1899–1903, 1994.
4. Bonkhoff H: Neuroendocrine cells in benign and malignant prostate tissue: morphogenesis, proliferation, and androgen receptor status. Prostate Suppl **8:** 18–22, 1998.
5. Angelsen A, Syversen U, Stridsberg M, *et al:* Use of neuroendocrine serum markers in the follow-up of patients with cancer of the prostate. Prostate **31:** 110–117, 1997.
6. Abrahamsson PA: Neuroendocrine differentiation in prostatic carcinoma. Prostate **39:** 135–148, 1999.
7. Abrahamsson PA: Neuroendocrine differentiation and hormone-refractory prostate cancer. Prostate Suppl **6:** 3–8, 1996.
8. Krijnen JL, Jansen PJ, Ruizeveld de Winter JA, *et al:* Do neuroendocrine cells in human prostate cancer express androgen receptors? Histochemistry **100:** 393–398, 1993.
9. Jongsma J, Oomen MHA, Noordzij MA, *et al:* Androgen independent growth is induced by neuropeptides in human prostate cancer cell lines. Prostate **42:** 34–44, 2000.
10. Kadmon D, Thompson T, Lynch G, *et al:* Elevated plasma chromogranin A concentrations in prostatic carcinoma. J Urol **146:** 358–364, 1991.
11. Krijnen JL, Bongdanowicz J, Seldenrijk CA, *et al:* The prognostic value of neuroendocrine differentiation in adenocarcinoma of the prostate in relation to progression of disease after endocrine therapy. J Urol **158:** 171–174, 1997.
12. Ahlgren G, Pedersen K, Lundberg S, *et al:* Regressive changes and neuroendocrine differentiation in prostate cancer after neoadjuvant hormonal treatment. Prostate **42:** 274–279, 2000.
13. Sciarra A, Voria G, Gentile V, *et al:* Effect of intermittent versus continuous androgen deprivation therapy on chromogranin A expression in prostate cancer cases. Prostate **55:** 168–179, 2003.
14. Union Internationale Contre le Cancer: *TNM klassification maligner Tumoren*, 5th ed. Berlin, Springer, 1997.
15. Iversen P, Tyrrell CJ, Kaisary AV, *et al:* Bicalutamide monotherapy compared with castration in patients with nonmetastatic locally advanced prostate cancer: 6.3 years of followup. J Urol **164:** 1579–1582, 2000.
16. See WA, McLeod D, Iversen P, *et al:* The Bicalutamide Early Prostate Cancer Program: demography. Urol Oncol **6:** 43–47, 2001.
17. Wirth M, Tyrrell C, Wallace M, *et al:* Bicalutamide (Casodex) 150 mg as immediate therapy in patients with localized or locally advanced prostate cancer significantly reduces the risk of disease progression. Urology **58:** 146–151, 2001.
18. Lightner DJ, Lange PH, Reddy PK, *et al:* Prostate specific antigen and local recurrence after radical prostatectomy. J Urol **144:** 921–926, 1990.
19. Berruti A, Dogliotti L, Mosca A, *et al:* Circulating neuroendocrine markers in patients with prostate carcinoma. Cancer **88:** 2590–2596, 2000.
20. Helpap B, Kollermann J, and Oehler U: Neuroendocrine differentiation in prostatic carcinomas: histogenesis, biology, clinical relevance, and future therapeutical perspectives. Urol Int **62:** 133–138, 1999.