Neuroendocrine Differentiation in Castration-Resistant Prostate Cancer: A Systematic Diagnostic Attempt

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Abstract

We propose a composite and reproducible neuroendocrine differentiation (NED)—assessing panel—including plasma chromogranin A (p-CgA), tissular CgA (t-CgA), somatostatin receptor 2 (SSTR2), and Ki-67—of needle biopsy specimens to be applied to patients with castration-resistant prostate cancer (CRPC). In our series, a high prevalence (85.1%) of NED was found. Neuroendocrine markers were associated with high prostate-specific antigen (PSA) levels, aggressive (high initial Gleason Score) and rapidly progressive disease, and consequent decreased overall survival (OS).

Background: Assessing the neuroendocrine (NE) pattern in castration-resistant prostate cancer (CRPC) may prove useful in selecting potential responders to target therapies such as somatostatin analogues. The aim of this study was to define a panel of markers or examinations appropriate to characterize NE differentiation (NED).

Methods: Forty-seven patients with CRPC underwent a systematic diagnostic attempt to characterize the NE phenotype using a plasma blood test for chromogranin A (CgA) and immunohistochemical staining of needle biopsy–obtained specimens (CgA, somatostatin receptor 2 [SSTR2], Ki-67, and androgen receptors). In a subgroup of 26 patients, somatostatin receptor scintigraphy using 111In-DTPA-d-Phe octreotide (octreotide scintigraphy; Octreoscan, Covidiens, Hazelwood, MO) was also performed. Results: NED was found in 85.1% of patients (if serum CgA, tissular CgA, and tissular SSTR2 were considered separately: 54%, 67%, and 58%, respectively). Only 15% of the 26-patient subgroup had an abnormal octreotide scintigraphy result. Although p-CgA and t-CgA were associated with more aggressive disease with a worse prognosis, patients with positive tissular SSTR2 staining had longer overall survival (OS).

Conclusion: This systematic approach to explore the NED in a quite homogeneous group of patients with CRPC seems reproducible and appropriate. Further investigations are required to validate this panel and better characterize potential responders to targeted therapy.
**Introduction**

The potential prognostic and therapeutic implications of neuroendocrine differentiation (NED) in prostate cancer is an important and timely topic. Neuroendocrine (NE) cells generally have a focal distribution in a population of predominantly adenocarcinoma cells (except for rare cases of total neuroendocrine differentiation (NED)—ie, small cell carcinoma or carcinoid—) and serum or tissue levels of NE cell products might reflect their activity. Chromogranin A (CgA) is the most widely used serum and tissue marker of NED. The reported detection rate of NED in patients with prostate cancer varies between 10% and 100%, depending on the definition, methods, and disease setting. Considering only castration-resistant prostate cancer (CRPC), Cabrespine and colleagues reported increased plasma CgA levels in 45% of patients, whereas Casella and associates, in examining tissue obtained from autopsies in a subgroup of 15 patients, demonstrated the presence of tissular CgA (t-CgA) in 80% of the patients in their series.

The NED and NE cell hyperactivation may play a key role in the pathogenesis of CRPC through bioactive neuropeptide production and secretion. Because chemotherapy minimally improves survival of patients with CRPC, there is increasing interest in exploring other therapies. In fact, new targeted chemotherapeutic agents based on somatostatin analogues and cytotoxic somatostatin conjugates are under development. As a result, careful determination of the presence of NED, with identification of subgroups of potential responders to these therapies, seems to be an important goal.

The role of NED markers as prognostic indicators in prostate cancer is controversial. Some authors have found an association between NED and a worse prognosis, whereas others failed to find this relationship. Previous studies trying to characterize NED in prostate cancer generally had some methodologic limitations: heterogeneous disease stage, a heterogeneous specimen method of collection (transurethral resection, core biopsy, radical prostatectomy), and a heterogeneous NED markers panel. As a result, it is difficult to simply transfer their conclusions to a CRPC setting. The objective of this study was to test the prevalence and the prognostic significance of NED using a simple panel of specific immunostaining of core needle biopsy specimens, plasma levels of CgA and somatostatin receptor scintigraphy (octreotide scintigraphy; Octreoscan, Covidi, Hazelwood, MO) in a group consisting of only patients with CRPC.

**Material and Methods**

From January 2003-December 2006, 47 patients with documented CRPC underwent transperineal, ultrasonographically guided sextant prostate biopsies and blood sample collection. CRPC was defined as 3 or more successive prostate-specific antigen (PSA) measurements rising past the nadir, with castrate testosterone levels after androgen withdrawal for at least 4 weeks and/or radiologic progression. All patients were maintained on androgen-deprivation therapy (ADT) with a luteinizing hormone–releasing hormone analogue. In all but 3 patients, oral estramustine plus etoposide-based chemotherapy was ongoing from the moment of study enrollment. Twenty-six patients underwent in vivo somatostatin receptor scintigraphy with octreotide. After NED evaluation, a subgroup of 16 patients in biochemical and/or clinical progression underwent somatostatin analogue plus oral estradiol associated therapy.

**Laboratory Findings**

p-CgA was measured with an enzyme-linked immunosorbent assay kit (Dako A/S, Glostrup, Denmark). The normal range of CgA concentrations was defined based on assays of blood samples from normal healthy individuals and corresponded to 20 U/L.

**Pathologic Examination**

Tissue specimens were obtained with a 16-gauge Tru-cut biopsy needle and were fixed in formalin and embedded in paraffin. For each case, archival hematoxylin and eosin sections and paraffin blocks were retrieved. Hematoxylin and eosin staining was used to determine the presence of prostate cancer cells, histologic type, and the extent of involvement by cancer (number of cores and percentage). Patients were subdivided into low, intermediate, and high grade according to the Gleason score (Gleason sum score: ≤ 6, 7, and ≥ 8, respectively).

**Immunohistochemical Evaluation**

Immunohistochemical study was performed on paraffin-embedded tissues of abnormal core biopsy specimens. A panel of commercially available primary monoclonal antibodies was used: CgA (Signet Laboratories, Dedham, MA), Ki-67 antigen (MB-1, Immunotech, Marseille, France), PSA (Immunotech, Marseille, France), androgen receptor (Oncogene Science, Cambridge, MA) and somatostatin receptor (BIOTREND Chemicals, Köln, Germany). After blocking endogenous peroxidase with 5% hydrogen peroxide for 12 minutes, dewaxed sections were caused to react with the primary antibodies for 30 minutes at room temperature using an automatic immunostainer (Dako Autostainer, Dako, Glostrup, Denmark) and then incubated with a high-sensitivity detection kit according to the manufacturer’s instructions (Dako EnVision+ System-HRP, Dako, Glostrup, Denmark). The specificity of all immunoreactions was double-checked by substituting the primary antibody with either a nonrelated, isotypic mouse immunoglobulin at a comparable dilution or with normal serum alone. Appropriate internal positive controls were also checked in all reactions.

The percentage of immunoreactive tumor cells for each marker (labeling index) was evaluated independently and blindly by counting (when present) at least 1000 tumor cells in representative fields of immunostaining using a standard light microscope. Evaluations of immunohistochemical staining were reviewed by 2 pathologists counting each sample 3 times; thus the highest score (ie, percentage) for each antibody and the highest score between the 2 observers were reported. Stromal chromogranin was reported as absent or present. The percentage of cytoplasmic/chromogranin–positive cells was evaluated semiquantitatively on a scale from 0 to 3 (0 = staining absent or observed in less than 5% of neoplastic cells; 1 = staining in 5%-25% of neoplastic cells; 2 = staining in 25%-50% of neoplastic cells; and 3 = staining in > 51% of neoplastic cells). Also, staining intensity and number of cells in each hot spot were recorded. The SST2R was recorded as positive if staining was identified in the cytoplasm or membrane of tumor cells and the percentages of positive cells were reported with a cutoff of 10%. We also verified the staining for SST2R within the stromal area.
Neuroendocrine Differentiation in CRPC Patients

**Somatostatin Receptor Scintigraphy**

Octreotide scintigraphy was performed on 26 patients (55%). The labeling of the Octreoscan was performed according to instructions provided by the manufacturer (Mallinckrodt Medical BV, Petten, Netherlands). Whole-body images were obtained at 4 and 24 hours after the injection of 110-150 MBq of $^{111}$In-[DPTA-d-Phe]$^1$-octreotide. A double-headed gamma camera, equipped with a medium-energy collimator was used (Infinia, GE Healthcare, Waukesha, WI). The collection of original data for single-photon emission computed tomography (SPECT) images was performed with a 64-step 360-degree rotation in a 64 × 64 word matrix. Energy windows of 171 and 245 keV + 20% were used. The collection time for each angle was 60 seconds, amounting to approximately 40,000 counts/angle. For the reconstruction of SPECT images, a Butterworth filter was applied to the original data.

The study was approved by the institutional review board of the hospital and all patients gave written informed consent.

**Statistics**

The $\chi^2$ test, the Fisher exact test, or the Mantel-Haenszel $\chi^2$ test for ordinal variables was used to compare percentages between groups. Medians were compared using the nonparametric 2-sample median test. If appropriate, log-transformation of values was performed in linear regression models. Overall survival was defined as the time interval from the date of hormone refractoriness to death or to last visit. It was plotted using the Kaplan-Meier method. The log-rank test was used to assess survival differences between groups in the univariate analysis. Multivariate Cox proportional hazards regression models were used to identify the prognostic independent clinicopathologic features associated with survival. All analyses were performed with SAS software (SAS Institute, Cary, NC). All tests were 2-sided.

**Results**

Patients’ clinical characteristics, plasma CgA (p-CgA) and immunohistochemical NED features are shown in Table 1. The median duration of the hormone sensitivity period was 16.5 months, whereas the median time from castration-resistant status to the NED evaluation was 8 months. Biopsy specimens showed tumors in 39 patients (83%).

**Prevalence**

If p-CgA was considered, 25 patients (54%) could be defined as having NED. Considering immunohistochemical findings, 26 patients (67% of patients with abnormal biopsy specimens) had positive tissue CgA (t-CgA) (Figure 1), whereas 22 (58% of patients with abnormal biopsy specimens) showed tissue presence of SSTR2 (Figure 2). Only half of this group (ie, 11 patients) had a relatively strongly (>10%) expressed SSTR2 pattern (Figure 2B); evidence of stromal presence of SSTR2 was noted in only 6 patients.

The androgen receptor was positive in neoplastic cells in 20 patients (53%) (Figure 3), and the median observed proliferation rate index Ki-67 was 10% (range 1-80) (Figure 4).

**Correlations Between NE Markers and Clinical Data**

Although elevated p-CgA was associated with high PSA values at the time of NED evaluation, with a median of 25 ng/mL for p-CgA ≤ 20 U/L and 50 ng/mL for p-CgA > 20 U/L (2P = .04), no other associations were found, even if PSA level and Gleason score at diagnosis, the presence of metastasis, or the duration of ADT were considered (Table 1).

The presence of cytoplasmic CgA-positive neoplastic cells (t-CgA) was associated with higher Gleason scores at diagnosis: 50% of patients with positive t-CgA cells had Gleason scores ≥ 8% vs. 11% in the group with negative t-CgA cells (2P = .03). Median duration of ADT was 36 months for patients with no t-CgA–positive cells, 18 months for patients with < 5% positive cells, and 23 months for those with ≥ 5% positive cells (level 2 + 3 on t-CgA positivity scale) (2P = .02, comparing level 0 with levels 1-3). Median duration of castration-resistant disease until the NED evaluation was 13 months for patients with no t-CgA–positive cells, 4 months for patients with the first level of t-CgA cell positivity, and 7 months for those with level 2 or 3 on the positivity scale (2P = .05, comparing no cells with scale 2-3 positive cells). The t-CgA was not associated with PSA at diagnosis, initial treatment, presence of metastasis, or actual PSA level.

The presence of SSTR2-positive neoplastic cells was not associated with any of the clinical evaluated parameters.

**Correlation Between NE Markers**

Association between p-CgA and immunohistochemical parameters is shown in Table 2.

Some correlation between the presence of t-CgA–positive cells and elevated (≥ 20 U/L) p-CgA levels was found, although statistical significance (2P = .09) was not reached. The percentage of positive t-CgA cells, staining intensity, number of cells per hot spot, and the presence of stromal CgA were not associated with the level of p-CgA.

The presence of SSTR2 showed no association with p-CgA levels or with t-CgA cell positivity.

There was a trend of positive correlation involving p-CgA and percentage of androgen receptor–positive neoplastic cells (2P = .064) (Figure 5).

**Correlation Between NE Markers and Clinical Course**

Median follow-up was 32 months (5-72 months) after the diagnosis of CRPC. Five-year overall survival (OS) was 50% if considered from the day of the beginning of ADT and 15% if considered from the onset of the CRPC. Considering a cutoff level of 20 U/L for p-CgA, the difference in OS after the diagnosis of CRPC did not reach statistical significance. Stratifying patients in quartiles (Q1 = CgA < 14; Q2 = 14 ≤ CgA < 24; Q3 = 24 ≤ CgA < 36; Q4 = CgA ≥ 36) values of p-CgA ≥ 36 U/L were associated with a modest decrease in OS (Figure 6), even when adjusted for age, PSA level, metastatic status, Gleason score, and the use of analogues of somatostatin (hazard ratio [HR] Q4 vs. others, 1.8; 95% confidence interval [CI], 1.3-2.5). The presence of cytoplasmic CgA-positive cells (t-CgA) was associated with a reduction in OS after CRPC diagnosis, but this difference did not reach statistical significance (Figure 7). In univariate analysis, tissue evidence of SSTR2 was associated with better survival in patients with CRPC. This tendency lost its statistical significance when data were adjusted according to age, PSA level, Gleason score, and metastatic status (HR, SSTR2-negative vs. SSTR2-positive, 2.0 (95% CI, 0.9-4.3) (Figure 8).
In Vivo NED Evaluation

Four of the 26 patients who underwent somatostatin receptor scintigraphy with octreotide (ie, 15%) showed in vivo presence of SSTR, matching bone and lymph node metastases in 3 patients and 1 patient, respectively. All patients with an abnormal result on scintigraphy had good expression of SSTR2 (20%, 40%, 40%, and 90%).

Discussion

Detection of NE activity and definition of the presence of NED in patients with prostate cancer remains controversial in the literature. NE cells are found among a predominant population of non-NE malignant cells, except for rare cases of total NED carcinomas, ie, small cell carcinoma or carcinoid.10 None of the patients in our series had a pure NE prostate carcinoma.

The prevalence of NED in prostate cancer as reported in the literature varies from 10%-100%.4-10 To our knowledge, this is the first study considering a composite panel of examinations, including blood sample, core needle biopsy, and somatostatin receptor scintigraphy with octreotide to define the presence of NED and its prognostic implications in a setting of only patients with CRPC.

According to this panel, the overall prevalence of NED in our series was 85.1% (35/47 having p-CgA > 20, 10/47 with some t-CgA positivity having normal p-CgA levels, and 5/47 showing only SSTR2 cell positivity). Somatostatin receptor scintigraphy revealed NED in 15% of the patients, but only in those having already showed SSTR2 positivity on biopsy specimens. This high prevalence of NED in patients with CRPC might prove useful for the development of new treatment modalities and strategies.

Table 1  Clinical Characteristics and Relationship With Serum and Immunohistochemical NED Analysis

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>Total N=47</th>
<th>Plasma CgAa</th>
<th>Cytoplasmic Positive CgAb</th>
<th>SSTR2a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 20 N = 21 (46%)</td>
<td>&gt; 20 N = 25 (54%)</td>
<td>P</td>
<td>≤ 5% N = 14 (36%)</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>66 (53–87)</td>
<td>65 (53–87)</td>
<td>66 (53–79)</td>
<td>.36</td>
</tr>
<tr>
<td>Initial PSA Levela</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>52 (4–2.500)</td>
<td>40 (4–1280)</td>
<td>56 (13–2500)</td>
<td>.17</td>
</tr>
<tr>
<td>Gleason Scorea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 6</td>
<td>11 (31)</td>
<td>6 (35)</td>
<td>5 (29)</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>10 (29)</td>
<td>4 (24)</td>
<td>5 (29)</td>
<td>.70</td>
</tr>
<tr>
<td>≥ 8</td>
<td>14 (40)</td>
<td>7 (41)</td>
<td>7 (41)</td>
<td>.17</td>
</tr>
<tr>
<td>Initial Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT + HT (%)</td>
<td>15 (32)</td>
<td>7 (33)</td>
<td>7 (28)</td>
<td>.70</td>
</tr>
<tr>
<td>HT (%)</td>
<td>32 (68)</td>
<td>14 (67)</td>
<td>18 (72)</td>
<td>.12</td>
</tr>
<tr>
<td>Duration of ADT (Months)a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>29 (9–198)</td>
<td>28 (13–95)</td>
<td>29 (9–198)</td>
<td>.87</td>
</tr>
<tr>
<td>Duration of hrpC Until NED Evaluation (Months)a</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median (Range)</td>
<td>8 (0–37)</td>
<td>9 (0–24)</td>
<td>8 (0–37)</td>
<td>.55</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>41 (87)</td>
<td>19 (90)</td>
<td>22 (88)</td>
<td>1.0</td>
</tr>
<tr>
<td>Actual PSA Level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>36 (2–5580)</td>
<td>25 (6–1175)</td>
<td>50 (4–5580)</td>
<td>.04</td>
</tr>
</tbody>
</table>

Abbreviations: ADT = androgen deprivation therapy; CgA = chromogranin A; CRPC = castration-resistant prostate cancer; HT = hormone therapy; NED = neuroendocrine differentiation; RT = radiation therapy; PSA = prostate-specific antigen; SSTR2 = somatostatin receptor subtype 2.

a One or more than 1 patient had missing data.
b Calculated on 39 patients with positive biopsy.
c 0 cells vs. ≥ 1 cell.
In our series consisting of only patients with CRPC, an elevated p-CgA level was associated with higher PSA values at the moment of NED evaluation. Previous studies failed to show this relationship in patients with CRPC. In fact, as reported by Sasaki et al, during endocrine therapy, p-CgA elevation and an increase in PSA levels are associated.

The appearance of CgA cell positivity in patients showing higher initial Gleason scores might indicate that tumors exhibiting more aggressive patterns initially are supposed to develop NED more easily, confirming data from previous studies, including patients in different stages of disease. In fact, patients with no evidence of t-CgA in our series had a better previous clinical course, which translated to longer duration of ADT and longer duration of the castrate resistance, whereas patients with disease that was rapidly progressing toward a castration-resistant stage extensively expressed NE features. As a consequence, it seems that the duration of ADT is more than an influence in determining a NED; it seems that the NED determines or characterizes a more aggressive prostate cancer pattern.

In our patients, the presence of t-CgA–positive neoplastic cells correlates somehow with the level of p-CgA even if it does not reach statistical significance. Angelsen et al demonstrated an association between serum and tissue markers of NED in a small series, with less than half of patients receiving ADT. By analyzing core needle biopsy specimens from patients with newly diagnosed prostate cancer, Bollito et al found a correlation between p-CgA and t-CgA, particularly in metastatic disease.

Prostatic NE cells are fully differentiated postmitotic cells with no proliferative activity and are negative for Ki-67. The proliferation index Ki-67 of cancer cells results were high (10%) in our series.
Proliferation index of neoplastic cells adjacent to NE cells is increased, as demonstrated in previous reports. Jongsma et al. showed that the proliferation of prostate cancer cell lines in conditions of androgen depletion can be modulated by neuropeptides that have mitogenic effects and are known to be produced by NE cells, thus justifying this high proliferation index. Grobholz et al. found a positive association between Ki-67 index and NED in prostatectomy specimens. In contrast, Ishida et al. and Casell et al. did not find this association in tissue obtained from radical prostatectomies and core needle biopsies of untreated patients.

Immunohistochemical studies showed that the androgen receptor is not expressed in prostatic NE cells, and NED has been proposed as a contributing factor for the development of CRPC. Besides this, high levels of androgen receptor expression and development of hypersensitive receptors has been recognized as another feature associated with the development of CRPC. The prevalence of androgen receptor–positive cells in our series is high (53%), confirming the hypersensitive/overexpression androgen receptor pathway for the development of CRPC. Interestingly, we found a positive relationship between p-CgA and the presence of androgen receptor–positive neoplastic cells, arguing for the possible proliferative action of factors produced by NE cells in adjacent exocrine (androgen receptor positive/overexpressed) neoplastic cells.

Data on the prognostic impact of NED in CRPC are conflicting. High CgA levels are associated with a poorer prognosis in patients with CRPC, as demonstrated by Taplin et al. and Berruti. However this finding was not confirmed by Cabrespine et al. Our results show that high levels of p-CgA were associated with a modest reduction in OS, with statistical significance. A more aggressive course of disease in patients with NED could be determined by the capacity of NE-differentiated prostate cancer cells to escape apoptosis. NED cells can survive and continue acting in the androgen-deprived environment, producing and secreting potent factors that promote cell proliferation.
neuropeptides that stimulate cell growth, differentiation, transformation, and invasion.39

The negative prognostic value of some NE tissue markers is well documented, even if obtaining tissue samples that adequately represent the entire prostate tumor might be a challenging and unsolvable issue. In fact t-CgA positivity was associated with a worse prognosis if considering tissues obtained from radical prostatectomies; core needle biopsy specimens from untreated patients.42

<table>
<thead>
<tr>
<th>Immunohistochemical Parametersa</th>
<th>Total No. 47</th>
<th>Plasma CgA ≤ 20 No. 21</th>
<th>Plasma CgA &gt; 20 No. 25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stromal CgA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>42 (89)</td>
<td>19 (90)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Present</td>
<td>5 (11)</td>
<td>2 (10)</td>
<td>3 (12)</td>
</tr>
<tr>
<td><strong>Cytoplasmic CgA Positive Cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 cells</td>
<td>13 (33)</td>
<td>9 (47)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>&lt;5%</td>
<td>14 (36)</td>
<td>6 (32)</td>
<td>8 (42)</td>
</tr>
<tr>
<td>&gt;5%</td>
<td>12 (31)</td>
<td>4 (21)</td>
<td>7 (37)</td>
</tr>
<tr>
<td><strong>SSTR2c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>16 (42)</td>
<td>7 (37)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>+</td>
<td>22 (58)</td>
<td>12 (63)</td>
<td>10 (56)</td>
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<td><strong>SSTR2-Positive Cellsc</strong></td>
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<tr>
<td>0</td>
<td>16 (42)</td>
<td>7 (37)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>&lt;10%</td>
<td>11 (29)</td>
<td>5 (26)</td>
<td>6 (33)</td>
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<tr>
<td>&gt;10%</td>
<td>11 (29)</td>
<td>7 (37)</td>
<td>4 (22)</td>
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<td><strong>Stromal SSTR2c</strong></td>
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<td>−</td>
<td>32 (84)</td>
<td>15 (79)</td>
<td>16 (89)</td>
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<tr>
<td>+</td>
<td>6 (16)</td>
<td>4 (21)</td>
<td>2 (11)</td>
</tr>
<tr>
<td><strong>Ki67 (%)c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>10 (1–80)</td>
<td>10 (1–31)</td>
<td>15 (1–80)</td>
</tr>
</tbody>
</table>

*Calculated on 39 patients with positive biopsy.
First category versus others.
One or more than one patient had missing data.
Abbreviations: CgA = chromogranin A; SSTR2 = somatostatin receptor subtype 2.
patients, those with metastatic disease, those with stage D2 disease, or patients with newly diagnosed prostate cancer. In our series as well, the presence of t-CgA characterized (statistically significant) a more rapid progression toward CRPC and was associated (without reaching a statistical significance) with a poorer prognosis. In this regard, our study seems the first to suggest a possible negative prognostic value of t-CgA in a setting of only CRPC patients. By contrast, the tissular evidence of SSTR2 correlated (without a statistical significance) with an improved survival, advocating once more for the existence of different aspects or phases of NED in CRPC.

Radiolabeled analogues of somatostatin, such as $^{111}$In-octreotide scintigraphy are used clinically for the localization of tumors expressing receptors for somatostatin. Studies on the use of somatostatin receptor scintigraphy with octreotide in CRPC are scarce. Nilsson et al found some positive results in 94% of 31 patients with CRPC, but only 37% of all bone metastases detected by $^{99m}$Tc-labeled bone scan presented some $^{111}$In-octreotide uptake. More recently, series of CRPC presented lower rates of octreotide positivity: 85% of 20 cases, all with low intensity uptake, and only 37% of 20 cases. We have found a low rate (15%) of octreotide positivity. It has to be noted that all patients with somatostatin receptor scintigraphy with octreotide positivity had good expression of SSTR2 in the neoplastic tissue, indicating that with a positive scan, a high expression of SSTR2 should be assumed.

Our study has some limitations, imposing caution in data interpretation. First, the patient sample size is small and because prostatic tissue was needed for immunohistochemical analysis, patients who underwent radical prostatectomy were excluded, thus adding another selection bias.

Second, as the aim of the study was to set up a composite, representative (ie, appropriate) but also easy to perform NE-assessing panel, we deliberately chose the prostatic core needle biopsy (despite the limited obtainable tissue) and excluded different NED markers, such as neuron-specific enolase, synaptophysin, or chromogranin B or others subtypes of the SSTR family. This choice (of performing prostate biopsy only) determined the lack of data comparing pathologic features of the primary site with those of the metastatic sites. The heterogeneity of bone and extrasosseous metastasis in end-stage prostate cancer is known and reported in the literature. This fea-
ture theoretically might be explained in 2 ways: (1) each "metastasis" is a "collision" of independent clones or (2) the final heterogeneity of the cell population arises from the acquired mutations of one castration-resistant clone. Since patients in our study did not have end-stage disease and assuming the second hypothesis, the previously mentioned heterogeneity should not have been expressed to a great extent at the time of inclusion in the study. Systematic data of pathologic examination of metastases would have been useful to support the hypothesis that tumor within the prostate reflects the castration-resistant clone. The correlation of somatostatin receptor scintigraphy with octreotide and a high percentage of RSST2-positive cells in the prostate supports this hypothesis, but this patient subgroup is quite small.

Finally, the fact that most of the patients were receiving oral chemotherapy may represent another bias. These patients were a subgroup of another series, studied prospectively. They started the oral chemotherapy no more than 4 weeks after having been given castration-resistant status. The duration of the castration resistance phase and that of the oral chemotherapy were thus proportional. Even if there are no reports about the possible effects of estramustine and etoposide on prostate cancer cells, some interference with NE cells might not be excluded. In fact, patients with no t-CgA had a longer duration of the castration resistant phase and as a consequence had to undergo more cycles of oral chemotherapy. Thus even if the absence of t-CgA in these patients might have been a consequence of the estramustine phosphate/etoposide oral chemotherapy, we believe that the presence or absence of NED should determine or characterize a more or less aggressive prostate cancer pattern with short or longer response to the androgen deprivation and consequently to the previously mentioned oral chemotherapy regimen.

**Conclusion**

In a setting of only patients with CRPC, a high prevalence (85.1%) of NED was found. NED markers were associated with high PSA levels, more aggressive disease (ie, high initial Gleason score), and disease that was rapidly progressing toward castration resistance, with consequent decreased OS.

NED was assessed using a composite and easy to perform multiindicator panel, including p-CgA immunohistochemical staining for CgA, SSTR2, and proliferation rate (Ki-67) of core needle biopsy specimens. Since somatostatin receptor scintigraphy with octreotide was positive in a very small subgroup of patients, its inclusion in this NE-assessing panel is questionable.

These findings need to be confirmed in larger studies to better understand the prognostic value of different NE markers. Moreover, further investigation should be addressed to point out potential candidates for somatostatin analogue–based target therapies.

**Clinical Practice Points**

- NED was assessed using a multidiagnostic panel, including p-CgA immunohistochemical staining for CgA, SSTR2, and proliferation rate (Ki-67) of core needle biopsy specimens. In more than half of the patients, somatostatin receptor scintigraphy with octreotide was carried out.
- In a setting of only patients with CRPC, a high prevalence (85.1%) of NED was found. NED markers were associated with high PSA levels, more aggressive (ie, high initial Gleason score) disease that was rapidly progressing toward castration resistance with consequent decreased OS.
- Since chemotherapy minimally improves survival of patients, new targeted chemotherapy agents based on somatostatin analogues and cytotoxic somatostatin conjugates are under development. A systematic, appropriate, and easy to perform panel of investigation (as the 1 presented in our study) addressed to define NED in patients with CRPC should improve identification of subgroups of potential responders to these therapies.

**References**


