Relationship between neuroendocrine features and prognostic parameters in human prostate adenocarcinoma

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Summary

Purpose: The biological behaviour of prostate cancer is highly variable and prediction by the commonly employed prognostic parameters is not sufficient. The concept of neuroendocrine (NE) differentiation in prostate adenocarcinoma has recently received increasing attention due to possible implications for prognosis and therapy.

Materials and methods: Core needle biopsies from 142 newly diagnosed patients were immunohistochemically examined for the coexistence of NE differentiation using an antibody against chromogranin A (CgA). Circulating CgA was available in 106 of these patients.

Results: NE differentiation was found in 64 (45.1%) tumors. Among them 29 (20.4%) had CgA positive cells scattered or focally distributed in less than 5% per mm³ of tumor tissues, 26 (18.3%) between 5% and 10% and 9 (6.4%) more than 10%, respectively. There was a significant correlation between the extent of NE features and either Gleason score \( P < 0.01 \) or stage of disease. Circulating CgA but not PSA correlated with immunohistochemical CgA \( P < 0.03 \) particularly in metastatic cases.

Conclusions: These data support the concept that NE differentiation in human prostate cancer has a negative prognostic significance. Circulating CgA levels reflect immunohistochemical findings.

Key words: chromogranin A, neuroendocrine differentiation, prostate carcinoma

Introduction

Prostate carcinoma exhibits remarkably variable biologic behaviour ranging from small, well-differentiated, localized carcinomas that are incidental findings at autopsy to large, high-grade carcinomas that frequently metastasize and cause death.

On these grounds, it is of paramount importance to establish validated prognostic indicators that could help clinicians in tailoring adequate treatment for each patient.

Histology grade, pathologic stage and prostate-specific antigen (PSA) serum levels are widely recognized prognostic factors for newly diagnosed prostate cancer patients. These indicators, however, are unable to predict the course of all diagnosed tumors, as many malignancies are not homogeneous for these variables and could exhibit marked clinical disparity. The development of further prognostic indicators is therefore desirable. New prognostic parameters, however, might be useful if applicable in core needle biopsies, the currently preferred diagnostic procedure.

Several authors during the past decades have demonstrated that a substantial proportion of primary and metastatic prostate tumors contains a dispersed subpopulation of malignant cells expressing neuroendocrine (NE) features [1-4]. The clinical significance of NE differentiation within classic prostate adenocarcinoma with acinar or ductal pattern is still a matter of debate. Many review articles by most experts in this field [5, 6] suggest a significant role for NE differentiation in prostate cancer progression, which is supported by the following evidence: 1) regulation of cellular proliferation and metastatic potential of prostate carcinoma by NE factors, 2) association of NE differentiation with androgen-independent prostate cancer, and 3) adverse influence of NE differentiation on patient survival. This evidence, notwithstanding a number of clinical studies, led to contradictory results. In two studies of clinically localized carcinoma of the prostate, for example, NE differentiation was not a statistically significant independent prognostic factor [7, 8]. This is in contrast with others studies that show prostate cancer with extensive NE differentiation to behave aggressively and to be associated with poor prognosis [9-12]. Noordzji et al. [7] did not detect any association between NE differentiation and tumor grade, but others have found the opposite [13, 14]. In two studies chromogranin A (CgA) expression was associated with an advanced stage of disease [15, 16], whereas other studies did not confirm...
these relations with any clinical or pathologic stage [13, 14]. Hitherto, published clinical studies of NE differentiation in prostate cancer, have generally involved relatively small numbers of cases; some studies included unselected cases while others restricted patient recruitment to those candidates for radical prostatectomy. Some tumors studied had undergone prior treatments while others did not. The limited sample size, as well as the heterogeneity of published series, may therefore have contributed to the discrepancies reported.

The NE cells in malignant prostate tissue can be identified immunohistochemically with the use of antibodies against a number of products by these cells. The majority of these products can also be released into the blood stream and measured by immunoassay techniques [16–18]. CgA is the most frequently employed marker used to detect NE features, either at the tissue level or in the general circulation.

In the present study CgA was evaluated either immunohistochemically or in the blood stream in a consecutive series of patients with newly diagnosed prostate cancer. The goals of this study were to search for a relationship between the immunohistochemical CgA expression and the commonly recognized prognostic variables, such as Gleason score, stage of disease and circulating PSA, and to correlate immunohistochemical CgA with CgA circulating levels.

Materials and methods

Patient population

The study was performed on 148 consecutive patients with newly diagnosed prostate cancer.

From 1997 to 1999 these patients presented at the Urologic Department of our hospital with the following features: 1) elevated PSA (> 10 ng/ml) regardless of whether it was associated with urologic symptoms; 2) PSA within the ‘grey zone’ (4–10 ng/ml); 3) PSA in the range of normality (<4 ng/ml) but with clinically suspected prostate cancer after digital rectal and/or ultrasonographic examination; and 4) evidence of metastatic prostate cancer.

In order to avoid possible endogenous interferences, patients with previous or concomitant other neoplastic history, adrenal incidentalomas, hepatic and/or renal impairment, and uncontrolled blood hypertension were excluded from the measurement of circulating CgA. Similarly, patients taking drugs known to alter the metabolism and secretion of CgA, such as nitrates and proton pump inhibitors, were not addressed to the assessment of plasma CgA. Informed consent for the diagnostic procedures was obtained from all patients. At the study entry, the patients underwent physical examination and routine blood chemistry including serum PSA. Bone scan followed by radiologic confirmation of hot spots, chest X-ray, and computed tomography of the whole abdomen were prescribed when indicated on the basis of clinical signs of metastatic disease or in relation to the PSA values. The disease stage was assessed according to the American Urologic Association criteria [19].

Histopathology and immunohistochemistry

Histological specimens of prostate needle-biopsy were examined. All cases were graded according to the Gleason grading system [20]. Immunohistochemistry was performed on paraffin-embedded tissues as follows: a few sections (1–3 for each case) including the neoplastic areas were cut at 5 μm and then rehydrated; paraffin was removed in xylene and graded alcohol. Endogenous peroxidase activity was blocked with 0.3% peroxide in methanol (15 min, at room temperature); the slides were then rinsed three times in distilled water. Primary anti-CgA monoclonal antibodies, clone LK2H10 (by Novocastra Laboratories, Newcastle Upon Tyne, UK), were applied according to the manufacturers’ instruction (1-h incubation at room temperature); the sections were then boiled for 15 min in 10 mmol/1 citrate buffer solution (pH 6.0) using a microwave oven for antigen retrieval, cooled and rinsed in PBS. Finally, slides were counterstained, dehydrated, cleared, and mounted for examination by light microscope with coverslip.

The mAb-antigen binding was demonstrated by means of Dako En Vision™ Plus System (by Dako Corporation, Carpinteria, California) which relies on a dextran polymer conjugated with goat anti mouse Ig and peroxidase. The peroxidase activity was demonstrated with 3,3’-diaminobenzidinetetrahydrochloride (Dako Corporation, Carpinteria, California). The negative control estimates were performed by omitting the use of the primary antibodies.

The positive controls were represented by paraffin sections from tissue samples with known immunoactivity for CgA (bronchial carcioid).

The percent of reactive cells was assessed referring to a total count of 1 × 10³ cells for each section using a standard light microscope equipped with a 10 × 10 square graticule. Reproducibility of counting was assessed by a second investigator.

An immuno-stain for mean-molecular-weight-cytokeratin (MMWCK panel including cytokeratin 13, 14, 15, 16, 17 according to Moll’s classification) was concomitantly performed to exclude NE cells from the count which were present in normal glands that were trapped from the tumor.

Circulating marker measurements

Blood specimens were obtained in the early morning after an overnight fast. Blood for CgA assessment was collected in a fasting state 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 values of CgA were obtained in a group of 87 age-matched healthy male subjects selected among those consecutively presented at the Urology Department of our hospital and having the following characteristics: 1) PSA within the normal range; 2) no clinical suspect of PC by means of digital rectal examination; 3) no hypertension, or controlled hypertension with therapy; and 4) no renal nor hepatic impairment. Normal values were therefore defined as less than or equal to 20 U/l.

Statistical analysis

Differences between groups were tested using the nonparametric Mann–Whitney U-test or Kruskal–Wallis analysis of variance when indicated. Differences in proportions were analyzed by the chi-square test. Multivariate analysis was performed by multiple regression. Statistical analysis was performed on an IBM-compatible personal computer using Statistics for Window software [21].

Results

Among the 148 examined cases, four cases were excluded because the size of the neoplasm visible on the ‘core-biopsy’ was too small for the execution of further sec-
Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age, median (range)</th>
<th>AUA stage</th>
<th>Gleason score</th>
<th>CgA positive cells</th>
<th>Plasma CgA (U/l)</th>
<th>Serum PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>142</td>
<td>69 (52–89)</td>
<td>A</td>
<td>13 (9.2%)</td>
<td>6 (2–10)</td>
<td>106 (74.6%)</td>
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<tr>
<td></td>
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<td>B</td>
<td>71 (50.0%)</td>
<td>6.5 (±1.4)</td>
<td>137 (96.5%)</td>
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<td></td>
<td></td>
<td></td>
<td>C</td>
<td>27 (19.0%)</td>
<td>11.4 (0.1–1110.0)</td>
<td>11.4 (0.1–1110.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D</td>
<td>31 (21.8%)</td>
<td>131 (96.5%)</td>
<td>137 (96.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td>78 (54.9%)</td>
<td>16.0 (3.0–10.0)</td>
<td>10.0 (49.2–333.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 5%</td>
<td>26 (18.3%)</td>
<td>6.5 (±1.34)</td>
<td>15.6 (±9.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5%–10%</td>
<td>29 (20.4%)</td>
<td>6.5 (±1.20)</td>
<td>21.0 (±12.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 10%</td>
<td>9 (6.3%)</td>
<td>7.2 (±1.25)</td>
<td>56.3 (±95.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.2 (±1.64)</td>
<td>123.8 (±255.8)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>5/9 (55.5%)</td>
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</table>

Table 2. Gleason score, plasma CgA and serum PSA according to immunohistochemical CgA expression.

| % CgA positive cells per mm² tumor area | 0% | < 5% | 5%–10% | > 10% | P-value*
<table>
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</thead>
<tbody>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>6.0 (2.0–10.0)</td>
<td>6.5 (4.0–8.0)</td>
<td>7.0 (5.0–9.0)</td>
<td>7.0 (5.0–9.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>6.5 (±1.34)</td>
<td>6.5 (±1.20)</td>
<td>7.2 (±1.25)</td>
<td>7.2 (±1.64)</td>
<td></td>
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<tr>
<td>CgA (U/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>13.0 (3.0–53.7)</td>
<td>18.2 (3.0–51.0)</td>
<td>28.1 (3.0–490.0)</td>
<td>30.0 (7.2–800.0)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>15.6 (±10.4)</td>
<td>21.0 (±12.8)</td>
<td>56.3 (±95.3)</td>
<td>123.8 (±255.8)</td>
<td></td>
</tr>
<tr>
<td>Supranormal values</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>16/52 (30.7%)</td>
<td>9/19 (47.4%)</td>
<td>15/26 (57.7%)</td>
<td>5/9 (55.5%)</td>
<td></td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>13.0 (0.9–1003.0)</td>
<td>10.0 (49.2–333.0)</td>
<td>15.0 (0.1–1110)</td>
<td>7.7 (0.1–716.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>49.1 (±147.1)</td>
<td>32.0 (±66.8)</td>
<td>103.0 (±243.0)</td>
<td>130.0 (±287.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Kruskal–Wallis analysis of variance.

b Chi-square 5.25 (P < 0.03) comparing 57.7% vs. 30.7%.

...cations. Two further cases were excluded due to the morphological and immunohistochemical appearance of 'small-cell cancer' (so-called 'pure NE prostate cancer').

The remaining 142 cases were fully evaluable and were included in the study. The characteristics of these fully assessable patients are outlined in Table 1. Most tumors were organ confined (stage A, B). The core needle biopsies revealed 34 low (23.9%, Gleason score 2–5), 77 intermediate (54.2%, Gleason score 6–7) and 31 high grade (21.8% Gleason score 8–10) prostate cancers.

In the samples of core-biopsy the immunohistochemical stains described above showed no aspect of NE differentiation in 78 cases (54.9%). In the other 64 (45.1%) we observed heterogeneous patterns and different percentages of positivity for CgA within the cancer. In 29 particular cases (20.4%) only rare scattered stained cells were found in percentage < 5% of the malignant population; while in 26 cases (18.3%) a positivity with focal distribution was detected in a range variant from 5% to 10% of the neoplastic cells; and finally in nine cases (6.4%) the positiveness resulted in wider foci representing beyond the 10% (10%–50%) of the tumor.

As shown in Table 2, there was a modest but significant relationship between the extent of NE differentiation and the tumor grade. The Gleason score depicted a slight stepwise increase with the increase in the percentage of CgA stained cells.

Appraising the clinical stage, we observed that most of the tumors with negative CgA staining were picked up in the early stage with minimal or organ-confined disease; the positive immunohistochemical results were achieved with higher frequency in locally advanced and particularly in disseminated tumors (P < 0.02) (Figure 1). It is noteworthy that NE differentiation was detected in the great majority of primary tumors with metastatic pattern ab initio.

PSA levels were available in 137 patients. Plasma samples for CgA assessment were not collected in the first 20 patients. Among the remaining 122 consecutive patients, CgA measurement was performed in 106. The reasons of non measurement of CgA plasma levels were severe hypertension in two cases, concomitant assumption of nitrates or proton pump inhibitors in eight, uncorrect plasma collection in six. Circulating CgA significantly correlates with immunohistochemical findings in terms of both plasma values and supranormal rates. No statistically meaningful correlation was found with the PSA levels and CgA assessed by immunohistochemistry in tissue sections (Table 2). The concordance...
Neuroendocrine differentiation in predicting the disease stage according to a multivariate regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>SE Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason score</td>
<td>0.241</td>
<td>0.075</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSA</td>
<td>0.288</td>
<td>0.073</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NE differentiation</td>
<td>0.335</td>
<td>0.071</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

PSA is a continuous variable. Stage of disease, Gleason score and Neuroendocrine differentiation are discrete ordinate variables. See Table 1 for categorization of both disease stage and number of scattered chromogranin A positive cells per mm³ tumor area.

(positve plus negative results) of immunohistochemical and circulating CgA was 62% in overall cases, and 81.8% (9/11 cases), 56.0% (28/50), 50.0% (10/20) and 76.0% (19/25) in patients with stage A, B, C and D prostate cancer.

The multivariate analysis showed that the Gleason score, the PSA serum levels and the immunohistochemical NE differentiation were independently associated with the disease stage (Table 3).

Discussion

In the present study we have documented NE differentiation in conventional prostate adenocarcinoma and assessed the relationship of the extent of NE status to the commonly recognized prognostic variables.

NE cells were found in 45% of the adenocarcinomas, a figure which is in line with the 50% reported by di Sant'Agnese [1] and by Krijnen et al. [22]. Other authors described smaller [9], or larger percentages [7, 13, 14]. Differences in tissue processing, types of tissue investigated and patient characteristics probably account at least in part for these discrepancies. The entrapment of benign NE cells in tumors might also partially account for the higher percentage of tumors with NE cells reported by some authors. Hitherto, we cannot exclude that in the present study the number of tumors with NE cells was underestimated, since only small tumor fragments were investigated. Due to the widely scattered distribution of NE cells in most tumors, in fact, the likelihood of obtaining positively stained cells most probably correlates with the tumor volume investigated.

In order to avoid these limitations a number of authors performed studies on tumor tissues coming from radical prostatectomy [7, 8]. These studies, however, may be biased by the fact that patients selected to be addressed to radical prostatectomy may be patients already destined to have a good prognosis even in the absence of surgical intervention, and are not representative of the entire patient population.

In our series NE differentiation appeared to significantly correlate to either the Gleason score or the tumor stage, and these data are in favor of the notion that the presence of NE feature could negatively influence patient outcome.

A significant relationship between tumor grade and NE differentiation was found in some studies [13, 23–25] but failed to be confirmed in others [7, 8]. It should be noted that in the present study the increase in Gleason score with the increase of CgA-positive cells was only modest, so that one could speculate that published studies involving a lower number of patients are not large enough to detect such a difference [7, 8].

As far as the stage of disease is concerned, our data showed that the extent of NE differentiation strongly correlates with the AUA stage, thus confirming previous reports [8, 15, 16]. The relationship of NE differentiation with the stage of disease was even maintained after adjusting for the Gleason score and the PSA serum levels in a multivariate analysis. Interestingly, the majority of patients diagnosed as having stage D1 or D2 disease had primary tumors with more than 5% scattered CgA-positive cells per mm³. This observation is consistent with the results obtained by Abrahamsson et al. [8] describing manifested NE differentiation in eight of nine patients with pathologic D1, and support the notion that NE features increase the metastatic potential of prostate cancer. The increased proliferative activity of neighboring tumor cells, as a result of paracrine effect by neurosecretory products [26], and the reported stimulation of neangiogenesis [27] may both be contributory to explaining the increased invasiveness of tumors with NE differentiation.

The relationship with the stage of disease, notwithstanding the percentage of scattered CgA positive cells, did not correlate with serum PSA. These data are consistent with a previous study by our group, showing that circulating CgA in prostate cancer patients did not correlate with serum PSA [28]. The absence of correlation between CgA and PSA expression suggest that NE cells behave differently with respect to the exocrine component of the tumor.

The follow-up period of our series is too short to obtain information on the role of NE differentiation in
predicting tumor progression or cancer-related death.

As far as circulating CgA is concerned, it should be noted that NE cells are found throughout the body so that one could question whether elevated circulating CgA levels truly come from prostate cancer or from other cells in the body. In a previous study we have observed that circulating CgA levels in prostate cancer patients are higher than those of patients with benign prostate disease and correlate with the stage of disease [28]. In the present series plasma CgA levels paralleled the immunohistochemical findings in primary tumor tissues. The data achieved by our group are consistent with those of Angelsen et al. [18] and suggest that, if patients with renal impairment or severe uncontrolled hypertension are excluded, circulating CgA is a reliable test in detecting the coexistence of NE differentiation within prostate adenocarcinoma.

It should be noted, however, that even if a correspondence exists between circulating and immunohistochemical CgA, it is not perfect and changes according to the stage of disease, (being maximal in metastatic cases). This probably expresses the differences between the two methods and their limits. Immunohistochemistry is highly specific but the sensitivity is hampered by the sampling error, conversely the diagnostic role of circulating CgA may be limited in patients with non metastatic disease conceivably due to the fact that the number of NE cells is not enough to raise the circulating levels. Circulating CgA may be advantageous in the case of distant metastases, since it corresponds to the entire primary tumor cell population and its associated metastases.

To conclude, this study further supports the concept that the presence of focal NE differentiation within classical prostate adenocarcinoma is predictive of poor prognosis, as it correlates with the Gleason score and the stage of disease.

CgA assessed immunohistochemically on tumor samples obtained by core biopsies is a reliable method for detecting the NE features within prostate adenocarcinoma. In our opinion, the risk of obtaining false negative results only applies to small numbers or small foci of positive cells that may not be of clinical significance. The circulating CgA reflects the immunohistochemical findings and represents a simple, reliable method for detecting NE features, particularly in metastatic cases.

References


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