Prognostic Value of Serial Tissue Prostate-specific Antigen Measurements during Different Hormonal Treatments in Prostate Cancer Patients

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ABSTRACT

To reveal the effects of different hormonal treatments directly on the prostate during treatment, the concentration of prostate-specific antigen in the tissue (T-PSA) was studied in 63 patients with untreated newly diagnosed carcinoma of the prostate (CaP). T-PSA measurements were performed in fine-needle aspiration biopsies at the time of diagnosis and 6, 12, and 24 months after initiation of treatment. Treatments modalities were bilateral orchidectomy, gonadotropin-releasing hormone (GnRH) agonists, or parenteral estrogens. Thirty-one (49%) of the patients died of CaP and 18 (29%) of other diseases. Fourteen of the patients (22%) were still alive at the end of the observation period (median follow-up time, 111.5 months; range, 98–128 months). In all of the 31 patients who died of CaP, T-PSA values increased during treatment. This increase was observed long before clinical signs of progression appeared (median of interval, 14 months). Twenty of these 31 patients showed an increase in T-PSA from pretreatment values at 6 months. At 12 months this increase was observed in 30 of 31 patients. In contrast, in all of the patients who responded to the hormonal regimen, T-PSA values decreased and remained low during treatment. Furthermore, the patients who did not die of CaP and received estrogen treatment had significantly higher T-PSA values compared with those who were treated with bilateral orchidectomy or GnRH agonists. This indicates that estrogens may stimulate PSA synthesis in tumor tissue in vivo in the presence of castration levels of testosterone.

Statistical evaluation showed that the T-PSA ratio between month 12 and month 0 had the most significant prognostic value for predicting the clinical outcome. This ratio was superior to clinical classifications, e.g., tumor stage and cytological grade, and also was higher than T-PSA at the time of diagnosis. This study has shown that aspiration biopsy material can be used to reveal biochemical changes in the tissue during treatment and that one specific marker (T-PSA) can predict the clinical outcome of endocrine treatment of CaP patients better than previously used methods. We believe that selected tissue markers or the protein pattern can help us to characterize the tumors and predict the clinical outcome so an optimal treatment can be chosen for every patient.

INTRODUCTION

The clinical course of men with prostate cancer may vary widely, even in men whose tumors are of similar clinical stage and cytological grade. Many efforts have focused on identifying factors that can predict the hormone sensitivity of CaP3 and thus the outcome of treatment. This should have special value for patients without metastases, because it is known that the cancer in these patients can develop both very slowly and very rapidly. At present, it is not possible to predict the clinical outcome in terms of time to progression and time to death. With the new screening programs many non–symptom-giving cancers will be discovered at an early stage. The use of a reliable prognostic marker should help the clinician to treat the patient in an optimal way. Such a marker also could be useful for early detection of both rare cases of primary hormone insensitivity and for patients who develop hormone insensitivity during treatment. For these two patient categories a more aggressive treatment should be considered.

S-PSA is widely used for monitoring treatment and also to some extent for diagnosis of CaP (1–3). After radical prostatectomy, increasing S-PSA values indicate residual disease and recurrence of tumor (4, 5). This is also true for endocrine treatment of CaP, including M1 patients. After initiation of treatment, S-PSA usually drops to normal and increases subsequently when the tumor becomes hormone insensitive (6). The prognostic value of total and free PSA has been discussed by many research groups (3, 7–9). Nevertheless, the usefulness of S-PSA analysis has not drastically improved in this respect.

In experimental studies we found lower PSA values in a cell line that was hormone resistant (LNCaP-r) compared with a hormone-sensitive cell line (LNCaP). On the basis of these results, we developed a method for quantitation of PSA in

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3 The abbreviations used are: CaP, carcinoma of the prostate; PSA, prostate-specific antigen; S-PSA, serum concentrations of PSA; T-PSA, PSA in the tissue; GnRH, gonadotropin-releasing hormone.
Computed fine-needle aspiration biopsies from patients with prostate diseases (T-PSA). T-PSA was shown to correlate negatively to clinical stage and cytological grade, that is, highly malignant tumors have low tissue concentrations of PSA (10–12). We also found an inverse correlation between pretreatment PSA concentrations in tumor tissue and in serum from patients with prostate cancer (13). In a 2-year follow-up study of hormonally treated CaP patients, we showed that T-PSA appeared to be a valuable adjunct to cytological grading for predicting progressive disease (12). In a recent study we retrospectively correlated pretreatment variables to the clinical outcome in 179 patients and showed that T-PSA was the single most important factor to predict time-to-progress and time-to-disease-specific death (14).

The major aim of this study was to reveal the effects of different hormonal treatments on PSA concentration in the prostate. The second aim was to evaluate retrospectively the prognostic value of serial T-PSA determinations during endocrine treatment of patients with CaP.

MATERIALS AND METHODS

Patients. The study comprised 63 patients (G2–G3; T2–T4; M0–M1) aged 54 to 87 years (median, 75 years). CaP was confirmed cytologically in all of the 63 patients and also histologically from transurethral resection prostate specimens in 18 patients. The diagnostic procedures and treatments of all of the patients were performed at the Department of Urology at Hud- dinge University Hospital. With exception of their specific disease, all of the patients were ambulatory, apparently healthy, and previously untreated for CaP. Fifty-one patients had somatic symptoms, e.g., anemia, bone pain, impaired renal function, or voiding disorders, that may have been related to CaP at time of diagnosis. Twelve patients without somatic symptoms were offered deferred treatment but decided to be treated for psychological reasons.

The patients were in a nonrandomized procedure allocated to three forms of hormonal treatment pending exclusively on patients decision. The following treatments were given: surgical castration by bilateral orchidectomy (30 patients) or medical castration with GnRH agonists [3.6 mg goserelin acetate (Zoladex; Zeneca, Wilmington, DE) s.c. every fourth week; 11 patients] or with parenteral depot estrogens [240 mg polyestradiol phosphate (Estradurin; Wyeth-Ayerst, Philadelphia, PA) i.m. per month; 22 patients]. Both regimens of medical treatment suppress circulating testosterone to castration levels (15, 16).

Follow-Up and Survival. The patients were included in the study from August 1986 until March 1988 and were recorded until April 1997. They were followed-up and evaluated according to the recommendations of European Organization for Research on Treatment of Cancer. Clinical examinations were performed every 24 weeks during the entire observation period. Objective progression of the disease was defined as an increase of T stage by two steps or more compared with the lowest T stage earlier recorded. The appearance of skeletal or nonskeletal metastases also was recorded as objective progression. All of the patients were followed-up until the end of the observation period or until death. Aspiration biopsies were taken before (0) and at 6, 12 and 24 months after diagnosis.

When this study was initiated, S-PSA assays were not routinely used in our departments. A comparison between S-PSA and T-PSA therefore was not possible in this study.

T Staging. T staging was performed exclusively by digital rectal examination throughout the entire period of investigation by the senior urologist (R.S.), according to Union International Contre Cancer (UICC) guidelines (17). Stages T1 and T2 are confined to the gland and correspond to stages A and B, respectively. In stage T1 the tumor is surrounded by palpably normal gland. Stage T2 has a smooth nodal deforming contour, without involvement of lateral sulci and seminal vesicles. Stages T3 and T4 are tumors extending beyond the capsule, corresponding to stage C. In stage T3 the tumor extends beyond the capsule with or without involvement of lateral sulci and/or seminal vesicles. In stages T4 the tumor is fixed or infiltrates neighboring structures. Ultrasound equipment for transrectal examination of the prostate was not available at our department when this study was initiated.

Fine-Needle Aspiration Biopsies. Fine-needle aspiration biopsies were obtained during routine examination according to the method of Franzén (18). All of the biopsies were taken by the same cytologist (B.L.R.). Four biopsies were obtained from the same tumor area. Two biopsies were prepared for morphological analyses and two for determination of T-PSA. To be able to perform the planned aspiration biopsies in the same tumor area, the pathologist did a meticulous depiction of the primary tumor location for each patient.

Cytology. Cytological grading of the aspirates was performed through the entire period of investigation by the same senior pathologist (B. L. R.) without knowledge of the biochemical data. Three grades of malignancy were defined based on six cellular properties: average nuclear size, average nucleolar size, variability in nuclear size, disturbance of nuclear arrangement, and cellular/nuclear dissociation (19).

Biochemical Analyses. Samples were stored at −70°C until analysis. Cytosols were prepared by sonication of the biopsies followed by centrifugation at 105,000 × g. During these procedures all of the samples were kept on ice or refrigerated. Cytosolic PSA was determined by RIA and DNA content

Table 1  Tumor stage, cytological grade, metastatic disease and T-PSA in 63 patients with CaP

<table>
<thead>
<tr>
<th>T stage</th>
<th>No.</th>
<th>T-PSA (µg PSA/µg DNA)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2</td>
<td>3</td>
<td>0.277 (0.062–0.53)</td>
</tr>
<tr>
<td>T2</td>
<td>40</td>
<td>1.400 (0.004–20.911)</td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>0.124 (0.002–0.804)</td>
</tr>
<tr>
<td>T3</td>
<td>31</td>
<td>1.785 (0.030–20.911)</td>
</tr>
<tr>
<td>T3</td>
<td>32</td>
<td>0.129 (0.002–0.632)</td>
</tr>
<tr>
<td>T4</td>
<td>52</td>
<td>1.112 (0.011–20.911)</td>
</tr>
<tr>
<td>T4</td>
<td>11</td>
<td>0.0885 (0.002–0.231)</td>
</tr>
</tbody>
</table>

aT-PSA values are shown as median with range in parentheses.
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in the biopsy material by fluorometry (10). The tissue content of PSA in the aspirates is given as μg PSA/μg of DNA.

Statistical Methods. Differences between groups were tested by Mann-Whitney U test and changes in T-PSA during treatment by ANOVA for repeated measurements. In the survival analyses the 11 M1 patients known to have an adverse prognosis were excluded. The categorical factors, T-stage and cytological grade, were analyzed separately in a Cox univariate analysis initially was carried out for these three prognostic variables, including the above mentioned categorical factors, was selected for stepwise Cox bivariate models to evaluate the most useful predictor (20, 21). Data are presented as arithmetic means ± SE or as median and range according to distribution.

RESULTS

Tumor Stage, Cytological Grade, and Metastatic Disease. After cytological examination, the tumors were graded: 31 as moderately differentiated (G2) and 32 as poorly differentiated carcinomas (G3). Three patients had T2 tumors, 40 had T3, and 20 had T4 tumors (Table 1). Tumors with a high malignancy grade were more frequently in stage T3. Fifty-two patients had M0 and 11 M1 disease at the time of diagnosis.

Table 2 T-PSA in 63 patients before and during treatment

<table>
<thead>
<tr>
<th>Time of sampling (months)</th>
<th>Group A (N = 32 patients)</th>
<th>Group B (N = 31 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.381 (0.050–20.911)</td>
<td>0.063 (0.002–0.723)</td>
</tr>
<tr>
<td>6</td>
<td>0.066 (0.002–10.699)</td>
<td>0.085 (0.007–1.488)</td>
</tr>
<tr>
<td>12</td>
<td>0.036 (0.002–7.681)</td>
<td>0.199 (0.025–2.398)</td>
</tr>
<tr>
<td>24</td>
<td>0.036 (0.001–4.922)</td>
<td>0.219 (0.099–2.687)</td>
</tr>
</tbody>
</table>

Change 0–24 months: Change 0–24 months: 
P = 0.023b P < 0.001b

"T-PSA values are shown as median with range in parentheses. Group A represents the patients who stayed alive during the observation period or died of causes other than CaP. Group B includes the patients who died of CaP.

aANOVA for repeated measurements.

Tissue PSA. T-PSA values at the time of diagnosis, expressed as μg PSA/μg DNA, in relation to tumor stage (T), cytological grade (G), and metastatic disease (M) are shown in Table 1. T-PSA values decreased with increasing tumor stage and cytological grade, except for the T2 stage tumors which was attributable to small sample size (3 patients). T-PSA levels also decreased significantly in the patients with metastatic disease.

Follow-up and Survival. For the patients who survived (n = 14) the median follow-up timet was 111.5 (range, 98–128 months). Corresponding values for the patients who died of CaP (n = 31) and the patients who died of causes other than CaP (n = 18) were 28.9 (6–70) and 48.3 months (18–109 months), respectively.

For analysis of outcome, patients were divided into two groups according to the metastatic situation at time of diagnosis. Twenty-one of the 52 M0 patients died of CaP, and 31 were alive at the end of the observation period or died of causes other than CaP (14 and 17 patients, respectively). Ten of the M1 patients died of CaP, and 1 patient for other reasons than CaP.

T-PSA Values before and during Treatment in Relation to Outcome. Figure 1 and Table 2 show T-PSA analyzed before and during hormonal treatment for all of the 63 patients. Group A represents patients who were alive at the end of observation period or died of causes other than CaP. Group B includes patients who died of CaP. Pretreatment values of T-PSA (time of sampling, 0 months) were significantly higher in group A compared with patients in group B [0.381 (0.050–20.911) versus 0.063 (0.002–0.723) μg PSA/μg DNA, P < 0.001]. On the other hand, there was no difference in pretreatment T-PSA values between M0 and M1 patients who died of CaP [0.067 (0.010–0.723) versus 0.043 (0.002–0.231) μg PSA/μg DNA, P > 0.3]. In group A the T-PSA values decreased significantly during treatment (P = 0.023). In contrast, T-PSA values of group B patients increased successively during treatment (P < 0.001).

Changes of T-PSA Values in Relation to Treatment. It has to be emphasized that in the subsequent description of our results, the 11 patients with metastatic disease were excluded. T-PSA values for the M0 patients (n = 52) before and during
treatment in relation to treatment regimen and clinical outcome (groups A and B) are given in Tables 3 and 4.

**Group A.** Thirty-one patients were alive at the end of the observation period or died of causes other than CaP (Table 3). Seventeen had been treated by orchidectomy or GnRH agonists and 14 with estrogens. Because surgical castration by bilateral orchidectomy and medical castration with GnRH agonists produce similar sex steroid conditions, these groups were combined in the statistical calculations. T-PSA values decreased significantly from pretreatment values and stayed at lower values in the orchidectomized/GnRH agonist–treated group (P = 0.031). In contrast, the T-PSA decrease in the group receiving estrogens showed no significance (P = 0.248). There was no difference in pretreatment T-PSA values between the two treatment groups (P > 0.4). In contrast, values during treatment were significantly higher in the patients treated with estrogens compared with the other group (0.001 ≤ P ≤ 0.005).

**Group B.** In this group 15 patients had been treated by bilateral orchidectomy or GnRH agonists and six patients with estrogens. All of the patients showed increasing T-PSA values during treatment. In the group treated with surgical castration or GnRH agonists the increase was significant (P = 0.002). Pretreatment values of T-PSA were significantly higher in patients receiving estrogen therapy (P < 0.005), but there was no influence of this treatment regimen on T-PSA values during treatment in group B (0.4 ≤ P ≥ 0.9).

In detail, at 6 months we observed a definite increase in T-PSA values in 11 patients. Only 2 of them developed clinical progression within this 6-month period (Table 5). The other 9 patients developed a later clinical progression (median, 26 months). One patient showed clinical progression after 4 months. In this case a definite T-PSA increase was seen first after 24 months (Table 5).

There was no difference in T-PSA values during treatment between M0 and M1 patients (data not shown).

The statistical calculations using Cox univariate analysis of M0 patients with respect to disease-specific death are shown in Tables 6 and 7. The change between the T-PSA values at 12 months and the initial values is the best predictive factor. Other competing factors of less importance were T-PSA value changes between M0 and M1 patients (data not shown).
Table 6  Cox univariate analysis of the rangordered T-PSA values with respect to survival: start of analysis and after 6, 12, and 24 months

<table>
<thead>
<tr>
<th>Continuous factor</th>
<th>RH(^a)</th>
<th>CI</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-PSA(_{6/0})</td>
<td>1.1</td>
<td>1.0–1.1</td>
<td>15.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-PSA(_{12/0})</td>
<td>1.1</td>
<td>1.1–1.2</td>
<td>25.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-PSA(_{24/0})</td>
<td>1.2</td>
<td>1.1–1.3</td>
<td>17.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\log_{10}) T-PSA(_{0})</td>
<td>0.1</td>
<td>0.04–0.3</td>
<td>19.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\) RH, relative hazards; 95% confidence interval CI; \(\beta = \ln(\text{RH})\).

Table 7  Survival analysis with respect to cancer-specific death for tumor stage and cytological grade

<table>
<thead>
<tr>
<th>Categorical factor</th>
<th>(n)</th>
<th>%</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0</td>
<td>10.3</td>
<td>0.006</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>28</td>
<td>8.8</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The present study has shown that aspiration biopsy material can be used to reveal biochemical changes in the tissue during treatment. We also have shown that estrogens affect T-PSA concentration and that the 12- to 0-month ratio of T-PSA can predict the clinical outcome of endocrine treatment of CaP patients better than hitherto clinically used methods.

For these studies it is of vital importance that the aspiration procedure is highly standardized and performed by an experienced cytologist. Using the fine-needle aspiration method for biopsies makes it possible to obtain tissue material from a circumscribed area of the gland a number of times during the treatment. The material is also rather specific, because studies by Tribukait et al. (22) showed that an average of 75% of all of the cells in fine-needle aspirations of prostate carcinomas are tumor cells. In well and poorly differentiated tumors, the average values were 66% and 85%, respectively, and the mean incidence of inflammatory cells was 3%.

A number of other research groups have studied intracellular markers, e.g., PSA, and correlated these results to the histological findings (23). Many of these studies are performed on tissue material obtained from operation specimens or core biopsies, which almost always contains a high degree of different components of prostate tissue. If therefore significant proportions of normal prostate cells are present in the material, the measured PSA values are overestimated. On the other hand, true values are underestimated if a significant proportion of stromal or inflammatory cells are present in the materials.

Our major goal was to reveal the effect of different endocrine treatment on T-PSA, and an interesting finding was that T-PSA levels during treatment in patients who responded to endocrine therapy were significantly higher in patients treated with estrogens than in those treated with orchidectomy/GnRH agonists and that estrogen treatment did not result in any pronounced decrease in T-PSA levels. The synthesis of PSA is considered to be androgen sensitive, and androgen depletion is known to decrease S-PSA levels in patients with hormone-sensitive CaP. The regulation of PSA, however, is complex, and other factors may affect both the synthesis and secretion. In vitro, besides androgens, estrogens also have been shown to stimulate PSA synthesis in LNCaP cells (24, 25). Our finding indicates that estrogens also may have this ability in vivo. The effect of estrogens also is of interest because there are indications for a role of estrogens in the etiology of CaP (Ref. 26 and references cited therein). Besides a hypothetic stimulatory effect of estrogens on cellular PSA synthesis, there also may be the possibility that estrogen treatment affects T-PSA levels by changing the relation between epithelium and stroma. However, investigation of such a relationship implies the microdissection technique, which was not available at our department at the time of our investigation.

In the patients who did not respond to endocrine therapy, the initial T-PSA levels were significantly lower than in the patients who responded to therapy but increased dramatically during treatment. Also, there were no differences related to therapy regimen in T-PSA levels during treatment in these patients. These findings clearly illustrate that other factors are responsible for the regulation of T-PSA levels in these tumors. One possibility is that the PSA synthesis has become androgen insensitive, but another possibility is that gene amplification of the androgen receptor occurred and that the adrenals stimulate PSA synthesis.

In previous studies we could relate a decrease in T-PSA values to an increase in malignancy grade and tumor stage (11), and we also have shown that T-PSA at the time of diagnosis is the best single prognostic factor when compared with serum total and free PSA, free-to-total serum PSA ratio, tumor stage, cytological grade, and DNA ploidy to predict the clinical outcome (12, 14). These results are confirmed in this study, and an even higher prediction could be found using the 12- to 0-month T-PSA ratio, which can be considered as a biochemical analysis of the changes that occurred in the tissue.

Compared with serum PSA, T-PSA is less dependent on factors not related to the production of protein in the tissue, such as volume of the PSA-producing tissue, transport of PSA into...
the blood, and clearance of the protein from the circulation. Any process that alters any of these factors will affect S-PSA levels (27). Therefore, T-PSA reflects the biochemical composition of the gland more physiologically, e.g., T-PSA levels are high in normal tissue and in hyperplastic prostate tissue (10).

We also observed that pretreatment T-PSA concentrations in patients with M0 or M1 disease at time of diagnosis are not different in the group of patients who died of CaP. This indicates that the factors involved in metastasizing do not affect the PSA concentration.

We initially found it strange that one single biochemical tissue marker can predict so well the clinical outcome. However, we consider today T-PSA as a marker for normal prostate tissue, and a decrease should be considered as sign of abnormal regulation. In line with this, we believe that it is possible to develop these methods further and to use the aspiration biopsy material to analyze other markers of interest or characterize the protein pattern by two-dimensional gel techniques. By doing this, we could learn much more about the biochemical changes that occur during the development of CaP at the same time, because we can explore the use of different markers for predicting the clinical outcome. In this way we also should be able to select the optimal treatment, which means both avoiding treatment when not necessary and treating aggressively when not necessary.

REFERENCES