The role of PCA 3 as a prognostic factor in patients with castration-resistant prostate cancer (CRPC) treated with docetaxel

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Purpose: To investigate potential fluctuations in PCA 3 scores in castration-resistant prostate cancer (CRPC) patients treated with docetaxel and investigate the assay as a potential prognostic factor.

Materials and Methods: This was a prospective observational cohort study. Inclusion criteria included patients on hormonal treatment that were recently diagnosed with CRPC. Exclusion criteria included patients previously having radical treatment (surgery or radiotherapy) and patients who have completed the first cycle of chemotherapy. All urine samples were collected and analyzed using the Progensa™ assay (Gen-Probe, San Diego, CA). Samples were collected before starting chemotherapy and at 12 months. A prospective database was created including routine blood tests, prostate staging and PSA levels throughout the study period. The effects of chemotherapy were also recorded.

Results: Between January 2010 and February 2013, 12 patients were included in the study out of an initial cohort of 23 patients with CRPC. Mean follow up was 14.8 months. Mean age at CRPC diagnosis was 73.8 years (+/- 3.6 SD). Mean Gleason score was 8, with PSA 84.23 ng/ml (+/- 158 SD). Mean duration of androgen deprivation treatment was 45.16 months (+/- 34.9 SD). Mean time to castrate resistant state was 46.58 months (+/- 35.3 SD). All twelve (n=12, 100%) patients had non-assessable PCA 3 scores at baseline and at 12 months follow up. As a direct consequence, statistical analysis did not take place as the anticipated change in PCA 3 scores was not identified and correlation between measurable dif-
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Introduction
PCA3 is a segment of non-coding messenger ribonucleic acid (mRNA) from chromosome 9q21-22 and is over expressed by a median of 66 times in prostate cancer tissue relative to benign tissue. It is not detected in extraprostatic tissues and unlike PSA, it is not affected by age, prostate volume or other prostatic diseases (e.g. prostatitis) nor affected by PSA. At a cutoff score of 35, PCA 3 has a sensitivity of 64% and specificity of 76% for detecting prostate cancer and presently is used to help determine the need for repeat prostate biopsies in men who have had a previous negative biopsy. Several studies have shown the superiority of PCA3 score to PSA in predicting biopsy outcome [Roobol et al. 2011] [de la Taille et al. 2011], as increasing scores correspond frequently to a positive biopsy. In 2012, the US Food and Drug Administration approved PCA 3 as a diagnostic adjunct to aid clinicians in decision-making regarding repeated biopsies. Furthermore, several studies have investigated the relation of PCA 3 to tumor volume and aggressiveness, with mixed results. A pooled analysis of two multi-center European trials that evaluated the significance of PCA 3 in predicting biopsy and prostatectomy specimen characteristics by van Poppel et al showed a relationship of higher PCA 3 scores with more significant cancers with respect to indolent ones. The authors conclude that PCA 3 should be included in the decision making process for active surveillance.

Overall, there is a large body of evidence to substantiate the integration of PCA 3 in risk stratification models for prostate cancer diagnosis. However, to this day, very little is known about the usefulness of PCA 3 as a marker of treatment response and its role in advanced disease. In this article we present our study of PCA 3 in a population of patients with advanced prostatic malignancy, aiming to identify any correlation with disease progression and treatment response.

Purpose
The primary end point of our study was to investigate potential fluctuations in PCA 3 scores in patients with castration-resistant prostate cancer (CRPC) treated with docetaxel and investigate the assay as a potential prognostic factor. Secondary outcome measures included correlation of PCA 3 with laboratory and cancer staging parameters in disease progression during chemotherapy.

Materials and Methods
This was a prospective observational cohort study, conducted in compliance with all relevant institutional, scientific and ethical committee review boards and local regulatory requirements. Informed consent was obtained from all patients. Inclusion criteria included adult patients diagnosed with locally advanced or metastatic prostate cancer (T3) who have not had any treatment other than androgen deprivation therapy (ADT), recently diagnosed with castration-resistant prostate cancer, as defined in Table 1, who were suitable for chemotherapy with docetaxel. Other inclusion criteria were ECOG score less than 2 and expected survival of more than 12 months. Exclusion criteria included patients previously having radical treatment (surgery or radiotherapy) and patients who have completed the first cycle of chemotherapy, as well as patients with other concomitant malignancy (urological extraprostatic, skin, liver, lung, gastrointestinal tract, brain, musculoskeletal). Patients were recruited from the existing pool of follow up appointments at our academic centre. All patients underwent DRE and prostatic massage to obtain urine samples that were collected.

Conclusions
All patients tolerated chemotherapy and completed the scheduled cycles with no serious adverse effects.

Conclusion: To our knowledge, this is the first prospective study that demonstrates lack of expression of PCA3 in castration-resistant prostate cancer, with the result apparently not influenced by chemotherapy. There appears to be a strong association between hormonal treatment and lack of PCA 3 expression. It is still unknown whether disease progression per se affects PCA 3 scores. The gradual reduction and eventual complete non expression of PCA 3 with ongoing treatment and disease progression provide an insight towards molecular pathways that may be connected to castration resistant state.
and analyzed using the Progensa® assay (Gen-Probe, San Diego, CA) at baseline (i.e. before starting chemotherapy) and at 12 months. PCA 3 scores were calculated as 1,000 x (PCA 3 m RNA copies/PSA m RNA copies) and all the samples were processed in the same accredited laboratory (Genekor SA®). A PCA 3 score cut-off of 35 was determined as diagnostically significant. Non-assessable or non-informative PCA 3 results were also considered. Baseline routine blood function tests and biochemistry, PSA and PSA ratio, prostate volume (assessed by TRUS), prostate cancer diagnosis and staging information (initial biopsy PSA and Gleason score, nodal status and evidence of visceral and bone metastases), as well as duration of androgen deprivation and side effects of chemotherapy were collected on presentation and included in a prospective database, that was updated at 12 months follow up.

Docetaxel chemotherapy was administered as the standard regimen of intravenous 75 mg/m2 three weekly along with prednisolone 10 mg/day. Up to 10 cycles of treatment were planned. Dose reduction was offered to patients with serious neutropenia and/or sepsis, along with granulocyte colony-stimulating factor. Adverse effects and additional management were recorded.

For statistical analysis, a repeated measure ANOVA test was selected in order to correlate PCA 3 values pre-and post chemotherapy, and also with variables that prognosticate disease response to treatment (i.e. post chemotherapy PSA, extent of visceral and bone metastases and worsening of symptoms). Level of statistical significance was considered as \( p < 0.05 \).

**Results**

Between January 2010 and February 2013, a total of 23 patients were identified with CRPC. Of those, 16 patients met the inclusion criteria and were originally included in the study. During the initial follow up period one patient unfortunately passed away and three more deteriorated significantly and could not continue on the study. The remainder 12 patients that met the inclusion criteria completed the initial 12 months and were followed up until disease progression (Figure 1). Mean age at CRPC diagnosis and inclusion in the study was 73.8 years (+/- 3.6 SD). Mean Gleason score at initial diagnosis was 8, with mean PSA 84.23 ng/ml (+/- 158 SD). Androgen deprivation treatment consisted of LHRH agonist (8 patients Goserelin acetate, 5 patients Leuprolrelin acetate, 3 patients Triptorelin) in combination with a non-steroidal antiandrogen for the first four weeks in all patients. Mean duration of androgen
deprivation treatment was 45.16 months (+/- 34.9 SD). Mean time to castrate resistant state from original diagnosis was 46.58 months (+/- 35.3 SD). Mean PSA at CRPC diagnosis was 230.51 ng/ml (+/- 296.75 SD), with free PSA 58.6 ng/ml (+/- 90.3 SD) and PSA ratio 0.26 +/- 0.11 SD. Mean prostate volume as measured by transrectal ultrasound was 40.1 cc (+/- 17.6 SD). Mean ECOG score was 1. Mean overall follow up was 14.8 months. Post chemotherapy PSA at 12 months follow up was 181.98 ng/ml +/- 306.2 SD, free PSA 66.55 ng/ml +/- 142.0 SD and PSA ratio 0.34 +/- 0.1 SD. A summary of the results is provided in Table 2.

All twelve (n=12, 100%) patients had non-assessable PCA 3 scores at baseline and at 12 months follow up. Of note, baseline PCA 3 was non assessable due to PSA m RNA levels below the level of detection in all sixteen (n=16) patients at baseline. As a direct consequence, statistical analysis did not take place as the anticipated change in PCA 3 scores was not identified and correlation between measurable differences was not possible. Integration of a time-to-event model was also not possible again because there was no measurable difference to begin with.

All patients tolerated chemotherapy well for the first three months, with no adverse effects, and bone pain improved significantly. In the following cycles, one patient experienced neutropenic sepsis that was successfully treated, three patients experienced low-grade fever, malaise and anorexia, which did not alter treatment and two patients experienced anorexia, diarrhea and vomiting. All patients completed the scheduled treatment cycles with significantly improved symptoms and no new metastases during the study follow-up period.

**Discussion**

Early efforts to identify the influence of medical intervention on PCA 3 scores have produced equivo-

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**Table 2**

<table>
<thead>
<tr>
<th>PATIENT (S/N)</th>
<th>AGE (YEARS)</th>
<th>TIME TO CRPC SINCE DX (MONTHS)</th>
<th>PSA AT DX</th>
<th>GLEASON SCORE</th>
<th>STAGE (TNM)</th>
<th>ADT DURATION (MONTHS)</th>
<th>PSA AT CRPC</th>
<th>PCA 3 AT BASELINE</th>
<th>PCA 3 AT 12 MONTHS</th>
<th>PSA AT 12 MONTHS</th>
<th>FOLLOW-UP (MONTHS)</th>
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<td>34</td>
<td>7</td>
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<td>Not assessable *</td>
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<td>17</td>
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<td>24.1</td>
<td>8</td>
<td>T4N1M1b</td>
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<td>Not assessable *</td>
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<td>46.58</td>
<td>84.23</td>
<td>8</td>
<td>-</td>
<td>45.16</td>
<td>230.51</td>
<td>-</td>
<td>-</td>
<td>181.98</td>
<td>14.8</td>
</tr>
</tbody>
</table>

* Non assessable: PSA m RNA < 7500 copies/ml.

CRPC: Castration Resistant Prostate Cancer, Dx: Diagnosis
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The disruption of prostatic architecture in higher grade tumors has also been postulated as explanation for low PCA 3 scores. The loss of glandular pattern in higher Gleason score cancer leads to obliteration of the ducts and lumen, making it difficult to express prostatic cells in the urine in order to achieve a measurable result. Histopathologic changes in hormonally treated prostate cancer cells are mainly characterized by cellular shrinkage with little cytoplasm, that translates to glandular shrinkage and an overall reduction in size. DRE in these patients often reveals a small, hard prostate. However, this hypothesis is contradicted by the fact that, although PCA 3 was not assessable, prostate cells were identified in the urine of all patients in our study, suggesting a molecular rather than a mechanistic mechanism.

According to our findings, another possible mechanism would be an ablative effect of androgen deprivation to PCA 3 expression, something also shown in the Triptocare study. Continuation of ADT in CRPC patients throughout the chemotherapeutic period constitutes common practice and probably relates better to our results than any effect of docetaxel treatment. In our study, PCA 3 was non-assessable both at baseline and at 12 months follow up after the completion of chemotherapy. Based on the above findings, further research is required to answer the questions of the true nature of PCA 3 mRNA in prostate cancer molecular pathogenesis pathways and its expression during the various stages of progression. The Triptocare investigators point out in addition that hormone naive patients with metastases produced significantly lower PCA 3 scores than men without metastases. In our study of exclusively metastatic patients, non-assessable PCA 3 was the rule, which substantiates further the hypothesis of declining PCA 3 expression with ADT and advancing disease.

In both studies, PCA 3 failed to prove as a marker for disease prognostication and treatment response. Nevertheless, paucity of PCA 3 expression with ADT has never been demonstrated previously and this discovery may have yet to reveal its full potential. In retrospect, the evident gradual decrease in PCA 3 detection with ongoing ADT and the eventual complete non-assessability demonstrated in our cohort appear to be sequential events. The EFFECT trial is another prospective open label single arm multicentre trial that is currently ongoing and investigates the effect of leuprorelin acetate at 6 months on PSA, PCA 3 and TMPRSS2-ERG-mRNA amongst other markers. In our cohort, 8 patients received Goserelin acetate, 5 patients Leuprorelin ac-
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Original Greek text:


te, 3 patients Triptorelin previously and continued throughout chemotherapy. The trial aims to recruit 50 patients and compare testosterone and PSA with the newer biomarkers to provide more evidence and much needed answers in the field of advanced prostatic malignancy

Conclusion

To our knowledge, this is the first prospective study that demonstrates lack of expression of PCA3 in castration-resistant prostate cancer, with the result apparently not influenced by chemotherapy. There appears to be a strong association between hormonal treatment and lack of PCA 3 expression, probably by means of an ablative effect to gene expression. Although preliminary evidence from other studies supports this theory, it is still unknown whether disease progression per se affects PCA 3 scores. The ongoing EFFECT trial will add to the existing evidence. The gradual reduction and eventual complete non expression of PCA 3 with ongoing treatment and disease progression provide an insight towards molecular pathways that may be connected to events leading to castration resistant state.

Acknowledgements

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Conflicts of interest

The authors declared no conflicts of interest.

Περίληψη

Ο πρωταρχικός σκοπός της μελέτης ήταν η ανεύρεση διακυμάνσεων του αθροίσματος του PCA 3 σε ασθενείς με προχωρημένη νόσο που μόλις μετέπεσαν σε ορμοαντοχή και υπεβλήθησαν σε αγωγή με δοσιταξέλη (docetaxel) και η πιθανή προγνωστική σημασία. Όλοι οι ασθενείς υπεβλήθησαν σε δακτυλική εξέταση και προστατική μάλλαξη ώστε να γίνει συλλογή δείγματος ούρων κατάλληλο για επεξεργασία και ανάλυση επιπέδων PCA 3 με τη χρήση του αντιδραστηρίου Progensa assay σύμφωνα με τις προδιαγραφές της κατασκευαστρικής εταιρείας (Gen-Probe, San Diego, CA, USA) κατά την ένταξη στη μελέτη και μετά από πάροδο 12 μηνών υπό χημειοθεραπεία. Συντάχτηκε έτσι μια προοπτική βάση δεδομένων που συμπεριλάμβανε το είδος και τη διάρκεια θεραπείας ορμοαποκλεισμού καθώς και τις παρενέργειες των θεραπευτικών σχημάτων καθ’ όλη τη διάρκεια της μελέτης.

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