

# Biochemical Markers for Monitoring Response to Therapy: Evidence for Higher Bone Specificity by a Novel Marker Compared with Routine Markers

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## Abstract

The aim of the present study was to compare a novel marker for high bone turnover with two routine markers for screening in prostate cancer patients. The markers were evaluated in two studies: (a) a cross-sectional study of 170 prostate cancer patients with local disease stratified by  $\pm$  lymph node metastases (N<sub>0</sub>, N<sub>1</sub>) compared with controls and (b) a longitudinal study of 40 hormone refractory prostate cancer patients stratified by skeletal involvement and followed during docetaxel (+/–BM) and zoledronate (+BM) treatment. Presence or absence of bone metastases (BM) was assessed by imaging techniques (magnetic resonance imaging or X-ray) and technetium-99m scintigraphy. The serum or urinary levels of alpha C-telopeptide of collagen type I ( $\alpha\alpha$ CTX), prostate-specific antigen (PSA), and total alkaline phosphatase (tALP) were assessed. PSA was elevated in both N<sub>0</sub> and N<sub>1</sub> patients

compared with controls, whereas  $\alpha\alpha$ CTX was elevated only in N<sub>1</sub> patients. tALP exhibited no difference in any of the groups. In the treatment study, PSA decreased with treatment in both the –BM and +BM groups compared with baseline values, showing similar effect of docetaxel or docetaxel/zoledronate treatment on this marker. On the contrary,  $\alpha\alpha$ CTX and tALP did not decrease with docetaxel treatment in the –BM group compared with baseline, whereas it decreased significantly with docetaxel/zoledronate treatment in the +BM group, already after 1 month of treatment for  $\alpha\alpha$ CTX. Results suggest that  $\alpha\alpha$ CTX is superior to PSA and tALP for identifying patients having a high risk of metastatic disease and for monitoring skeletal progression in +BM prostate cancer patients during treatment. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1269–76)

## Introduction

Prostate cancer is one of the most common cancer types, representing 19% of all cancers diagnosed in 2002, with 679,000 new cases in the western world (1). Bone metastases are common in prostate cancer patients and arise when the primary tumor metastasizes to the bone, causing a lesion of high bone remodeling, which destroys the bone structure. The consequences are devastating symptoms such as severe bone pain, pathologic bone fractures, increased mortality, hypocalcaemia, spinal cord compression, etc. (2, 3).

Bone metastases occur in >50% to 60% of patients with advanced cancer disease (4) and are incurable. Osteoblasts and osteoclasts are affected by the invasive tumor cells in the bone metastases causing increased number, activity, and survival of these bone remodeling cells (5), a phenomenon known as the vicious cycle. Prostate cancer is characterized mainly by sclerotic bone lesions especially in men failing androgen therapy (6).

To initiate successful therapy, it is crucial that bone metastases are detected as early as possible. Early-stage prostate cancer can usually be cured by surgery or radiation therapy, but this is not effective for metastatic disease (6). Treatment is changed radically when a patient develops bone metastases by adding treatment targeting the skeleton. Bisphosphonates are some of the most used bone-targeting drugs for these patients, and effectively inhibits osteoclasts (7). Soft-tissue metastases are, at present time, normally targeted by chemotherapy or by docetaxel (6).

**Biomarkers for Detection of Bone Metastases.** Bone markers are noninvasive, inexpensive, and potentially serve as helpful tools for detecting bone metastases. In our lab, we previously investigated the relation of eight biomarkers to the extent of skeletal involvement in a group of prostate, lung, and breast cancers (8). From this clinical study, we concluded that collagenous markers were the best discriminators in general. The most sensitive marker for bone metastases was a novel marker of high bone turnover alpha-telopeptide collagen type I ( $\alpha\alpha$ CTX), which increased the most with increasing number of bone metastases. This marker provides an index of bone resorption of newly formed bone (8-10) by measuring the release of the nonisomerized form of the CTX epitope. The resorption of mature bone can be

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assessed by measuring the release of isomerized CTX ( $\beta\beta$ CTX); however, this is not as sensitive for the detection of bone metastases as  $\alpha\alpha$ CTX. The CTX epitope is generated by cathepsin K released by osteoclasts, thus being specific for bone resorption (11).

A characteristic of prostate cancer is the high levels of prostate-specific antigen (PSA) due to the increased numbers of prostate epithelial cells. PSA has become the routine marker for screening for prostate cancer, detection of recurrence, bone metastases, and response to treatment (6). Total alkaline phosphatase (tALP) is a bone formation marker, used as a routine marker for skeletal involvement; however, recent publications have pointed to the fact that bone-specific ALP is a better choice as an index of bone formation due to its higher specificity for bone (12-14).

**Objective.** The use of PSA and tALP as routine markers has become a topic for urologist whether these are the appropriate markers for detection of bone metastasis. Therefore, the aim of this study was a head-to-head comparison between these two routine markers and the novel marker  $\alpha\alpha$ CTX in two different prostate cancer groups: (a) a cross-sectional study of newly diagnosed patients with clinically localized prostate cancer before radical prostatectomy (these patients were negative for bone metastases yet some had lymph node metastases and were stratified accordingly) and (b) a prospective study of hormone refractory stage prostate cancer stratified by bone metastases. All received docetaxel as palliative treatment but only patients with bone metastases were additionally administered zoledronate. Furthermore, immunohistochemistry was done on metastatic lymph nodes, nonmetastatic lymph nodes, and bone metastases, using the  $\alpha$ CTX antibody, to investigate whether the epitope was detectable in such tissues.

## Materials and Methods

### Patients and Study Design

**Cross-Sectional Study.** In this prospective study, a total of 170 histologic confirmed clinically localized prostate cancer patients were referred to the Department of Urology and Paediatric Urology, Philipps-University Marburg between January 2004 and July 2005 for having a radical retropubic prostatectomy (including regional lymphadenectomy). Sixty-eight age-matched men with benign urologic disorders served as controls. Physical examination, abdominal ultrasonography, and bone scanning using technetium-99 scintigraphy, together with X-ray or magnetic resonance imaging whenever appropriate, were used for clinical staging and to verify the absence of bone metastases.

Second void urine samples and serum samples were collected after fasting prior to radical retropubic prostatectomy and stored at  $-80^{\circ}\text{C}$ . After surgery, the disease was pathologically staged by the Institute of Pathology, Philipps-University Marburg according to the 2002 Union Internationale Contra Cancrum classification (15). The study was done in accordance with the Helsinki Declaration II and Standards of Good Clinical Practice.

**Longitudinal Study.** A total of 40 hormone refractory prostate cancer patients were referred to the Department of Urology and Paediatric Urology, Philipps-University

Marburg between December 2003 and July 2005 to receive taxan-based chemotherapy. All patients underwent bone scanning in the same manner as in the cross-sectional study to verify the presence or absence of bone metastases. Patients received four treatment cycles each lasting 4 weeks: 35 mg/m<sup>2</sup> docetaxel in weeks 1, 2, and 3, and no treatment in week 4. Patients with bone metastases additionally received 4 mg zoledronate in week 1 of every cycle. Second void urine samples and serum samples were collected after overnight fasting at baseline and at each cycle in week 1 before treatment and stored at  $-80^{\circ}\text{C}$ . The study was done in accordance with the Helsinki Declaration II and Standards of Good Clinical Practice and approved by an institutional review board at the Medical School, Philipps-University Marburg.

**Quantification of Biochemical Markers.** Samples were stored at  $-80^{\circ}\text{C}$  until assaying. Urine samples were used for estimation of high bone turnover; bone resorption by assessing the level of  $\alpha\alpha$ CTX by urinary ALPHA CrossLaps (ref. 9; Nordic Bioscience). Urinary excretion was corrected for creatinine levels measured by an automated urine analyzer (Hitachi-912, Roche). tALP was measured in heparin plasma on a clinical chemistry analyzer (Hitachi 917, Roche Diagnostics). The concentration of PSA was measured in serum samples on the Elecsys 2010 analyzer (Roche Diagnostics). All samples were tested in a blinded manner.

**Statistical Analysis.** Data shown are mean  $\pm$  SD, unless otherwise indicated. Baseline demographic characteristics were compared with Student's *t* test for unpaired observations. The values of  $\alpha\alpha$ CTX and PSA were logarithmically transformed to obtain normality. Comparison between levels of each marker in controls, patients with, and patients without lymph node metastasis was done by ANOVA using the General Linear Models Procedure of the Statistical Analysis System (SAS). The same statistical procedure was used for comparison of levels in patients in the longitudinal study during each treatment cycle. Differences and associations were considered statistically significant if  $P < 0.05$ .

**Immunohistochemistry on Lymph Node Tissue.** The analyses were done on paraffin-embedded pelvic lymph node sections from six prostate cancer patients purchased for immunohistology at Biocat GmbH. Three cases were lymph node positive for prostate cancer ( $N_1$ ) and three cases were negative ( $N_0$ ).

Paraffin was removed and antigen retrieval was done by overnight incubation of the sections in a Tris-EDTA buffer (pH 9) at  $60^{\circ}\text{C}$ . Sections were blocked in TBS containing 0.5% casein and incubated overnight at  $4^{\circ}\text{C}$  in a moist atmosphere with primary antibody diluted in TBS containing 0.5% casein or control. The primary antibodies used were monoclonal: F44 raised against the  $\alpha$ CTX epitope (Nordic Bioscience; 1:45,000 dilution of a 3.18 mg/mL stock) and another raised against cytokeratin against pan-cytokeratin (KL1 Dako; 1:400 diluted from a 0.13 mg/mL stock). A control for  $\alpha$ CTX specificity was done by overnight incubation at room temperature of F44 and 50-fold excess of antigen before use. After incubation, the sections were thoroughly washed in TBS and incubated with secondary peroxidase-labeled antibody (Mouse EnVision, Dako-Cytomation) for 30 min at room temperature. Finally,

**Table 1. Demographic data on controls and prostate cancer patients stratified by  $\pm$  lymph node metastases**

Group	Controls	PCa N <sub>0</sub>	PCa N <sub>1</sub>
<i>n</i>	68	146	24
Mean age (range), y	62.8 (48-77)	65.5 (46-71) <sup>ns</sup>	62.6 (21-79) <sup>ns</sup>
T stage (pct)	—	2 (81), 3 (18), 4 (1)	2 (25), 3 (67), 4 (8)
Gleason score (pct)	—	4 (4), 5(62), 6(16), 7(16), 8(2)	5 (16), 6 (21), 7 (42), 8 (8), 9 (13)

NOTE: N<sub>0</sub>, lymph node negative; N<sub>1</sub>, lymph node metastases; T-stage, tumor node metastasis; pct, percentage of total number of patients. Abbreviations: PCa, prostate cancer; ns, not significant different from control.

the peroxidase activity was visualized with 3,3'-diaminobenzidine+ (Sigma), and the nuclei were counterstained with Harris acidified hematoxylin. The lymph node sections were then dehydrated through a gradient of alcohol (70-99%) and toluene and mounted in dibutyl polystyrene xylene. Pictures were taken using an Olympus BX-60 light microscope equipped with an Olympus DP71 digital camera using the Cell A software from Olympus.

**Immunohistochemistry on Bone.** The analyses were done on six iliac crest or transpedicular bone metastasis bone biopsies of the spine of metastatic prostate cancer patients from archive material from the University Hospital Hamburg-Eppendorf, Hamburg, Germany, kindly provided by Günter Dellling. Specimens were fixed in buffered formalin (4%), decalcified by ultrasonic sound in combination with constant temperature at 24°C, and embedded in paraffin for preparation of 5- $\mu$ m-thick sections.

Immunohistochemistry on the bone tumor was done in the same manner as described for the lymph node immunohistochemistry. F44 was diluted 1:45,000 from a 3.18 mg/mL stock. Osteoclasts were visualized by their specific tartrate-resistant acid phosphatase activity.

## Results

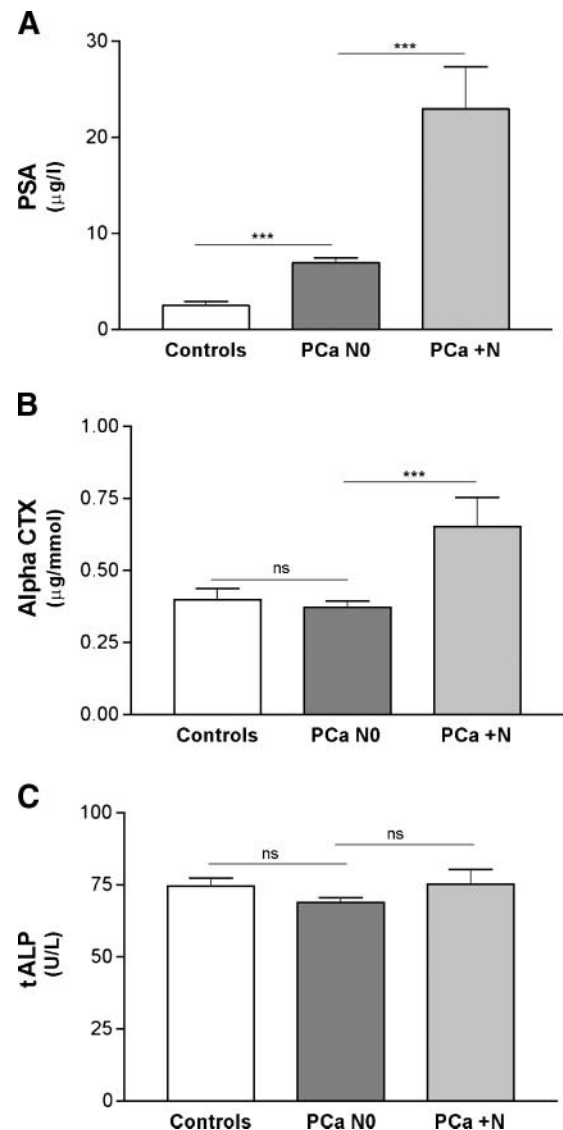
### Relation to the Extent of Lymph Metastatic Disease.

Patients were stratified by the metastatic involvement of their lymph nodes: 24 patients were lymph node positive (N<sub>1</sub>) and 146 patients were lymph node negative (N<sub>0</sub>). Demographic data are shown in Table 1. There was no significant difference in age between the groups. As expected, the tumor node metastases (T stage) and Gleason score showed that patients in the N<sub>1</sub> group had more progressed disease than those in the N<sub>0</sub> group (Table 1).

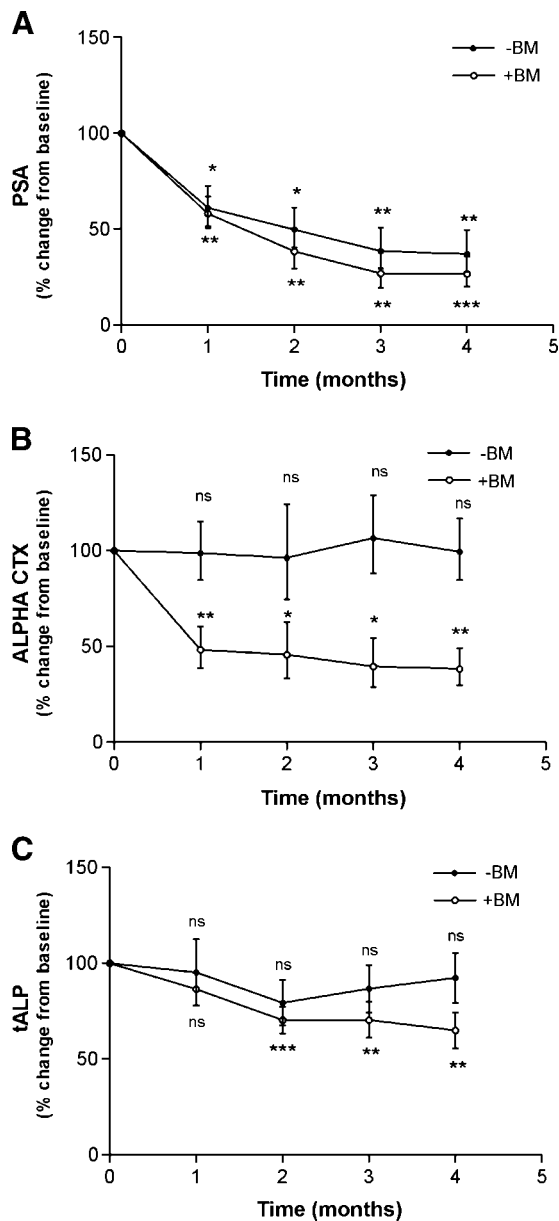
The mean levels of PSA,  $\alpha$ CTX, and tALP were stratified according to metastatic disease in the lymph nodes compared with controls, as seen in Fig. 1. PSA increased significantly in both the N<sub>0</sub> group compared with controls ( $P < 0.001$ ) and in the N<sub>1</sub> group compared with the N<sub>0</sub> group ( $P < 0.001$ ; Fig. 1A).  $\alpha$ CTX was not elevated in N<sub>0</sub> patients compared with controls (Fig. 1B), whereas it was highly elevated in N<sub>1</sub> patients compared with N<sub>0</sub> ( $P < 0.001$ ). No difference could be detected in the tALP across the three groups (Fig. 1C).

**Monitoring Response to Therapy.** Patients were stratified by the metastatic involvement of their bones: 26 patients were positive for bone metastases (+BM) and 14 patients were negative for bone metastases (-BM). Six +BM patients did not participate in follow-up due to

discontinuing of treatment. Ten patients were lymph node positive in the -BM group. Hormonal therapy was unchanged during the observation period. Mean age was 72 years (range, 42-87 years).



**Figure 1.** Levels of PSA (A),  $\alpha$ CTX (B) and (C) tALP in prostate cancer patients (PCa) without bone metastases stratified by +N and N<sub>0</sub> and controls. Asterisks indicate significant difference between the two groups; ns, not significant different between the two groups.



**Figure 2.** Levels of PSA (A),  $\alpha$ CTX (B), and tALP (C) in prostate cancer patients with (+BM) or without (-BM) bone metastases at each visit. Asterisks indicate significant decrease in the level of each marker in patients +BM compared with baseline.

Relative levels of PSA,  $\alpha$ CTX, and tALP were calculated at each visit as the percentage change of the marker level in relation to the baseline level (Fig. 2). PSA (Fig. 2A) showed a significant decrease in both the docetaxel and docetaxel/zoledronate groups at each visit. In contrast, the relative decrease in  $\alpha$ CTX (Fig. 2B) in patients +BM receiving docetaxel/zoledronate was significantly decreased compared with baseline at all time points ( $P < 0.01$  at months 1 and 4;  $P < 0.05$  at months 2 and 3), indicating a response to zoledronate treatment detected by  $\alpha$ CTX.  $\alpha$ CTX was not affected

by the docetaxel treatment in the -BM group. For tALP, the levels were decreased significantly in the docetaxel/zoledronate +BM group at visits 2 to 4 ( $P > 0.001$  at month 2, and  $P > 0.01$  at months 3 and 4) but not after 1 month.

The absolute mean values for all three markers at each visit are seen in Table 2.

**Immunolocalization of the  $\alpha$ CTX Epitope in Lymph Nodes.** Immunohistochemistry was done on pelvic lymph node biopsies from six prostate cancer patients with (N<sub>1</sub>) or without (N<sub>0</sub>) metastatic disease. Figure 3A and B shows staining with cytokeratin and  $\alpha$ CTX, respectively, on N<sub>0</sub> lymph nodes. It was verified that the primary cancer had not spread to the lymph nodes due to the absence of cytokeratin staining in the tissue. In adjacent sections, no staining by  $\alpha$ CTX was observed. Cytokeratin staining was detected in the N<sub>1</sub> lymph node, confirming the presence of metastatic cancer (Fig. 3C). Staining by  $\alpha$ CTX in the N<sub>1</sub> lymph node was observed as island-like staining in the tissue (Fig. 3D). The negative control for  $\alpha$ CTX on lymph node sections showed no staining (data not shown).

**Immunolocalization of the  $\alpha$ CTX Epitope in Bone Metastases.** Immunohistochemistry was done on bone metastasis biopsies from six prostate cancer patients to investigate whether the  $\alpha$ CTX epitope was present. Figure 4A to D shows adjacent sections from bone invaded by prostate cancer cells. Cytokeratin staining and areas of hyperchromatic nuclei confirmed the presence of tumor cells in the bone metastases (Fig. 4A). Numerous osteoclasts were revealed by tartrate-resistant acid phosphatase staining showing abnormally high numbers of osteoclasts in the bone tumor (Fig. 4B). In the proximity of tumor cells and osteoclasts, a diffuse  $\alpha$ CTX staining was observed (Fig. 4C) with intense staining at sites of high bone turnover. Control showed no staining (Fig. 4D).

## Discussion

This is the first treatment study revealing longitudinal data for the novel high bone turnover marker  $\alpha$ CTX compared with two routinely used markers in patients with different stages of prostate cancer. Furthermore, these are first data on  $\alpha$ CTX in prostate cancer patients stratified by  $\pm$ lymph node metastasis supported by immunohistochemistry. We made a head-to-head comparison between the two routine markers PSA and tALP used for determining the progression of prostate cancer and  $\alpha$ CTX in a cross-sectional study of prostate cancer patients stratified by lymph node metastasis as well as in a longitudinal treatment study with patients treated with docetaxel and zoledronate.

Briefly, the findings were as follows: (a) crosssection tALP was not elevated in any of the groups compared with controls, whereas PSA was elevated in both cancer groups compared with controls or lymph node-negative patients. In contrast,  $\alpha$ CTX was significantly elevated in lymph node-positive prostate cancer patients compared with lymph node-negative patients, but not in lymph node-negative patients compared with controls. (b) The percentage decrease in  $\alpha$ CTX and tALP in patients +BM, but not in the -BM group, was significant when

**Table 2. Marker values on prostate cancer patients stratified by  $\pm$  bone metastases and visit from the treatment study**

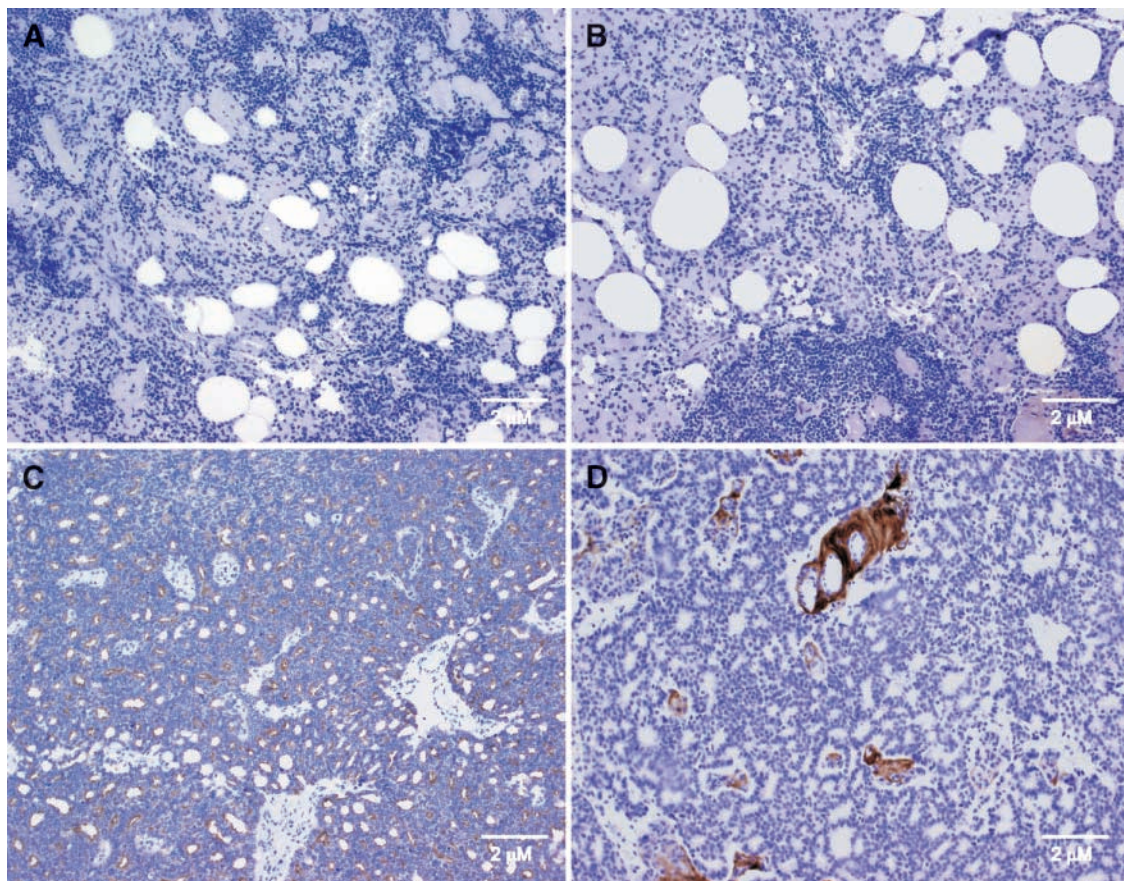
	BM	Visit				
		0	1	2	3	4
$\alpha$ CTX ( $\mu$ g/mmol; $\pm$ SD)	-	0.25 (0.17-0.19)	0.23 (0.18-0.22) <sup>ns</sup>	0.21 (0.18-0.23) <sup>ns</sup>	0.23 (0.17-0.20) <sup>ns</sup>	0.24 (0.18-0.21) <sup>ns</sup>
	+	0.89 (0.64-0.79)	0.43 (0.25-0.29)**	0.35 (0.23-0.27)*	0.31 (0.20-0.25)*	0.33 (0.19-0.22)**
tALP (units/L; $\pm$ SD)	-	89 (54-62)	76 (39-45) <sup>ns</sup>	70 (35-40) <sup>ns</sup>	76 (60-73) <sup>ns</sup>	73 (38-43) <sup>ns</sup>
	+	246 (178-221)	185 (142-178) <sup>ns</sup>	125 (92-115)***	111 (78-95)**	120 (65-76)**
PSA ( $\mu$ g/L; $\pm$ SD)	-	96 (170-263)	120 (189-263)*	115 (201-329)*	56 (133-285)**	44 (94-177)**
	+	115 (142-212)	119 (141-205)**	74 (109-179)**	34 (57-103)**	45 (67-111)***

Asterisks: Significant difference between PCa + BM or PCa - BM compared to baseline.

compared with baseline level. This was not the case for PSA, which decreased in both the -BM and +BM groups during treatment. (c) Immunohistochemistry on lymph nodes showed staining for  $\alpha$ CTX in metastatic lymph node. (d) Immunohistochemistry on bone metastasis from prostate cancer patients revealed staining for  $\alpha$ CTX in proximity of tumor cells and high number of osteoclasts.

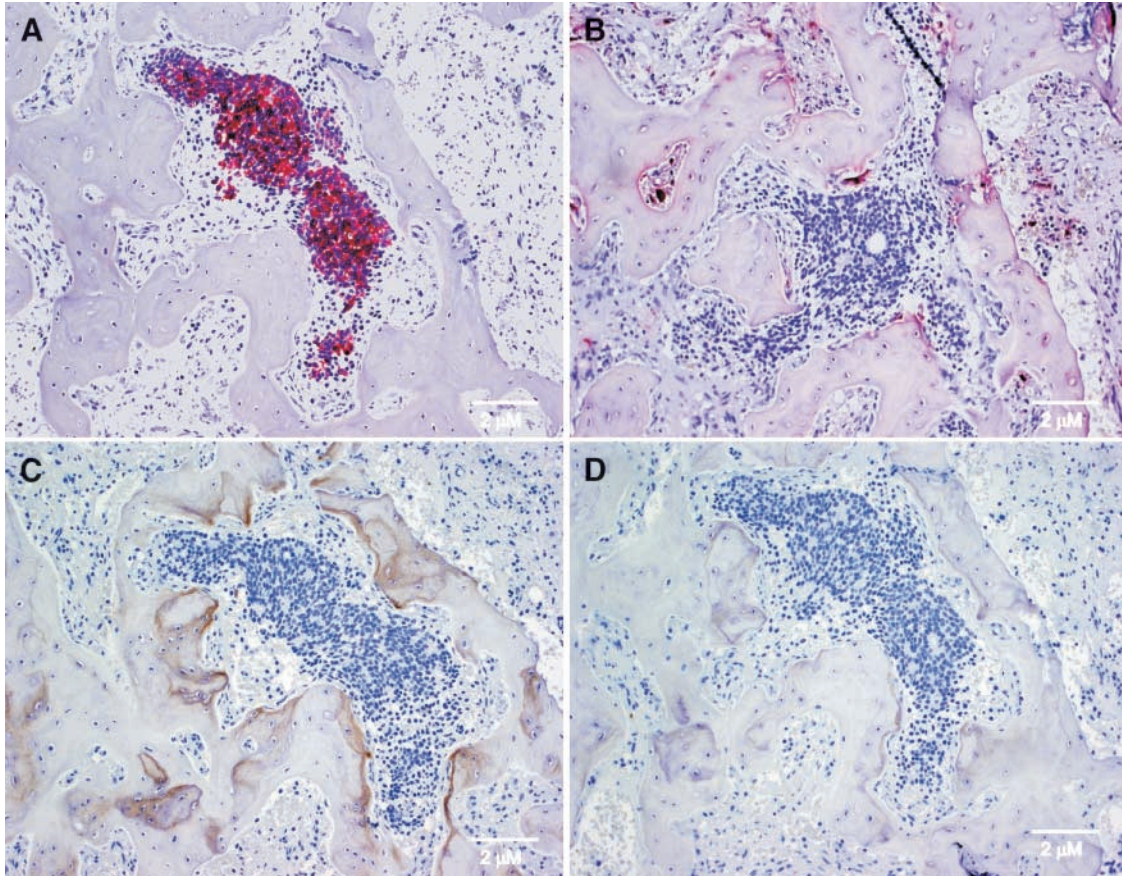
**Biomarkers for Routine Use in Prostate Cancer Patients.** Emerging evidence suggests that biochemical markers have the potential for early detection of bone

metastases compared with traditional imaging techniques (8, 13, 14, 16-19). PSA is the most extensively used biomarker for determining prostate cancer stage and for following the response to systemic treatment of metastatic disease. The general use and confidence in PSA as a marker has become controversial because studies have revealed a high occurrence of false-negative diagnosis of prostate cancer done from PSA assessments (20, 21). This may also compromise the use of this marker for determining skeletal involvement.



**Figure 3.** Immunohistochemistry on pelvic lymph node biopsies from six prostate cancer patients. **A** and **B**, lymph node-negative adjacent sections; **C** and **D**, lymph node positive. **A** and **C**, pan-cytokeratin staining (KL1); **B** and **D**,  $\alpha$ CTX CTX staining (F44). Magnification,  $\times 10$  (**A-D**).





**Figure 4.** Immunohistochemistry and histology on bone tumor tissue secondary from six primary prostate cancer patients. **A**, pan-cytokeratin (CK-MNF); **B**, tartrate-resistant acid phosphatase–positive staining of osteoclasts; **C**,  $\alpha$ CTX CTX staining (F44); **D**, control (adjacent sections). Magnification,  $\times 10$  (**A–D**).

Physiologically, it is not anticipated that PSA is specific for bone metastases. PSA is highly expressed by prostate cancer cells as well as by normal prostate epithelial cells, and thus is helpful for following tumor behavior, but its use is compromised as indicator for bone metastases in which osteoclasts and osteoblasts are the main cells involved in the vicious cycle. Total ALP has been the most used marker for detecting increased bone formation in metastatic prostate cancer (12), being highly elevated as they mainly develop sclerotic bone lesions. tALP consists of a pool of isoforms originating from various tissues such as liver, kidney, and bone, and in healthy individuals only  $\sim 50\%$  of the tALP originates from bone remodeling (12).  $\alpha$ CTX is a highly bone-specific resorption marker released during the pathology of bone metastases. Here the normal bone turnover is disassociated, and bone resorption occurs on newly formed bone. Although the bone lesions are mainly sclerotic, a high level of bone resorption is ongoing within these lesions together with the fact that many patients will have a mix of sclerotic and lytic lesions (22).

**Biomarkers in Lymph Node Metastatic Patients.** The data represented here show that PSA could discriminate between patients with and without metastatic disease in the lymph nodes; however, the elevation in patients without

lymph node metastasis compared with controls revealed the nonspecificity for metastases. In contrast,  $\alpha$ CTX was more related to the metastatic disease than were PSA and tALP. We speculate that patients with a high risk of having developing or hidden bone metastases with the first cancer cells spread to the skeleton could be identified by  $\alpha$ CTX.

A limited amount of information is available on markers assessed in prostate cancer patients stratified by lymph node metastases. Part of the author group has previously investigated PSA compared with a number of bone markers in which they found the same pattern for PSA (17). However, they reported that tALP was significantly elevated in patients with localized disease and in lymph node–positive patients, which was not observed in the present cohort. The reason for this discrepancy is unclear. To further elucidate the role of tALP, a study with a higher number of patients should be performed. Another group (13) additionally investigated the ability of a formation marker to diagnose and predict metastatic spread in prostate cancer patients stratified by lymph node and bone metastasis compared with nine other serum markers. They showed that bone formation markers, a bone resorption marker, and two osteoclastogenesis markers were elevated in patients with bone

metastases, of which the osteoclastogenesis marker had the best discriminating power.

**Biomarkers in Bone Metastatic Patients.**  $\alpha\alpha$ CTX and ALP were unaffected by the docetaxel treatment in the –BM group and simultaneously affected by the zoledronate treatment in the +BM group. However, it is clearly seen that there is a trend that tALP decreases with docetaxel treatment of –BM patients, whereas this is not the case for  $\alpha\alpha$ CTX. Additionally, it was observed that  $\alpha\alpha$ CTX decreased significantly after the first month of treatment with docetaxel/zoledronate, in contrast to tALP, which was significantly decreased after 2 months of treatment. This observation is in line with the knowledge that resorption measurements are more dynamic than formation measurements, as well as the fact that a formation cycle follows a resorption cycle. This implies higher specificity for monitoring of the bone-targeting treatment of bone metastases by  $\alpha\alpha$ CTX. Monitoring by PSA and tALP seemed to be influenced by the docetaxel treatment; especially for PSA, the levels were similar in the two patient groups, which complicates the evaluation of skeletal involvement and response to treatment of bone metastases. The inclusion of control groups would have been interesting to further investigate the sensitivity of  $\alpha\alpha$ CTX for bone metastases; however, for ethical reasons such a group did not exist.

$\alpha\alpha$ CTX mean levels in the +BM group reached those of the –BM group at visit 2 subsequent to 2 months of docetaxel/zoledronate therapy, indicating a normalization of the bone turnover of young bone within the bone metastases in these patients at this point. Mean levels of PSA were at the same level in both treatment groups at all visits. This suggests that PSA can be used for monitoring docetaxel treatment of the primary tumor cells without any reflection on bone turnover. Normalization was also seen in tALP, although the percentage decrease relative to baseline was the same in the two different treatment groups.

Other groups have compared bone markers with PSA and tALP. Five bone turnover markers were compared with PSA and tALP in a prostate cancer cohort stratified by  $\pm$ BM treated with zoledronate (23). In this group, none of the markers were significantly elevated at baseline in the progression group compared with patients without progression. During treatment, the CTX marker for mature bone resorption ( $\beta\beta$ CTX) decreased the most among all markers. As we have assessed the  $\alpha\alpha$ CTX, we cannot directly compare the results, but still it seems that in this cohort a collagenous marker was superior to PSA and tALP. In another study, the markers tartrate-resistant acid phosphatase 5b, matrix metalloproteinase-2, and matrix metalloproteinase-9 were compared with PSA and tALP in a prostate group stratified by  $\pm$ BM (24). Here tALP and PSA were the only markers significantly increased in patients +BM compared with –BM. The matrix metalloproteinase markers were not recommended for detection of bone metastases. This illustrates that not all markers are superior to PSA and tALP. The matrix metalloproteinase-derived collagenous marker COOH-terminal telopeptide of type I collagen was additionally compared with PSA and tALP in a group of prostate cancer patients and evaluated on the relation to number of bone metastases (14). Here, COOH-terminal telopeptide of

type I collagen was not well correlated to bone metastases and was not a superior marker for bone metastases compared with PSA and tALP. Because COOH-terminal telopeptide of type I collagen is degraded by cathepsin K produced by osteoclasts, it was, however, not expected to be bone specific but rather specific for matrix metalloproteinase activity (25).

**Immunohistochemical Results.** The lymph node–negative stainings revealed no staining for cytokeratin and  $\alpha\alpha$ CTX as expected. Cytokeratin staining was positive in the metastatic lymph nodes showing an invasion by prostate cancer cells; however, the positive staining for  $\alpha\alpha$ CTX was not expected due to its cathepsin K specificity. Some groups have revealed that prostate and breast cancer cells are able to produce cathepsin K (26, 27), which could explain why  $\alpha\alpha$ CTX is located in the presence of tumor cells. Nevertheless, 10 lymph node–positive patients from a total of 14 were included in the –BM group in the treatment study, and  $\alpha\alpha$ CTX did not decrease with docetaxel treatment. The docetaxel treatment should have a diminishing effect on the tumor cells that have metastasized to the lymph nodes, and thus should decrease  $\alpha\alpha$ CTX during this treatment. This suggests that the  $\alpha\alpha$ CTX assessed in urine measurements originates from osteoclastic bone resorption and thus is specific for bone.

The bone staining showing cytokeratin, tartrate-resistant acid phosphatase, and  $\alpha\alpha$ CTX stainings within the bone metastases showed that  $\alpha\alpha$ CTX could be found in the proximity of tumor cells invading bone tissue, as previously shown in bone metastasis biopsies from breast cancer patients (10).

## Conclusion

$\alpha\alpha$ CTX was the only marker unaffected by the primary cancer and elevated in metastatic disease, indicating a potential for identification of patients at high risk of having hidden bone metastases. In addition, the marker was unaffected by docetaxel treatment in patients without bone metastases, whereas it decreased with docetaxel and zoledronate treatment in patients with bone metastases, suggesting specificity for bone-targeting therapy. The epitope, furthermore, was located in the proximity of invading prostate cancer cells in the lymph nodes and bone.

## Disclosure of Potential Conflicts of Interest

P. Qvist, M. Karsdal, D. Leeming, and I. Byrjalsen: Nordic Bioscience employees.

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## References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;55:74–108.
2. Oefelein MG, Ricchiuti V, Conrad W, Resnick MI. Skeletal fractures

- negatively correlate with overall survival in men with prostate cancer. *J Urol* 2002;168:1005–7.
3. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev* 2001;27:165–76.
  4. Yin JJ, Pollock CB, Kelly K. Mechanisms of cancer metastasis to the bone. *Cell Res* 2005;15:57–62.
  5. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med* 2004;350:1655–64.
  6. Williams SA, Singh P, Isaacs JT, Denmeade SR. Does PSA play a role as a promoting agent during the initiation and/or progression of prostate cancer? *Prostate* 2007;67:312–29.
  7. Lester J, Coleman R. The use of bisphosphonates in breast cancer. *J Br Menopause Soc* 2005;11:12–7.
  8. Leeming DJ, Koizumi M, Byrjalsen I, Li B, Qvist P, Tanko LB. The relative use of eight collagenous and noncollagenous markers for diagnosis of skeletal metastases in breast, prostate, or lung cancer patients. *Cancer Epidemiol Biomarkers Prev* 2006;15:32–8.
  9. Cloos PA, Lyubimova N, Solberg H, et al. An immunoassay for measuring fragments of newly synthesized collagen type I produced during metastatic invasion of bone. *Clin Lab* 2004;50:279–89.
  10. Leeming DJ, Delling G, Koizumi M, et al. Alpha CTX as a biomarker of skeletal invasion of breast cancer: immunolocalization and the load dependency of urinary excretion. *Cancer Epidemiol Biomarkers Prev* 2006;15:1392–5.
  11. Henriksen K, Leeming DJ, Byrjalsen I, et al. Osteoclasts prefer aged bone. *Osteoporos Int* 2007;18:751–9.
  12. Fohr B, Dunstan CR, Seibel MJ. Clinical review 165: markers of bone remodeling in metastatic bone disease. *J Clin Endocrinol Metab* 2003;88:5059–75.
  13. Jung K, Lein M, Stephan C, et al. Comparison of 10 serum bone turnover markers in prostate carcinoma patients with bone metastatic spread: diagnostic and prognostic implications. *Int J Cancer* 2004;111:783–91.
  14. Kataoka A, Yuasa T, Kageyama S, et al. Diagnosis of bone metastasis in men with prostate cancer by measurement of serum ICTP in combination with alkaline phosphatase and prostate-specific antigen. *Clin Oncol (R Coll Radiol)* 2006;18:480–4.
  15. Wittekind C, Meyer HJ, Bootz F, editors. *TNM klassifikation maligner tumoren*. 6th ed. Berlin, Heidelberg, New York: Springer; 2003.
  16. Jung K, Stephan C, Semjonow A, Lein M, Schnorr D, Loening SA. Serum osteoprotegerin and receptor activator of nuclear factor- $\kappa$ B ligand as indicators of disturbed osteoclastogenesis in patients with prostate cancer. *J Urol* 2003;170:2302–5.
  17. Hegele A, Wahl HG, Varga Z, et al. Biochemical markers of bone turnover in patients with localized and metastasized prostate cancer. *BJU Int* 2007;99:330–4.
  18. Garnero P, Buchs N, Zekri J, Rizzoli R, Coleman RE, Delmas PD. Markers of bone turnover for the management of patients with bone metastases from prostate cancer. *Br J Cancer* 2000;82:858–64.
  19. Akimoto S, Furuya Y, Akakura K, Ito H. Comparison of markers of bone formation and resorption in prostate cancer patients to predict bone metastasis. *Endocr J* 1998;45:97–104.
  20. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level  $\leq$  4.0 ng per milliliter. *N Engl J Med* 2004;350:2239–46.
  21. Fang J, Metter EJ, Landis P, Chan DW, Morrell CH, Carter HB. Low levels of prostate-specific antigen predict long-term risk of prostate cancer: results from the Baltimore Longitudinal Study of Aging. *Urology* 2001;58:411–6.
  22. O'Keefe RJ, Guise TA. Molecular mechanisms of bone metastasis and therapeutic implications. *Clin Orthop Relat Res* 2003;(415 Suppl): S100–4.
  23. Lein M, Wirth M, Miller K, et al. Serial markers of bone turnover in men with metastatic prostate cancer treated with zoledronic acid for detection of bone metastases progression. *Eur Urol* 2007;52:1381–7.
  24. Salminen EK, Kallioinen MJ, Ala-Houhala MA, et al. Survival markers related to bone metastases in prostate cancer. *Anticancer Res* 2006;26:4879–84.
  25. Garnero P, Ferreras M, Karsdal MA, et al. The type I collagen fragments ICTP and CTX reveal distinct enzymatic pathways of bone collagen degradation. *J Bone Miner Res* 2003;18:859–67.
  26. Littlewood-Evans AJ, Bilbe G, Bowler WB, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. *Cancer Res* 1997;57:5386–90.
  27. Brubaker KD, Vessella RL, True LD, Thomas R, Corey E. Cathepsin K mRNA and protein expression in prostate cancer progression. *J Bone Miner Res* 2003;18:222–30.



# BLOOD CANCER DISCOVERY

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