

# Prognostic Value of PINP, Bone Alkaline Phosphatase, CTX-I, and YKL-40 in Patients With Metastatic Prostate Carcinoma

Klaus Brasso,<sup>1</sup> Ib Jarle Christensen,<sup>2,3</sup> Julia S. Johansen,<sup>4,5\*</sup> Børge Teisner,<sup>6</sup> Patrick Garnero,<sup>7</sup> Paul A. Price,<sup>8</sup> and Peter Iversen<sup>1</sup>

<sup>1</sup>Department of Urology, H:S Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup>Finsen Laboratory, H:S Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

<sup>3</sup>Department of Surgical Gastroenterology, H:S Hvidovre Hospital, Hvidovre, Denmark

<sup>4</sup>Department of Rheumatology, H:S Hvidovre Hospital, Hvidovre, Denmark

<sup>5</sup>Department of Rheumatology, Herlev Hospital, University of Copenhagen, Herlev, Denmark

<sup>6</sup>Department of Immunology and Microbiology, University of Odense, Odense, Denmark

<sup>7</sup>Molecular Markers, Synarc, Lyon, France

<sup>8</sup>Department of Biology, University of California San Diego, La Jolla, California

**BACKGROUND.** To examine the prognostic value of markers of bone metabolism (serum PINP, BAP, and CTX-I) and serum YKL-40 in metastatic prostate carcinoma (PC).

**METHODS.** The biomarkers were determined by ELISAs in 153 metastatic PC patients before treatment with parenteral estrogen or total androgen ablation. The median follow-up was 4.9 years. One hundred fifteen patients died.

**RESULTS.** The biomarkers were increased in the patients compared to controls ( $P < 0.001$ ), and related to performance status and Soloway score (except YKL-40), but not to T-category and WHO tumor grade. PINP was elevated in 87%, BAP (55%), CTX-I (33%), and YKL-40 (43%). Univariate analysis showed an association to survival: PINP (HR = 1.6,  $P < 0.0001$ ), BAP (HR = 1.4,  $P < 0.0001$ ), CTX-I (HR = 1.7,  $P < 0.0001$ ), and YKL-40 (HR = 1.4,  $P = 0.004$ ). In multivariate Cox analysis performance status, WHO grade, Soloway score, PINP, and YKL-40 were independently predictive factors.

**CONCLUSIONS.** High serum PINP, BAP, CTX-I, and YKL-40 are associated with poor outcome of metastatic PC patients. *Prostate* 66: 503–513, 2006. © 2005 Wiley-Liss, Inc.

**KEY WORDS:** biomarkers; bone metastases; CHI3L1

## INTRODUCTION

Prostate carcinoma (PC) is the most frequent cancer in males and is estimated to be responsible for 29,900 deaths in United States in 2004 [1]. In patients diagnosed at an early time in the natural history of the disease, a number of prognostic factors including prostate-specific antigen (PSA), tumor grade, and clinical stage can be used to predict outcome of primary treatment and prognosis. However, in patients in later stages (i.e., patients developing a hormone refractory PC) the prognostic significance of PSA declines [2,3].

Part of the results has previously been presented at the ASCO meeting in Chicago 2003, abstract number 1525.

Grant sponsor: Dagmar Marshalls Foundation; Grant sponsor: Danish Medical Research Council; Grant sponsor: Else og Mogens Wedell-Wedellsborg Fond; Grant sponsor: Michaelsen Fonden; Grant sponsor: Sigvald and Edith Rasmussens Legat.

\*Correspondence to: Julia S. Johansen, MD, Department of Rheumatology Q107, Herlev Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark. E-mail: julia.johansen@post3.tele.dk

Received 25 February 2005; Accepted 24 May 2005

DOI 10.1002/pros.20311

Published online 21 December 2005 in Wiley InterScience

(www.interscience.wiley.com).

New prognostic biomarkers<sup>1</sup> may add to our knowledge and predict, which PC patients might benefit from additional therapy. The high frequency and poor prognosis of patients with metastatic PC emphasize the need for both additional and better biomarkers. Identification of clinically useful biomarkers could add to earlier diagnosis of metastatic PC and by their possible prognostic function, influence the treatment regimes.

Metastatic tumors in the bone interfere with normal bone remodeling by the local release of cytokines, growth factors, and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) that increase osteoclast and osteoblast activity [4,5]. This metabolic disruption results in increased bone destruction (osteolysis), increased bone formation (osteosclerosis), or both. A number of peptides and growth factors that are normal participants in the process on bone remodeling have been implicated in the development of bone metastases. Measurement of these factors in blood or urine are suggested as potential biomarkers of bone remodeling in patients with metastatic PC [5–8]. None of these biomarkers are disease-specific but reflect alterations in skeletal metabolism independently of the underlying cause.

Bone formation markers are direct or indirect products of active osteoblasts. The most commonly used markers of bone formation are total alkaline phosphatase (AP), bone alkaline phosphatase (BAP), osteocalcin, and propeptides of human procollagen type I [5–9]. BAP is present on the surface of osteoblasts, but the mechanism of its release into the circulation is unclear. Increased serum BAP are found in patients with advanced PC with bone metastases compared to patients with local disease and patients with benign prostatic hyperplasia [8–14]. Recently two large studies of PC patients with bone metastases demonstrated that patients with elevated serum BAP had shorter survival compared to patients with low serum BAP [8,9]. High serum BAP could also predict early skeletal-related events and disease progression in patients with bone metastases secondary to prostate-, lung-, renal cell-, head and neck-, and thyroid cancer [9].

Collagen type I, the most abundant collagen in most soft tissues, accounts for more than 90% of the organic bone matrix and is produced by osteoblasts in bone. During the formation of type I collagen the amino (N)- and carboxy (C)-terminal extension propeptides (PINP and PICP) are cleaved from the procollagen molecules, and serum PINP and PICP reflect the formation rate of

type I collagen molecules. Elevated serum levels of PICP and PINP are found in PC patients with bone metastases compared to patients without bone metastases or with benign prostatic hyperplasia [8,13–16]. Patients with PC and bone metastases [8,14] or metastatic breast carcinoma [17] and high serum PINP had shorter survival than patients with normal serum PINP.

The collagen type I telopeptides form short, non-helical stretches at the N- and C-termini of the collagen molecule. Most markers of bone resorption are degradation products of type I collagen [5–7], like the N- and C-terminal peptide fragments of various sizes and cross-link content. An eight-amino-acid sequence from the C-telopeptide of type I collagen (CTX-I) can be measured in serum or urine [18,19] and has been found to be a marker of bone resorption [5–8,20]. Patients with PC and bone metastases had higher serum and urine CTX-I levels compared to controls [20], and a single dose of pamidronate induced a decrease in serum and urine CTX-I in many patients within 15 days [5,20]. It has also been reported that the pretreatment level of urine CTX-I, the magnitude of the decrease in urine CTX-I after treatment, and the level it reached after treatment were predictive factors of the efficacy of pamidronate in reducing bone pain in patients with bone metastases from prostate and breast carcinoma [21]. Recently, it has been shown that serum CTX-I and NTX-I [8] and urine NTX [9] were prognostic biomarkers of survival in patients with metastatic PC, and high urine NTX could also predict early skeletal-related events and disease progression in patients with bone metastases secondary to prostate-, lung-, renal cell-, head and neck-, and thyroid cancer [9].

YKL-40<sup>2</sup> [22,23], a growth factor for connective tissue cells [24] and a migration factor for endothelial cells [25], is expressed by several types of solid human carcinoma, including PC (*dbest* database at the National Center for Biotechnology Information). In vitro, YKL-40 is secreted by the human osteosarcoma MG63 [22] and prostate cancer cell lines DU-145 and PC-3 (personal observation). The gene for YKL-40 is known (CHI3L1) [26] and its crystal structure is described [27], but the site and mode of binding to cell surface receptors is not yet known. Increased serum levels of YKL-40 are found in patients with primary and metastatic carcinoma of the breast (including patients with bone metastases) [28,29], colorectal [30], ovary [31,32], and lung [33]. The studies also showed that high serum YKL-40 was related to short recurrence-free interval and short survival and serum YKL-40 was

<sup>1</sup>Biomarker (biological marker): A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

<sup>2</sup>The protein has several names: “YKL-40” [22]; “Human Cartilage glycoprotein-39 (HC gp39)” [23]; “Breast regressing protein 39 Kd (brp-39)””; “38-kDa heparin-binding glycoprotein (Gp38k)””; “CHI3L1” [26]; “Chondrex” [39]; and “Chitinase 3-like protein” [24].

independent of other prognostic variables [28–33]. The prognostic value of serum YKL-40 in patients with local or advanced PC has never been evaluated.

The purpose of this study was to compare the performance of three biomarkers of bone turnover (serum PINP, BAP, CTX-I) and the growth factor YKL-40 in identifying the increased osteosclerotic activity of patients with advanced PC and to examine their clinical usefulness for prediction of poor prognosis.

## MATERIALS AND METHODS

### Patients

The study included 153 men (median age 72, range 54–89 years) with metastatic PC (Stage T1–4, Nx, M+, Soloway score 1–3) and a WHO performance status of 0–2. The patients were from 11 Danish hospitals and included in the SPCG5 study of patients with metastatic PC treated with parenteral estrogen versus total androgen ablation [34]. The patients were enrolled in the period from November 1993 to June 1996 and randomized to treatment with either total androgen ablation (either bilateral orchiectomy or triptorelin 3.75 mg every month combined with the antiandrogen flutamide 250 mg three times daily) or parenteral estrogen (intramuscular injections of 240 mg polyestradiol phosphate every second week for the first 8 weeks (five doses) followed by a maintenance dose of 240 mg every month). The choice between orchiectomy and triptorelin was at the discretion of the clinician and patient. Staging was based on histologic and/or cytologic findings, digital rectal examination, and the assessment of metastases to bone at time of diagnosis was based on plain X-ray skeletal surveys and radioisotopic bone imaging. Extent of osseous dissemination was graded according to Soloway [35]. Patients previously given systemic treatment for PC, previously diagnosed with another malignant disease, with a myocardial or cerebral infarction within the past month, with previous or present liver disease, or not believed capable of following the study rules were excluded.

The patients were followed in the out-patient clinic until death or up to 31 January 2000. The median follow-up was 4.9 years (3.7–9.2). Time to death was measured from the date of starting treatment. At each follow-up examination, the assessment included medical history, clinical examination, a full blood count, liver enzymes, and PSA. Two hundred fourteen Danish patients were included in the study. However, serum samples for measurements of PINP, BAP, and YKL-40 concentrations were only available from 150, 151, and 152 patients, respectively at inclusion and from 137 patients for serum CTX-I with complete marker data was available from 136 patients. At time of follow up,

115 (76%) of the patients had died and the median survival time after diagnosis of advanced PC was 25 months (95% confidence interval (CI) 20–31 months). Eighty one of the patients died of PC, 14 died probably of PC, 3 died of cardiovascular causes, 8 of other diseases, and 9 died of unknown cause. Further details about the study are described elsewhere [34].

The study was performed in accordance with the Helsinki II declaration, and the patients were informed about the possibility of withdrawing from the study at any time. The Central Ethics Committee and The Danish Board of Health approved the research protocol.

### Healthy Controls

The normal range of serum BAP in 126 healthy men older than 25 years was provided by the manufacturer (Quidel, Santa Clara, CA). The median serum BAP in these controls was 23 U/L (range 15–41 U/L, cut-point set as 41 U/L). The normal range of serum CTX-I in 115 healthy men aged 41–80 years was provided by Synarc Laboratory (Lyon, France), since the serum samples from the PC patients were measured at Synarc Laboratory. The median serum CTX-I in controls was 0.300 µg/L (range 0.07–0.72 µg/L, 95th percentile was 0.638 µg/L and set as cut-point). The normal ranges of serum PINP and YKL-40 were determined in 93 healthy Danish men older than 25 years (median age of 51 years, range 26–73 years). These men were all healthy, were not taking any medicine, and had no signs or clinical symptoms of cancer, joint, liver, metabolic, or hormonal disease [36]. The median serum PINP in these controls was 49 µg/L (range 24–143 µg/L, 95th percentile was 66 µg/L and set as cut-point). The median serum YKL-40 in the controls was 47 µg/L (range 20–184 µg/L, upper 95th percentile was 104 µg/L). Serum YKL-40 increases with age in normal subjects and the age-adjusted 95th percentile was set as cut-point (see Statistical analyses).

### Biochemical Analysis

Serum total AP (upper range 275 U/L) and PSA (upper range 4 µg/L) levels were determined by routine methods. Blood samples, non-fasting, were collected at the time of inclusion. The serum was separated from the blood cells within 3 hr after venipuncture by centrifugation at 3,000 rpm for 10 min. Serum samples were stored in aliquots at –80°C until analysis. Serum BAP concentration was determined by ELISA (ALKPHASE-B ELISA, Quidel) [37]. The sensitivity of the assay was 0.7 U/L, and the intra- and inter-assay variations were <5.8% and 7.6%, respectively. Serum PINP was determined by an in-house ELISA, which measures both molecular forms of PINP [38].

The detection threshold of the assay was 0.5 µg/L, and the intra- and inter-assay variations were less than 5.3%. Serum CTX-I was determined by a two-site immunoassay [19] (Serum CrossLaps, Roche Diagnostic, Mannheim, Germany). The sensitivity of the assay was 0.01 µg/L, the intra- and inter-assay variations were less than 4% and 6%, respectively. Serum YKL-40 was determined by ELISA (YKL-40 ELISA, Quidel) [39]. The sensitivity of the assay was 10 µg/L, and the intra- and inter-assay variations were 3.6% and 5.3%.

### Statistical Analyses

The SAS<sup>®</sup> software package (version 8.2; SAS Institute, Cary, NC) was used to manage patient data and to perform all statistical analyses. Linear regression on the log scale was used to estimate the age dependence of serum YKL-40 in healthy controls. A normal reference region was calculated as described by Royston [40] on the log transformed ( $\log_e$ ) serum YKL-40 values of the healthy controls adjusting for age, and the 95% percentile was chosen as the cut-point. The serum PINP, BAP, CTX-I, and YKL-40 concentrations were  $\log_e$  transformed and treated as continuous variables for the uni- and multivariate analyses of survival. In addition, serum concentrations of PINP, BAP, CTX-I, and YKL-40 dichotomized by their respective cut-points as described above were analyzed for association to survival. The endpoint for survival analysis was death of all causes. The Kaplan–Meier method was used to estimate survival probabilities, and the log-rank test was used to test for equality of strata. The Cox proportional hazards model was applied for univariate analysis of covariates as well as for multivariate analysis. The assumption of proportional hazards was verified graphically. A concordance index [41] for 2-year survival was calculated. In addition, stepwise backwards selection was used to select covariates. Rank statistics were used to calculate correlation coefficients and to test hypotheses on location. Tests of independence were done using the chi-square test. The significance level was set to 5%.

### RESULTS

The median serum concentrations of PINP (166 µg/L, range 32–5198), BAP (52 U/L, 9–2420), CTX-I (0.368 µg/L, 0.015–5.16), and YKL-40 (112 µg/L, 20–2080) in the patients with advanced PC were significantly ( $P < 0.001$ ) higher compared to the levels in healthy men. Eighty-seven percent (130/150) of the patients had elevated serum PINP, 55% (83/151) elevated serum BAP, 33% (45/137) elevated serum CTX-I, and 43% (66/152) had elevated serum YKL-40. The median serum PSA was 270 µg/L (range 10–7730) and all patients had elevated serum PSA (i.e.,

>4 µg/L). The median serum total AP was 371 U/L (range 12.3–4390) and 64% (97/152) had elevated serum total AP (i.e. >275 U/L). Table I gives the serum concentrations of PINP, BAP, CTX-I, and YKL-40 in the PC patients according to performance status, T-category, WHO tumor grade, and the extent of bone metastases (Soloway score) [35] before treatment. All four biomarkers increased with increasing performance status. Serum PINP, BAP, and CTX-I increased with higher Soloway score, whereas serum YKL-40 was not related to this score (Table I). Figure 1 illustrates the individual serum concentrations of PINP, BAP, CTX-I, and YKL-40 in the patients according to Soloway score. None of the biomarkers were related to T-category and WHO tumor grade.

In these patients with metastatic PC positive correlations were found between serum PINP and BAP ( $R_S = 0.87$ ,  $P < 0.0001$ ), but not between serum YKL-40 and BAP ( $R_S = 0.04$ ) and PINP ( $R_S = 0.10$ ). Serum CTX-I correlated with serum PINP ( $R_S = 0.73$ ,  $P < 0.0001$ ), BAP ( $R_S = 0.61$ ,  $P < 0.0001$ ), and YKL-40 ( $R_S = 0.18$ ,  $P = 0.04$ ). Serum PSA correlated with serum PINP ( $R_S = 0.35$ ,  $P < 0.0001$ ), BAP ( $R_S = 0.35$ ,  $P < 0.0001$ ), CTX-I ( $R_S = 0.25$ ,  $P = 0.004$ ), and YKL-40 ( $R_S = 0.16$ ,  $P = 0.05$ ). Total AP correlated with PINP ( $R_S = 0.72$ ,  $P < 0.0001$ ), BAP ( $R_S = 0.83$ ,  $P < 0.0001$ ), and CTX-I ( $R_S = 0.63$ ,  $P < 0.0001$ ) but not to YKL-40 ( $R_S = 0.09$ ,  $P = 0.26$ ).

In the original paper [34], no difference was found in overall survival in the two treatment groups. In our subgroup of patients, we found survival to be independent of treatment, thus all patients were evaluated together for the survival analysis. Figure 2 illustrates the survival plots when the patients were grouped by tertiles according to their pretreatment serum levels of PINP (Fig. 2a), BAP (Fig. 2b), CTX-I (Fig. 2c), and YKL-40 (Fig. 2d). Significantly shorter survival was found between the three groups of patients according to increasing tertiles for serum PINP, BAP, and CTX-I levels. Patients with normal or a small increase in serum YKL-40 had similar survival, whereas patients with serum YKL-40 levels in the highest tertile had significantly shorter survival compared to the other two groups. Serum PINP dichotomized by its cut-point showed poor survival for patients with elevated levels (HR = 2.4, 95% confidence interval (CI): 1.2–4.6,  $P = 0.007$ ). Similar analyses of serum YKL-40, BAP, and CTX-I were demonstrated (YKL-40: HR = 1.4, 95% CI: 1.0–2.0,  $P = 0.07$  (Wilcoxon's test  $P = 0.008$ ); BAP: HR = 1.8, 95% CI: 1.3–2.7,  $P = 0.002$ ; and CTX-I: HR = 2.7, 95% CI: 1.8–4.1,  $P < 0.0001$ ).

Univariate analysis of the serum levels of PINP, BAP, CTX-I, and YKL-40 (logarithmically transformed and treated as continuous variables) showed highly significant associations between overall survival and

**TABLE I. Serum PINP, BAP, CTX-I, and YKL-40 Levels in Patients With Metastatic Prostate Carcinoma According to Performance Status, Tumor Stage, T-category, and Soloway Score Before Treatment**

Characteristics	Serum PINP $\mu\text{g/L}$	Serum BAP U/L	Serum CTX-I $\mu\text{g/L}$	Serum YKL-40 $\mu\text{g/L}$
Performance status <sup>a</sup>				
0	130 (32–3112) <sup>76</sup>	38 (9–2420) <sup>75</sup>	0.28 (0.015–3.19) <sup>69</sup>	103 (22–975) <sup>77</sup>
1	256 (60–3412) <sup>54</sup>	68 (13–1180) <sup>55</sup>	0.55 (0.05–5.16) <sup>51</sup>	108 (20–1060) <sup>54</sup>
2	296 (50–5198) <sup>20</sup>	109 (9–1040) <sup>20</sup>	0.61 (0.13–5.05) <sup>16</sup>	242 (30–2080) <sup>20</sup>
	$P = 0.0002$	$P = 0.005$	$P = 0.002$	$P = 0.04$
T-category <sup>a</sup>				
T1	135 (64–2197) <sup>5</sup>	17 (15–1180) <sup>5</sup>	0.58 (0.10–5.16) <sup>5</sup>	340 (69–828) <sup>5</sup>
T2	109 (34–1175) <sup>18</sup>	36 (13–1040) <sup>18</sup>	0.28 (0.09–1.87) <sup>16</sup>	103 (35–1060) <sup>19</sup>
T3	189 (32–3412) <sup>62</sup>	72 (9–1040) <sup>62</sup>	0.46 (0.015–3.62) <sup>61</sup>	109 (20–975) <sup>62</sup>
T4	168 (37–5198) <sup>64</sup>	51 (9–2420) <sup>65</sup>	0.32 (0.05–5.04) <sup>54</sup>	115 (22–2080) <sup>65</sup>
	$P = 0.51$	$P = 0.33$	$P = 0.48$	$P = 0.26$
WHO tumor grade <sup>b</sup>				
1	133 (37–1402) <sup>28</sup>	56 (9–700) <sup>29</sup>	0.43 (0.015–2.51) <sup>25</sup>	161 (37–975) <sup>29</sup>
2	169 (42–3412) <sup>55</sup>	55 (10–2420) <sup>55</sup>	0.32 (0.05–5.04) <sup>52</sup>	106 (20–2080) <sup>56</sup>
3	188 (32–5198) <sup>61</sup>	50 (12–1180) <sup>61</sup>	0.58 (0.07–5.16) <sup>55</sup>	116 (22–1060) <sup>61</sup>
	$P = 0.42$	$P = 0.66$	$P = 0.55$	$P = 0.40$
Soloway score <sup>a</sup>				
1	71 (32–1700) <sup>43</sup>	20 (9–440) <sup>44</sup>	0.24 (0.07–1.53) <sup>40</sup>	99 (26–860) <sup>44</sup>
2	221 (42–5198) <sup>94</sup>	75 (13–2420) <sup>94</sup>	0.45 (0.015–5.16) <sup>86</sup>	115 (20–2080) <sup>96</sup>
3	523 (304–2279) <sup>11</sup>	296 (38–830) <sup>11</sup>	1.46 (0.62–2.53) <sup>9</sup>	146 (38–380) <sup>10</sup>
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.69$

Values are medians (range)<sup>number</sup>.

<sup>a</sup>One patient is missing.

<sup>b</sup>Six patients are missing.

each of the four biomarkers (Table II, left). As expected performance status, WHO grade, Soloway score, and serum AP were also highly significantly associated with overall survival (Table II, left). Age, T-category, and serum PSA were not related to overall survival.

Multivariate Cox regression analysis of overall survival was performed including age, performance status, T-category, WHO grade, Soloway score, and serum PSA and each of the four biomarkers separately (log transformed and treated as continuous variables). Serum PINP (HR = 1.4, 95% CI: 1.2–1.7,  $P = 0.0007$ ), serum BAP (HR = 1.2, 95% CI: 1.0–1.5,  $P = 0.05$ ), serum CTX-I (HR = 1.3, 95% CI: 1.0–1.7,  $P = 0.02$ ), and serum YKL-40 (HR = 1.3, 95% CI: 1.0–1.6,  $P = 0.03$ ) were found to be independent prognostic variables of short survival when tested separately in the multivariate analysis. Serum AP was also significant when entered into the model separately (HR = 1.4, 95% CI: 1.1–1.9,  $P = 0.01$ ). The concordance index for 2-year survival was 0.78 suggesting that the markers have reasonable predictive value.

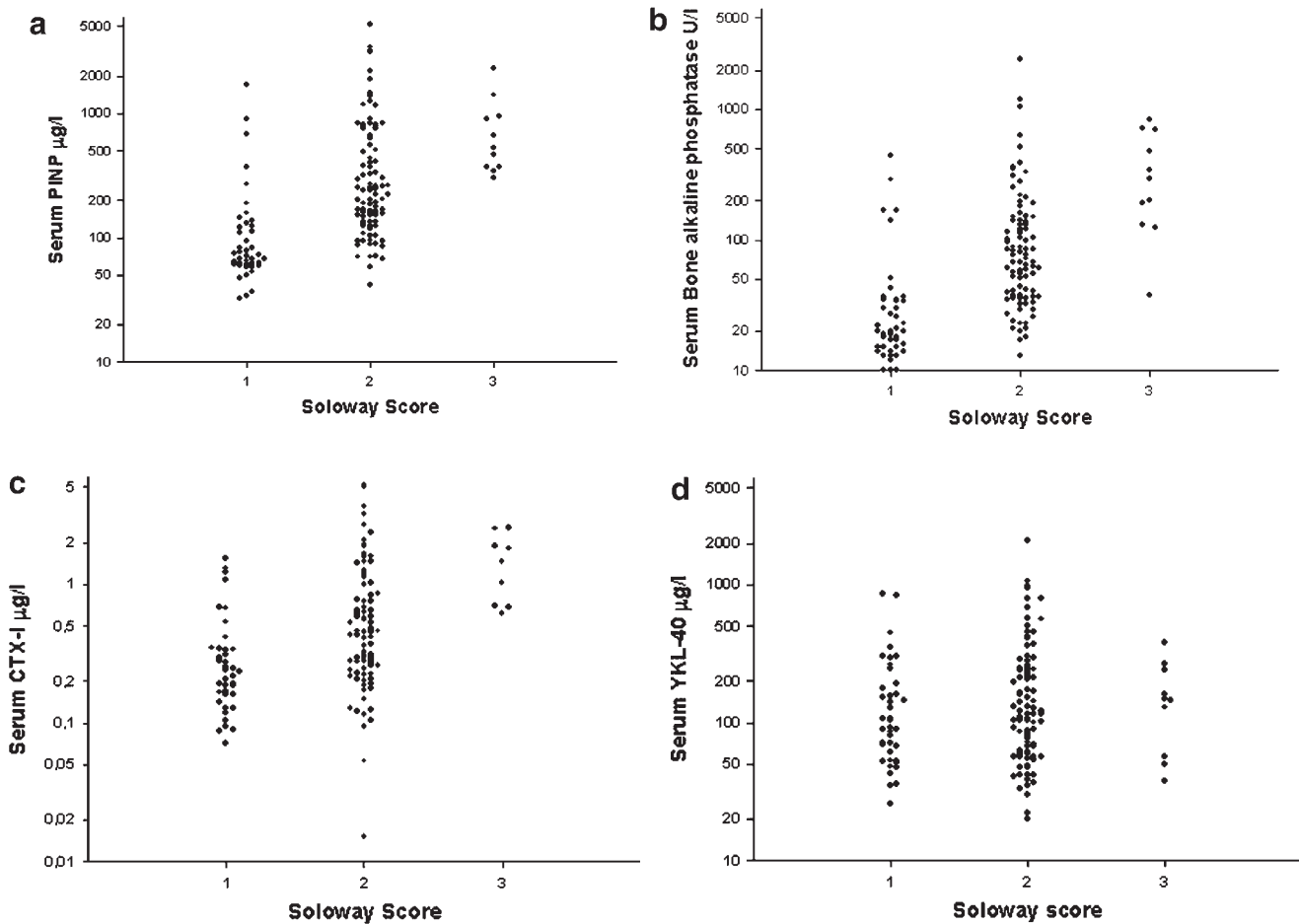
Multivariate Cox regression analysis of overall survival was performed including age, performance status, T-category, WHO tumor grade, Soloway score, serum PSA, PINP, CTX-I, and YKL-40 (Table II, right). The serological parameters were log transformed and

treated as continuous variables. Serum BAP and AP were not included simultaneously as serum PINP, total AP, and BAP are closely correlated. Excluding serum PINP and including serum total AP or BAP indicated significance for total AP ( $P = 0.01$ ), whereas BAP was not significant ( $P = 0.48$ ). Including all of the above covariates in a multivariate model and using backwards selection resulted in serum PINP (HR = 1.4, 95% CI: 1.2–1.7,  $P = 0.0001$ ) and serum YKL-40 (HR = 1.4, 95% CI: 1.1–1.8,  $P = 0.01$ ) being retained in the model along with T-category, WHO tumor grade, and Soloway score.

The hazard ratio between a patient with serum PINP and YKL-40 level equal to the third quartile respectively compared to a patient with levels equal to the first quartile was 2.5. Patients with one marker at the level of the third quartile had a 1.5–1.7 higher risk of death compared to a patient with serum PINP and YKL-40 levels equal the first quartile.

## DISCUSSION

In accordance with earlier studies [5–16,20,21], we found elevated serum PINP, BAP, and CTX-I levels in PC patients with bone metastases. Nearly 90% of the patients had elevated serum PINP, whereas only half of

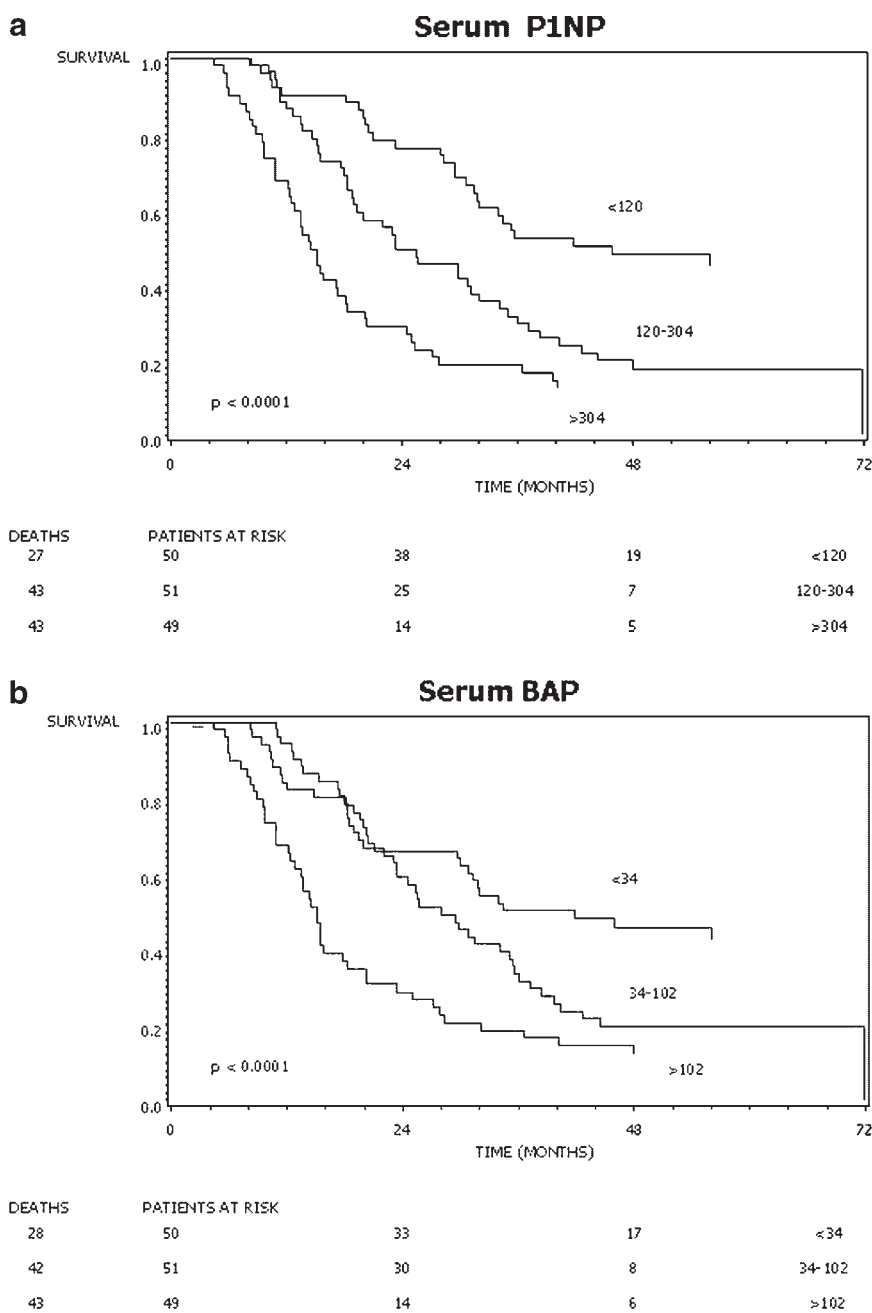


**Fig. 1.** The individual serum concentrations of PINP (a), BAP (b), CTX-I (c), and YKL-40 (d) in the patients with metastatic prostate carcinoma according to Soloway score. The y-axis is a logarithmic scale. Median values and differences between the groups are given in Table I.

the patients had elevated serum BAP, and a third had elevated serum CTX-I. All three biomarkers of bone remodeling were related to the burden of metastatic PC in the skeleton determined by the Soloway score. Bone metastases in patients with PC are typically referred as “slerotic” characterized by increased osteoblastic activity [42]. Serum concentration of PINP, a marker of type I collagen formation by osteoblasts, therefore seems to reflect the degree of osteosclerosis in the skeleton of PC patients with bone metastases. Osteoblast growth factors like TGF- $\beta$  and platelet-derived growth factor have been purified from prostate cancer cells [42] and in patients with PC and bone metastases the new bone formation is not necessarily preceded by bone resorption [42]. It is, therefore, not surprisingly that fewer patients had elevated serum CTX-I, a marker of bone resorption.

Univariate Cox regression analysis demonstrated that the patients with elevated serum PINP, BAP, and CTX-I at time of diagnosis of metastatic PC had significantly shorter overall survival than patients with normal levels of these biomarkers. Furthermore, multi-

variate Cox regression analysis showed that serum PINP in combination with serum YKL-40, performance status, WHO grade, and Soloway score were independent prognostic parameters of poor prognosis. A combination variable of high serum PINP and YKL-40 more than double the risk of early death compared to patients with normal levels. Earlier studies have also found that high serum PINP and PICP were prognostic markers of short survival in patients with metastatic PC [8,14] and breast carcinoma [17]. A high serum AP level has been found to be related to short progression-free survival and overall survival in patients with metastatic PC [43], and high serum BAP has recently been shown in a large study of patients with bone metastases from PC, non-small cell lung cancer, and other solid tumors to be a predictor of short time to first skeletal-related event and short overall survival [9]. Jung et al. [8] compared 10 serum bone remodeling biomarkers (including PINP, BAP, and CTX-I) in patients with localized and metastatic prostate cancer. It was found that serum osteoprotegerin was best to discriminate between patients with or without bone



**Fig. 2.** The impact of serum PINP (a), BAP (b), CTX-I (c), and YKL-40 (d) level on overall survival of patients with metastatic prostate carcinoma. Patients were divided into three groups according to serum PINP, BAP, CTX-I, and YKL-40 obtained pretreatment. The number of events are shown for each group at the left, and the number of patients at risk are shown for 0, 24, and 48 months. a: Group 1: serum PINP <math>< 120 \mu\text{g/L}</math>; Group 2: serum PINP  $\geq 120$ , and  $\leq 304 \mu\text{g/L}</math>; and Group 3: serum PINP  $> 304 \mu\text{g/L}</math>. b: Group 1: serum BAP  $< 34 \text{ U/L}</math>; Group 2: serum BAP  $\geq 34$  and  $\leq 102 \text{ U/L}</math>; and Group 3: serum BAP  $> 102 \text{ U/L}</math>. c: Group 1: serum CTX-I  $< 0.25 \mu\text{g/L}</math>; Group 2: serum CTX-I  $\geq 0.25 \mu\text{g/L}$  and  $\leq 0.62 \mu\text{g/L}</math>; and Group 3: serum CTX-I  $> 0.62 \mu\text{g/L}</math>. d: Group 1: serum YKL-40  $< 81 \mu\text{g/L}</math>; Group 2: serum YKL-40  $\geq 81 \mu\text{g/L}$  and  $\leq 177 \mu\text{g/L}</math>; and Group 3: serum YKL-40  $> 177 \mu\text{g/L}</math>.$$$$$$$$$$$

metastases, and in multivariate analysis, only serum osteoprotegerin and bone sialoprotein were independent prognostic parameters of short survival.

High serum CTX-I and NTX-I [8] and urine NTX [9] are also reported as prognostic biomarkers of survival in patients with bone metastases from PC, and high

urine NTX could predict early skeletal-related events and disease progression in patients with bone metastases (including PC) [9]. NTX levels in urine in patients with metastatic PC seemed to be superior to serum BAP as a prognostic biomarker of time to first skeletal event and time to death. Monitoring urine NTX and serum

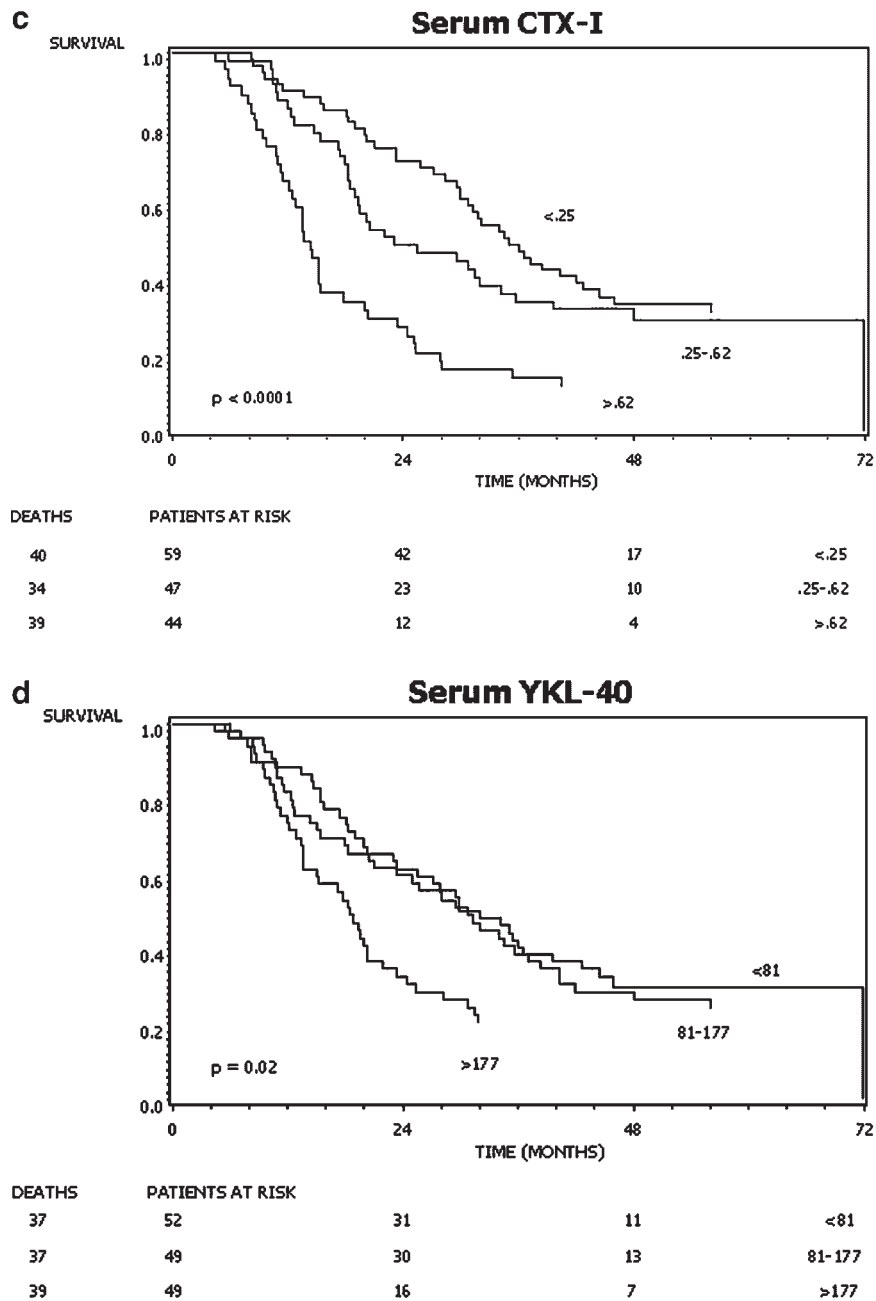


Fig. 2. (Continued)

BAP in untreated patients after diagnosis of metastatic PC also showed that these two biomarkers were significant predictive of skeletal-related events, time to first skeletal-related event, disease progression, and death [9]. Unfortunately CTX-I and NTX concentrations in urine could not be analyzed in the present study, since urine was not collected from the patients.

This is the first report on serum concentration of YKL-40 in patients with metastatic PC, and we found that 43% of the patients had elevated serum YKL-40 compared to healthy controls. In contrast to the biomarkers of bone remodeling, the serum YKL-40

level was not related to Soloway score. However, high serum YKL-40 at time of diagnosis of metastatic PC was an independent prognostic parameter of short survival. This prognostic value was similar to earlier studies in patients with primary and recurrent breast, colorectal, ovarian, renal cell, and small cell lung carcinoma [28–33].

The mechanism by which serum YKL-40 reflects cancer aggressiveness is not known, but it is likely that YKL-40 has a function in progression of malignant diseases. YKL-40 is a growth factor for fibroblasts and chondrocytes, and acts synergistically with IGF-1 [24].



**TABLE II. Univariate and Multivariate Cox Regression Analyses of Potential Clinical and Serological Parameters of Survival in Patients With Metastatic Prostate Carcinoma**

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (in years)	1.00	0.97–1.03	0.90	1.01	0.98–1.05	0.46
Performance status (>0)	2.2	1.5–3.2	<0.0001	1.8	1.1–2.8	0.02
T-category T3/T1-T2	1.1	0.6–2.0	0.74	0.6	0.3–1.2	0.16
T-category T4/T1-T2	1.3	0.7–2.2	0.39	0.9	0.5–1.8	0.85
WHO grade 1 versus 2	1.2	0.7–2.1	0.48	1.1	0.6–2.1	0.76
WHO grade 3 versus 2	2.1	1.4–3.3	0.0006	2.1	1.2–3.6	0.009
Soloway score 1 versus 2	0.5	0.3–0.7	0.001	0.6	0.3–1.0	0.06
Soloway score 3 versus 2	2.9	1.5–5.5	0.001	3.9	1.6–9.7	0.003
Serum PSA	1.1	0.9–1.3	0.12	0.9	0.8–1.1	0.36
Serum AP	1.7	1.4–2.1	<0.0001		Not included, see results	
Serum BAP	1.4	1.2–1.6	<0.0001		Not included, see results	
Serum PINP	1.6	1.4–1.9	<0.0001	1.4	1.0–1.8	0.03
Serum CTX-I	1.7	1.4–2.1	<0.0001	1.0	0.8–1.4	0.88
Serum YKL-40	1.4	1.1–1.7	0.004	1.3	1.0–1.7	0.02

HR, relative hazard ratio; CI, confidence interval.

The serum levels of PSA, total alkaline phosphatase (AP), bone alkaline phosphatase (BAP), the amino terminal propeptide of type I procollagen (PINP), CTX-I, and YKL-40 were log transformed and treated as continuous variables.

N = 129, 95 deaths.

YKL-40 initiates MAP kinase and PI-3K signaling cascades in fibroblasts leading to the phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase, and protein kinase B (AKT)-mediated signaling cascades [24,44], which are associated with the control of mitogenesis. The PI-3K pathway, and in particular the phosphorylation of AKT, is strongly associated with cell survival. Upregulated YKL-40 expression is found in a human glioblastoma cell line by genotoxic and micro-environmental stress (e.g., hypoxia, ionizing radiation) [45], and human astrocytes transfected with YKL-40 had increased resistance to radiation and increased invasion capacity in vitro [46]. This suggests that YKL-40 plays a role in the malignant phenotype as a cellular survival factor. Furthermore YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 also has a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells [25]. Furthermore, YKL-40 acts as a chemo-attractant for endothelial cells, stimulates their migration, and promotes migration and adhesion of vascular smooth muscle cells [25,47].

To conclude, it is unlikely that a single biomarker of bone remodeling has sufficient diagnostic or prognostic value in patients with bone metastases. However, the combination of several biomarkers with imaging techniques is likely to improve the clinical assessment

of patients with metastatic PC. Our study suggests that serum PINP and a panel of other biomarkers of bone remodeling (e.g., serum or urine NTX and CTX-I) and growth factors (e.g., YKL-40) may be useful in combination with imaging techniques to diagnose skeletal metastases in patients with PC. However, large prospective studies of prostate cancer patients are needed to identify which of the growing number of potential biomarkers of bone remodeling and tumor growth have a role at time of diagnosis of primary local PC in predicting skeletal events and early death, and if these biomarkers can be used to monitor patients with local PC as has been suggested for BAP and urine NTX in patients with metastatic PC [9]. Hopefully the biomarkers could help the selection of patients for early, prophylactic use of bisphosphonates. It should also be evaluated if the biomarkers are useful to monitor the efficacy of a given dose and schedule of bisphosphonate in an individual PC patient with bone metastases or if they can provide an early assessment of response to endocrine therapy and to identify patients who relapse during the course of the treatment.

#### ACKNOWLEDGMENTS

We thank the SPCG5 study group for giving access to the original data and the investigators participating in the SPCG5 study group. The deceased Inger Aakard,

Department of Rheumatology, Hvidovre Hospital is greatly appreciated for the measurement of serum YKL-40 and BAP. Jette Brandt and Anette Kliem, Department of Immunology and Microbiology, University of Odense are thanked for the measurement of serum PINP; and Fabrice Juillet, Molecular Markers, Synarc for the measurement of serum CTX-I. Quidel provided the study with YKL-40 and BAP ELISA kits. Patients included were part of a Scandinavian Study (SPCG5) supported by Pharmacia and Upjohn Sverige AB, Lund, Sweden, Schering-Plough AB, Stockholm, Sweden, and Ferring AB, Malmø, Sweden.

## REFERENCES

- Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. Cancer statistics, 2004. *CA Cancer J Clin* 2004;54:8–29.
- Khan MA, Partin AW. Management of patients with an increasing prostate-specific antigen after radical prostatectomy. *Curr Urol Rep* 2004;5:179–187.
- Hittelman AB, Purohit RS, Kane CJ. Update of staging and risk assessment for prostate cancer patients. *Curr Opin Urol* 2004;14:163–170.
- Mundy GR. Metastasis to bone: Causes, consequences and therapeutic opportunities. *Nat Rev Cancer* 2002;2:584–593.
- Fohr B, Dunstan CR, Seibel MJ. Clinical review 165. Markers of bone remodeling in metastatic bone disease. *J Clin Endocrinol Metab* 2003;88:5059–5075.
- Garnero P. Markers of bone turnover in prostate cancer. *Cancer Treat Rev* 2001;27:187–192.
- Demers LM. Bone markers in the management of patients with skeletal metastases. *Cancer* 2003;97(3 Suppl):874–879.
- Jung K, Lein M, Stephan C, von Hösslin K, Semjonow A, Sinha P, Loening SA, Schnorr D. Comparison of 10 serum bone turnover markers in prostate carcinoma patients with bone metastatic spread: Diagnostic and prognostic implications. *Int J Cancer* 2004;111:783–791.
- Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, Lee K-A, Zheng M, Hei Y-J, Coleman RE. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. *J Natl Cancer Inst* 2005;97:59–69.
- Cooper EH, Whelan P, Purves D. Bone alkaline phosphatase and prostate-specific antigen in the monitoring of prostate cancer. *Prostate* 1994;25:236–240.
- Morote J, Lorente JA, Encabo G. Prostate carcinoma staging. Clinical utility of bone alkaline phosphatase in addition to prostate specific antigen. *Cancer* 1996;78:2374–2378.
- Lorente JA, Valenzuela H, Morote J, Gelabert A. Serum bone alkaline phosphatase levels enhance the clinical utility of prostate specific antigen in the staging of newly diagnosed prostate cancer patients. *Eur J Nucl Med* 1999;26:625–632.
- Akimoto S, Furuya Y, Akakura K, Ito H. Comparison of markers of bone formation and resorption in prostate cancer patients to predict bone metastases. *Endocrine J* 1998;45:97–104.
- Akimoto S, Furuya Y, Akakura K, Shimazaki J, Ito H. Inability of bone turnover marker as a strong prognostic indicator in prostate cancer patients with bone metastases: Comparison with the extent of disease (EOD) grade. *Prostate* 1999;38:28–34.
- Diaz-Martin MA, Traba ML, De La Piedra C, Guerrero R, Mendez-Davila C, Garcia De La Pena E. Aminoterminal propeptide of type I collagen and bone alkaline phosphatase in the study of bone metastases associated with prostatic carcinoma. *Scand J Clin Lab Invest* 1999;59:125–132.
- Koizumi M, Yonese J, Fukui I, Ogata E. The serum level of the amino-terminal propeptide of type I procollagen is a sensitive marker for prostate cancer metastasis to bone. *BJU International* 2001;87:348–351.
- Jensen BV, Johansen JS, Skovsgaard T, Brandt J, Teisner B. Extracellular matrix building marked by the N-terminal propeptide of procollagen type I reflect aggressiveness of recurrent breast cancer. *Int J Cancer* 2002;98:582–589.
- Bonde M, Garnero P, Fledelius C, Qvist P, Delmas PD, Christiansen C. Measurement of bone degradation products in serum using antibodies reactive with an isomerized form of an 8 amino acid sequence of the C-telopeptide of type I collagen. *J Bone Miner Res* 1997;12:1028–1034.
- Garnero P, Borel O, Delmas PD. Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of type I collagen in osteoporosis. *Clin Chem* 2001;47:694–702.
- Garnero P, Buchs J, 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;82:858–864.
- Vinholes JJ, Purohit OP, Abbey ME, Eastell R, Coleman RE. Relationships between biochemical and symptomatic response in a double-blind randomised trial of pamidronate for metastatic disease. *Ann Oncol* 1997;8:1243–1250.
- Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. *J Bone Miner Res* 1992;7:501–512.
- Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;268:25803–25810.
- Recklies AD, Chantal W, Ling H. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. *Biochem J* 2002;365:119–126.
- Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJT. Gp38k, a protein synthesized by vascular smooth muscle cells stimulates directional migration of human umbilical vein endothelial cells. *Exp Cell Res* 1999;250:168–173.
- Rehli M, Krause SW, Andreesen R. Molecular characterization of the gene for human cartilage gp-39 (CHI3L1), a member of the chitinase protein family and marker for late stages of macrophage differentiation. *Genomics* 1997;43:221–225.
- Houston DR, Recklies AD, Krupa JC, van Aalten DMF. Structure and ligand-induced conformational change of the 39-kDa glycoprotein from human articular chondrocytes. *J Biol Chem* 2003;278:30206–30212.
- Johansen JS, Christensen IJ, Riisbro R, Greenall M, Han C, Price PA, Smith K, Brünner N, Harris AL. High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. *Breast Cancer Res Treat* 2003;80:15–21.
- Jensen BV, Johansen JS, Price PA. High levels of serum HER-2/neu and YKL-40 independently reflect aggressiveness of metastatic breast cancer. *Clin Cancer Res* 2003;9:501–512.

30. Cintin C, Johansen JS, Christensen IJ, Price PA, Sørensen S, Nielsen HJ. Serum YKL-40 and colorectal cancer. *Br J Cancer* 1999;79:1494–1499.
31. Høgdall EVS, Johansen JS, Kjaer SK, Price PA, Christensen L, Blaakaer J, Bock JE, Glud E, Høgdall CK. High plasma YKL-40 level in patients with ovarian cancer stage III is related to shorter survival. *Oncol Rep* 2003;10:1535–1538.
32. Dupont J, Tanwar MK, Thaler HT, Fleisher M, Kauff N, Hensley ML, Sabbatini P, Anderson S, Aghajanian C, Holland EC, Spriggs DR. Early detection and prognosis of ovarian cancer using serum YKL-40. *J Clin Oncol* 2004;22:3330–3339.
33. Johansen JS, Drivsholm L, Price PA, Christensen IJ. High serum YKL-40 level in patients with small cell lung cancer is related to early death. *Lung Cancer* 2004;46:333–340.
34. Hedlund PO, Henriksson P, the Scandinavian prostatic cancer group (SPCG)-5 trial study. Parenteral estrogen versus total androgen ablation in the treatment of advanced prostate carcinoma: Effects on overall survival and cardiovascular mortality. *Urology* 2000;55:328–333.
35. Soloway MS, Hardeman SW, Hickey D, Raymond J, Todd B, Soloway S, Moinuddin M. Stratification of patients with metastatic prostate cancer based on extent of disease on initial bone scan. *Cancer* 1988;61:195–202.
36. Johansen JS, Hvolris J, Hansen M, Backer V, Lorenzen I, Price PA. Serum YKL-40 levels in healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. *Br J Rheumatol* 1996;35:553–559.
37. Gomez B Jr., Ardakani S, Ju J, Jenkins D, Cerelli MJ, Daniloff GY, Kung VT. Monoclonal antibody assay for measuring bone-specific alkaline phosphatase activity in serum. *Clin Chem* 1995;41:1560–1566.
38. Ørum O, Hansen M, Jensen CH, Sørensen HA, Jensen LB, Hørslev-Petersen K, Teisner B. Procollagen type I N-terminal propeptide (PINP) as an indicator of type I collagen metabolism: ELISA development, reference interval, and hypovitaminosis D induced hyperparathyroidism. *Bone* 1996;19:157–163.
39. Harvey S, Weisman M, O'Dell J, Scott T, Krusemeier M, Visor J, Swindlehurst C. Chondrex: New marker of joint disease. *Clin Chem* 1998;4:509–516.
40. Royston P. Constructing time-specific reference ranges. *Statist Med* 1991;10:675–690.
41. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: Issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996;15:361–387.
42. Coleman RE. Metastatic bone disease: Clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev* 2001;27:165–176.
43. Smith JA, Lange PH, Janknegt RA, Abbou CC, DeGery A. Serum markers as a predictor of response duration and patient survival after hormonal therapy for metastatic carcinoma of the prostate. *J Urology* 1997;157:1329–1334.
44. Ling H, Recklies AD. The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. *Biochem J* 2004;380:651–659.
45. Junker N, Johansen JS, Hansen LT, Lund EL, Kristjansen PEG. Regulation of YKL-40 expression during genotoxic or micro-environmental stress in human glioblastoma cells. *Cancer Sci* 2005;96:183–190.
46. Nigro JM, Misra A, Zhang L, Smirnov I, Colman H, Griffin C, Ozburn N, Chen M, Pan E, Koul D, Yung WKA, Feuerstein BG, Aldape KD. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. *Cancer Res* 2005;65:1678–1686.
47. Nishikawa KC, Millis AJT. gp38k (CHI3L1) is a novel adhesion and migration factor for vascular cells. *Exp Cell Res* 2003;287:79–87.