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ORIGINAL ARTICLE

Type I collagen biomarkers in the diagnosis of bone metastases in breast cancer, lung cancer, urinary bladder cancer and prostate cancer. Comparison to CEA, CA 15-3, PSA and bone scintigraphy

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Summary

Purpose: *In this study we evaluated the clinical usefulness of serum pro-I collagen peptide (PICP) and I collagen telopeptide (ICTP) as indicators of early bone metastases in patients with breast (BC), lung (LC), urinary bladder (UBC) and prostate cancer (PC).*

Patients and methods: *305 patients were examined. 145 had histologically confirmed BC (92 with bone metastases), 20 UBC (6 with bone metastases), 11 LC (3 with bone metastases) and 129 PC (68 with bone metastases). In BC patients we compared the PICP and ICTP levels with those of CA 15-3, CEA and bone scintigraphy. Patients with LC and UBC had PICP and ICTP measurements, PC patients had serum PICP, prostate specific antigen (PSA) measurements and bone scans. 104 healthy individuals served as controls.*

Results: *ICTP and CA 15-3 levels were significantly higher in patients with BC and bone metastases in comparison to patients without metastases ($p < 0.05$), while PICP and CEA were only marginally higher. Significant correlation was observed between existence of bone metastases and ICTP levels ($p < 0.05$). The sensitivity of PICP, ICTP, CEA and CA 15-3 was 28.1, 48.6, 42, and 78%, respectively and specificity was 83.9, 94, 65 and 86%, respectively. ICTP and CA 15-3 were the most reliable markers for early diagnosis of bone metastases in BC. PICP alone or with ICTP were not sensitive enough. Only CA 15-3 showed sensitivity 78% and specificity 86%. When combined CA 15-3, ICTP and CEA the sensitivity and specificity increased to 82% and 96%, respectively. Furthermore, PICP and PSA levels were significantly higher in patients with PC and bone metastases in comparison to patients with benign prostate hyperplasia (BPH) ($p < 0.0001$) or in patients with PC without bone metastases ($p < 0.0005$ for PICP and $p < 0.0001$ for PSA). The co-evaluation of PICP and PSA improved the sensitivity (78%), specificity (96%), accuracy (97%) and positive predictive value (97%). In LC patients, ICTP levels differed significantly between patients with and without bone metastases ($p = 0.025$). In UBC patients, PICP levels differed significantly between patients with and without bone metastases ($p = 0.017$).*

Conclusion: *ICTP and CA 15-3 are the most reliable markers for early diagnosis of bone metastases in BC patients. PICP could be useful for diagnosing early bone metastases of PC and combined with PSA and bone scan can be an additional tool in the follow-up of PC patients. For LC patients, ICTP showed a significant difference in the discrimination of patients with and without bone metastases. In UBC patients, PICP showed a significant difference in the discrimination of patients with and without bone metastases.*

Key words: bone metastases, cancer, ICTP, PICP

Introduction

Bone metastases are divided in 3 categories: osteoblastic, osteolytic and mixed. It has been reported that bone involvement in patients with BC and the identification of the nature of bone metastases play

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an important role in the therapeutic decision-making [1]. Nowadays, the diagnosis of bone metastases in patients with BC is based, besides the clinical evaluation, on bone scintigraphy, computerized tomography (CT), magnetic resonance imaging (MRI) and on the evaluation of various tumor biomarkers. Nevertheless, it is common knowledge that early and accurate detection of bone metastases is still difficult by assessing the clinical symptoms or the available imaging techniques, serum tumor markers and biochemical markers measurements. Among the tumor markers CEA and CA 15-3 are the most widely used [2]. But still the early and accurate detection of bone metastases remains difficult since the specificity of the above mentioned methods is not always high. Therefore, there is a necessity for new biomarkers for the early detection of bone metastases in BC. Although bone scintigraphy is considered to be very powerful in the initial diagnosis having a high sensitivity, its specificity is low, since several hot spots (in skull or cervical spine) are commonly interpreted as false-positive areas on bone scans, indicating the limitations of this technique. The flare phenomenon (paradoxical deterioration) has also been reported on bone scans after the start of anticancer treatment (estrogens, LHRH agonists, mitomycin). In addition, bone scintigraphy is not suitable for monitoring short-term response of bone metastases to therapy. Moreover, bone scintigraphy is costly, time-consuming and entails a radiation hazard when performed during follow-up. As for x-ray imaging, it is effective only when metastases are more than 1-2 cm in diameter and about 30% of bone mineral has been lost or increased. CT and MRI can show lytic, mixed or blastic lesions but do not yield information about the actual interaction between neoplastic and normal bone cells. In addition bone micrometastases can not be easily detected.

Although PC frequently metastasizes to bones, the accurate detection of bone metastases remains problematic since all imaging methods, such as x-ray, ultrasound and CT can only detect the long-term effects of bone metastases (osteolytic and/or osteoblastic lesions). Before the wide application of PSA measurement as a first-choice screening test, most PC were detected at T2 or T3 stages, therefore untreatable. Nowadays, with the wide use of PSA many PC are detected in early stages and therefore can be treated either surgically or by drugs. But 50% of men with "normal" PSA levels have PC [3]. Therefore, the early detection of bone metastases is considered to be very important and decisive for the prognosis of patients with PC.

As far as LC is concerned, serum and/or tissue tumor markers bear prognostic significance in some situations [4-6]. Similarly, the use of genetic testing of the tumor to detect specific mutations or to determine specific gene expression profiles may provide estimates of disease behavior [7,8]. Serum tumor markers have not been shown to have broad clinical utility in patients with NSCLC and their routine use is not recommended.

A large number of studies for evaluation of multiple urinary biomarkers including DNA ploidy and qualitative fluorescence image analysis, molecular cytogenetics, telomerase expression, tumor associated intracellular or secreted products, oncogene mutations, microsatellite alterations, and markers of apoptosis are ongoing regarding BC [9-17] . Several have been approved in the USA (e.g. BTA Stat, BTA TRAK, UroVysion tests) for the detection of recurrent BC, but none is approved for widespread screening, initial diagnosis, or risk assessment [18]. None of these tests have shown sufficient diagnostic reliability to eliminate the need for cystoscopy for either primary or recurrent UBC [19].

Lately, some biochemical markers of bone turnover have been developed with the hope of reflecting the disease activity of bone metastases. As it is difficult to estimate directly the actual production of paracrine substances involved in bone remodelling, the evaluation of biochemical markers connected with changes in the rates of bone formation and degradation has been an alternative approach to the diagnosis and follow-up of patients with BC. Unfortunately, many studies have reported that most of these markers have serious limitations, lacking either sensitivity or specificity or both. These markers include products that reflect bone synthesis, such as alkaline phosphatase (ALP) and its bone isoform BALP, bone GLA-protein (osteocalcin/OC) and carboxyterminal propeptide of type I procollagen (PICP), and bone degradation such as urine pyridinoline (Pyr)/deoxypyridinoline (D-Pyr) and carboxyterminal telopeptide of type I collagen (ICTP). The bone synthesis markers lack sensitivity in metastatic bone disease in BC because the type of bone disease is mostly lytic. On the other hand pyridinolines and deoxypyridinolines are elevated in most patients with metastases but they lack specificity as they are also elevated in patients without metastases. Immunoassays for measuring the circulating products of both type 1 collagen synthesis and degradation have been developed and used for the early detection of the bone metastases in patients with BC, PC, LC and UBC [20]. The major structural protein in bone is collagen type 1, which is synthesized by osteoblasts and accounts for about 90% of the organic matrix of bone [21]. The PICP is a globular trimer glycoprotein, which is cleaved extracellularly from the carboxyterminus of procollagen. The amount

of PICP released into the circulation is directly related to the rate of *de novo* synthesis of collagen molecules, since PICP normally does not come from soft tissues. PICP is not incorporated into the bone matrix, and thus circulating levels of PICP indicate the rate of bone collagen synthesis and consequently osteoblastic activity [22,23]. The monitoring, therefore, of the collagen type 1 metabolism can be used to detect the activity of bone metastases [24]. PICP is cleared through mannose receptors in the liver. ICTP on the other hand, is a degradation product of type I collagen of mature extracellular matrix and circulates in blood as a 12 KD peptide after being cleaved from the collagen fibers. ICTP is cleared from circulation through the kidneys. It is a sensitive marker of bone resorption in disorders like hyper- and hypoparathyroidism, hypothyroidism and thyreotoxicosis, corticosteroid treatment, Paget's disease, osteoporosis, growth hormone deficiency, rheumatoid arthritis, multiple myeloma and PC. PICP and ICTP levels are indicators of osteoblastic and osteolytic activity and hence their evaluation is expected to contribute to the early diagnosis of bone metastases in patients with PC, BC, LC and UBC by providing adequate information about the nature of metastases and to be used as follow-up indicators of the treatment applied.

The aim of our study was the evaluation of the clinical usefulness of serum ICTP and PICP as indicators of bone metastases in patients with BC in combination with bone scan and CA 15-3 measurements, as well as the usefulness of PICP and ICTP in patients with LC and UBC. In addition, we aimed to study the usefulness of PICP as an indicator of early bone metastases in patients with PC in combination with PSA measurements and bone scintigraphy.

Patients and methods

Patients

Examined were 305 patients. From those, 169 patients had bone metastases. BC had 145 patients (92 with bone metastases), 20 had UBC (6 with bone metastases), 11 had LC (3 with bone metastases) and 129 had PC (68 with bone metastases). All of them had histological diagnosis of their malignancies. We also examined 104 healthy individuals (blood donors / control group). Patients with BC underwent bone scans, CA 15-3, CEA, PICP and ICTP measurement. All patients were normocalcemic. Neither patients nor controls had a history of disease such as bone fractures, osteomalacia, Paget's disease, renal or hepatic failure, or use of drugs (steroids, calcitonin, biphosphonates) which could affect bone metabolism. Patients with bisphosphonate treatment, radiation therapy and with predominantly sclerotic lesions in X-rays were excluded. Serum parathormone-related protein (PTHrP) was within normal range in all patients and controls. Patients who had surgery within 2 months were also excluded. Situations such as menopausal status and oestrogen therapy that influence collagen metabolism were taken into consideration. All subjects gave informed consent. Patients with UBC and LC underwent PICP and ICTP measurements and those with PC PICP and PSA measurement.

Methods

Diagnosis of bone involvement was performed with bone scintigraphy (tomographic γ- camera type MPR Milennium GE, USA), followed by confirmation with plain radiographies. MRI was performed to discriminate lesions that appeared positive on scintigraphy and negative on radiographies. All sera were sampled before 10.00 a.m. after overnight fasting and were stored at -20° C.

All cancer patient categories and control subject characteristics are summarized in Table 1. Serum levels of all markers were assayed with immunoradiometric assay (IRMA); ICTP and PICP with kits of Orion Farmos Diagnostic, Finland, CEA and CA 15-3 with kits of CIS BioInternational, France, and serum PSA with Tandem-R PSA assay (Hybritech Inc, San Diego, Calif, USA).

The reference value for PICP was 37-177 μg/l, for ICTP 0.76 - 4.6 μg/ml, for CA 15-3 up to 30 ng/ml and for CEA up to 7 ng/ml. The range, mean values, standard deviations and intermediate values are displayed on Table 2.

In our analysis we adopted the following patient grouping: group A included patients without bone metastases, group B patients with 1-3 bone metastases, and group C patients with >3 bone metastases.

Statistics

Data were analyzed using the statistical software program SPSS version 12.0 (SPSS Inc., Chicago IL). Statistical significance was set at 0.05. Groups were compared using Kruskal-Wallis, Mann-Whitney and Kolmogorov-Smirnov test, as appropriate. Receiver operating characteristics (ROC) curve analysis was applied for PICP and ICTP to compare their diagnostic accuracy.

Table 1. Demographic and clinical characteristics of the study cohort

Characteristics	*Patients with breast cancer*	*Patients with lung cancer*	*Patients with urinary bladder cancer*	*Patients with prostate cancer*	*Control group*
Age range/years, (mean)	50-66 (58)	48-62 (56)	53-69 (61)	58.7-67.3 (63)	20-59 (48.5)
Number of patients	145	11	20	129	104
Patients with bone metastases	92	3	6	68	
>3 bone metastases	73	2	3	46	
<3 bone metastases	19	1	3	22	
Concomitant metastatic sites in patients with bone metastases					
Lymph nodes	35	3	6	68	
Lungs	6	–	6	–	
Liver	4	–	5	–	
Treatment in patients with bone metastases					
Chemotherapy	56	3	6	–	
Endocrine therapy	36	–	–	42	
Surgical castration	–	–	–	26	
Breast cancer patients with bone metastases					
Premenopausal	49				
Postmenopausal	43				
Stage IV	12				
Breast cancer patients without bone metastases	53				
Premenopausal	26				
Postmenopausal	27				
Stage 0	7				
Stage I	36				
Stage IIα	10				

Table 2. Range value, mean value, standard deviation and intermediate value of PICP, ICTP, CA 15-3 and CEA in control group and in patients

	PICP (μg/l)		*ICTP (μg/ml)*		*CA 15-3 (ng/ml)*		*CEA (ng/ml)*		*PSA (ng/ml)*	
	Range	*Mean ±SD (intermediate)*	*Range*	*Mean ±SD (intermediate)*	*Range*	*Mean ±SD (intermediate)*	*Range*	*Mean ±SD (intermediate)*	*Range*	*Mean ±SD (intermediate)*
Control BC patients (n=52)	25.3±180	97.9404± 39.14442	0.7±4.2	1.7808± 0.87605	0.4±21.6	6.6346± 5.24082	0.1±5.8	1.2731± 1.13191	–	–
Control PC patients (n=52)	31.4-195.8	86.5942± 33.83419	–	–	–	–	–	–	0.1-7.1	1.2058± 1.36575
BC patients (n =145)	93-359	164.8655± 54.66644	0.8-67	7.3006± 7.50408	3-156.7	37.8786± 31.94388	0.2-26.5	5.3979± 5.94587	–	–
LC patients (n =11)	58.4-161.4	118.5091± 32.37639	0.7-5.1	2.2182± 1.57405	–	–	–	–	–	–
UBC patients (n=20)	48.9-185.6	108.725± 42.42349	0.6-20.3	3.955± 5.05709	–	–	–	–	–	–
PC patients (n=129)	49.5-396.1	165.7054± 60.28671	–	–	–	–	–	–	0.1-182	10.2659± 20.45585

For abbreviations see text

Results

Breast cancer

Levels of ICTP and CA 15-3 were significantly higher in patients with BC and bone metastases in comparison to patients without metastases ($p < 0.05$), while PICP and CEA were only marginally higher. A statistically significant correlation was observed among the existence of bone metastases and ICTP serum levels ($p < 0.05$). The sensitivity of PICP, ICTP, CEA and CA 15-3 was 28.1, 48.6, 42 and 78%, respectively, and their specificity was 83.9, 94, 65 and 86%, respectively. ICTP and CA 15-3 were the most reliable markers for the early diagnosis of bone metastases in BC. PICP alone or with ICTP were not sensitive enough. Only CA 15-3 showed a sensitivity of 78% and specificity of 86%. With combined CA 15-3, ICTP and CEA measurement the sensitivity and specificity rose to 82% and 96%, respectively.

Prostate cancer

Levels of PICP and PSA were significantly higher in patients with PC and bone metastases in comparison to patients with BPH ($p < 0.0001$) or in patients with PC without bone metastases ($p < 0.05$ for PICP and $p < 0.0001$ for PSA). The co-evaluation of PICP and PSA improved the sensitivity (78%), specificity (96%), accuracy (97%) and the positive predictive value (97%).

Lung cancer

In patients with bone metastases PICP levels were abnormal in 0/3 cases (sensitivity 0%). In patients without bone metastases PICP levels were normal in 8/8 cases (specificity 100%, negative predictive value 73%). ICTP levels in patients with bone metastases were abnormal in 2/3 cases (sensitivity 67%, positive predictive value 100%). In patients without bone metastases ICTP levels were normal in 8/8 cases (specificity 100%, negative predictive value 89%). Mean levels for PICP of all LC patients were 118.5091 ± 32.37639 ng/ml, while for ICTP they were 2.2182 ± 1.57405 ng/ml. No significant difference was observed between patients with and without bone metastases in relation with PICP level ($p=0.54$). A significant difference was observed between patients with and without bone metastases regarding ICTP levels ($p=0.025$).

Urinary bladder cancer

In patients with bone metastases the levels of PICP were abnormal in 1/6 cases (sensitivity 17%, positive predictive value 50%). In patients without bone metastases the PICP levels were normal in 13/14 cases (specificity 93%, negative predictive value 72%). In patients with bone metastases the ICTP levels were abnormal in 0/6 cases (sensitivity 0%, positive predictive value 0%), whereas in those without bone metastases the ICTP levels were normal in 10/14 cases (specificity 71%, negative predictive value 63%). Mean levels for PICP for all UBC patients were 108.7250 ± 42.42349 ng/ml , while for ICTP they were 3.9550 ± 5.05709 ng/ml . A significant difference was observed between patients with and without bone metastases regarding the levels of PICP expression ($p=0.017$). No significant difference was observed between patients with and without bone metastases regarding ICTP expresion levels ($p=0.20$).

Analysis including breast and prostate cancer patients

PICP

When we compared the levels of PICP between groups A, B, and C of patients a significant difference was observed in all comparisons ($p < 0.05$) (Figure 1A). In group A patients the mean PICP value was 123.103 ± 28.8194 ng/ml, in group B it was 173.166 ± 33.2436 ng/ml, while in group C it was 203.033 ± 56.9414 ng/ml. In group B patients PICP levels were abnormal in 26/41 of them (sensitivity 63%, positive predictive value 21%). In group C patients PICP levels were abnormal in 89/119 of them (sensitivity 75%, positive predictive value 72%). In group A patients PICP levels were normal in 106/114 patients (specificity 93%, negative predictive value 70%).

ICTP

When we compared the levels of PICP between A, B and C groups a significant difference was observed in all comparisons ($p < 0.05$) (Figure 1B). In group A patients the mean ICTP value was 3.322 ± 9.0014 ng/ml, in group B it was 6.400 ± 3.5471 ng/ml, and in group C it was 10.423 ± 5.4044 ng/ml. In group B patients ICTP levels were abnormal in 11/19 of them (sensitivity 56%, positive predictive value 15%). In group C ICTP levels were abnormal in 60/73 patients (sensitivity 82%, positive predictive value 80%), and in group A patients ICTP levels were normal in 49/53 patients (specificity 92%, negative predictive value 70%).

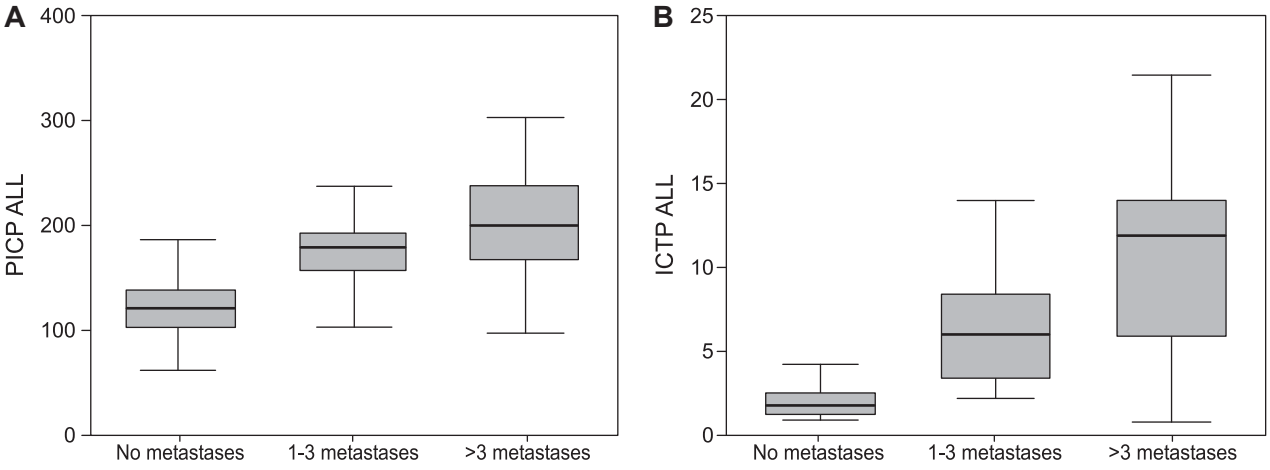

Figure 1. PICP and ICTP levels in breast and prostate cancer patients without bone metastases and in groups with 1-3 or >3 bone metastases: **A:** PICP levels; **B:** ICTP levels.

Analysis including all types of cancers

PICP

A significant difference was observed between patients with and without bone metastases regarding PICP expression levels when all types of cancers were studied ($p < 0.05$) (Figures 2A, 3A, 4A, 5). In patients without bone metastases the mean value of PICP was 119.6713 ± 31.2293 ng/ml, while in those with bone metastases it was 192.2911 ± 54.0311 ng/ml ($p < 0.05$). In patients with bone metastases PICP levels were abnormal in 109/169 patients (sensitivity 64%, positive predictive value 93%) and in those without bone metastases PICP levels were normal in 128/136 patients ($p < 0.05$; specificity 94%, negative predictive value 68%).

ICTP

A significant difference was observed between patients with and without bone metastases regarding ICTP expression levels when all types of cancers were studied ($p < 0.05$; Figures 2B, 3B, 4B, 5). In patients without bone metastases the mean value of ICTP was 3.328 ± 8.0001 ng/ml, while in those with bone metastases it was 9.035 ± 5.3888 ng/ml ($p < 0.05$). In patients with bone metastases ICTP levels were abnormal in 72/101 patients (sensitivity 71%, positive predictive value 86%) and in those without bone metastases they were normal in 63/75 of them ($p < 0.05$; specificity 84%, negative predictive value 68%).

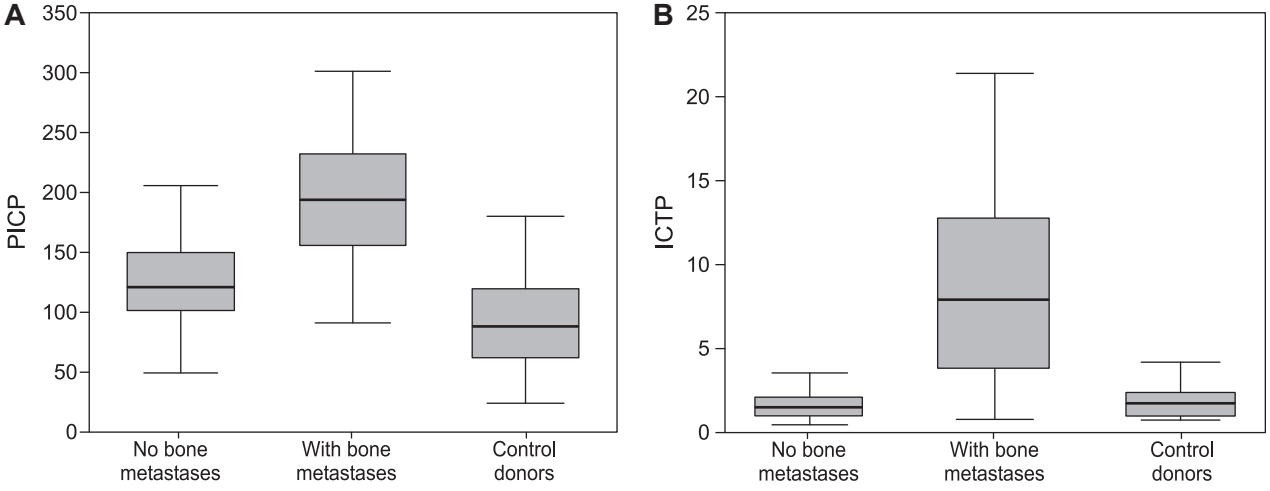

Figure 2. PICP and ICTP levels in patients with and without bone metastases in comparison to control group: **A:** PICP levels; **B:** ICTP levels.

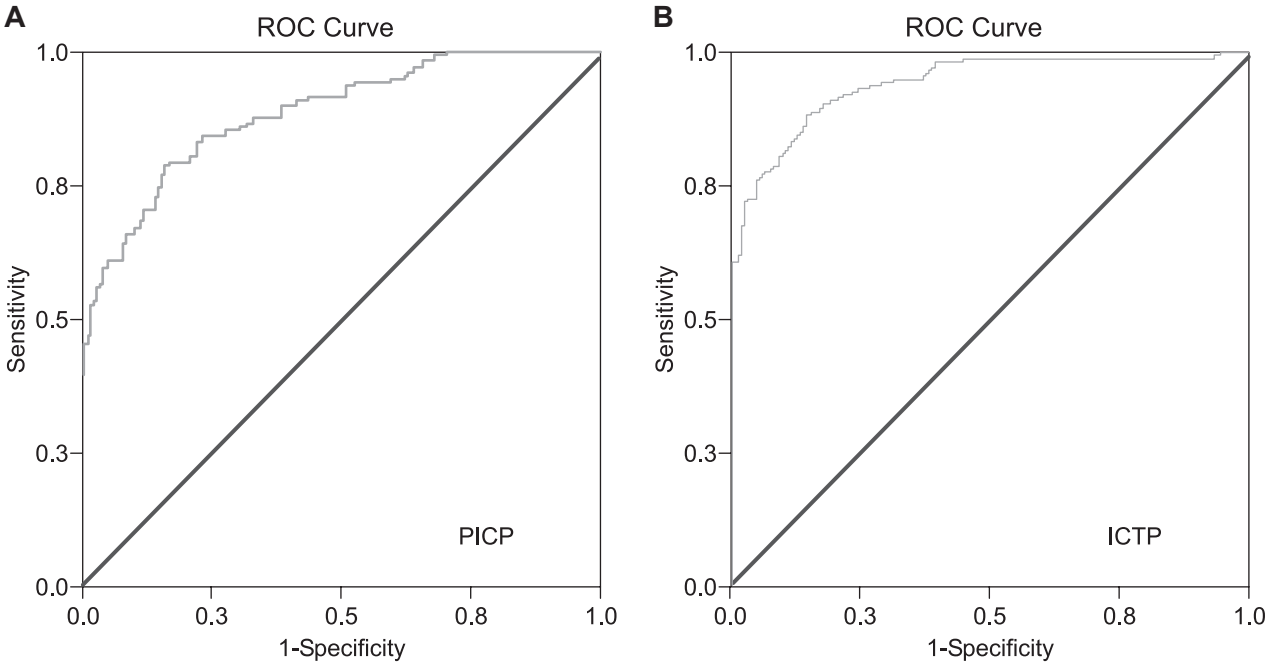

Figure 3. ROC curve analysis of PICP and ICTP. **A:** PICP ROC curve analysis; **B:** ICTP ROC curve analysis.

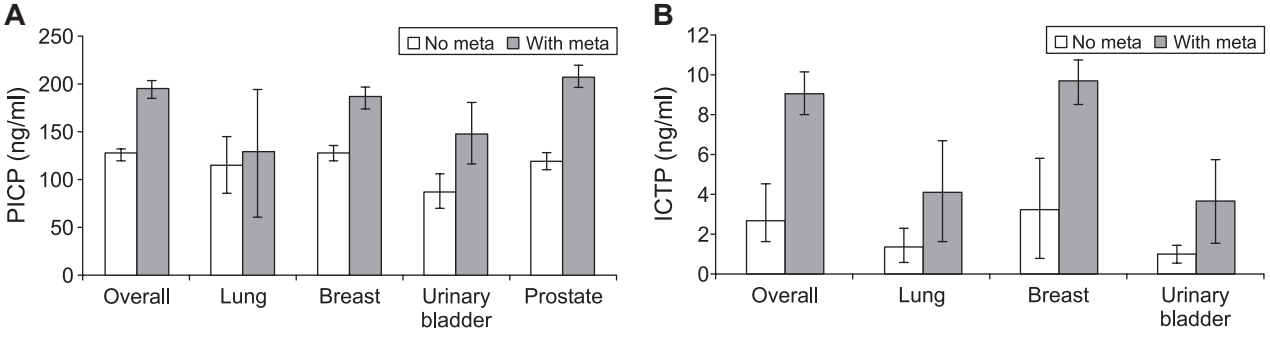

Figure 4. PICP and ICTP levels in breast cancer, lung cancer, urinary bladder cancer and prostate cancer. **A:** PICP levels; **B:** ICTP levels.

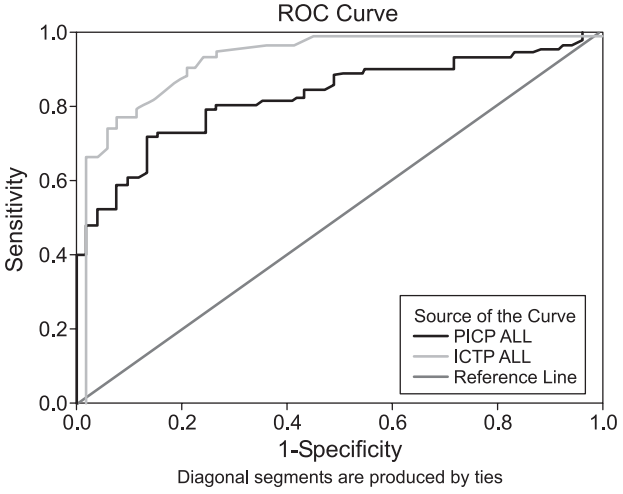

Figure 5. ROC curve of PICP and ICTP for all patients with bone metastases.

Discussion

Collagen type I is the most abundant collagenic protein in the organism. Not only is it an important constituent of soft connective tissue, but it also comprises most of the organic matrix of bone. Procollagen type I is the biosynthetic precursor of collagen type I. It is larger in size and contains additional aminoacidic sequences at both ends with respect to its final product. These aminoacid fragments are cleaved by specific proteases before the final collagen molecule is assembled. One of these fragments is the carboxyterminal propeptide of PICP. Its level in the serum can be evaluated. Igawa et al. concluded that both serum levels of PICP and ICTP were significantly higher in patients with extent of disease, grade 2 or 3 bone metastases than in patients without bone metastases [25] . Thus, PICP

and ICTP are not as useful at first detection of bone metastases as bone scintigram. The above findings are in accordance with our results. The same investigators showed that, in patients with high serum levels of PICP or ICTP before initial treatment, the changes in the concentrations of these markers may be helpful in evaluating response to treatment or disease progression. Unfortunately, we didn't have any data regarding this issue. Our study excluded patients with liver metastases, since we aimed to investigate the difference in behavior between PICP and other bone markers in patients with only bone metastases. Indeed, it is known that only important diseases of the liver (e.g. alcoholic hepatitis and cirrhosis) influence the circulating levels of PICP [26]. Obviously, in patients with both liver disease and bone metastases PICP will appear to be less useful than in patients without liver disease. However, we observed a significant reduction of PICP levels in some patients with liver and bone metastases (excluded from this study) when a remission of the bone metastases occurred. Chybowski et al. reported that PSA is by far the best index among many factors (prostatic acid phosphatase/PAP, total acid phosphatase, local clinical stage, and tumor grade), for confirming the results of bone scans in PC [27]. The present findings reconfirm the superiority of PSA for predicting bone metastases, and show that PICP and ICTP are the next most reliable markers of changes in bone metabolism. Regarding PC Westerhuis et al. showed that the diagnostic values of the new markers were generally comparable with those of alkaline phosphatase, although carboxyterminal cross-linked telopeptide of type I collagen yielded better results, but those with carboxyterminal propeptide of type I procollagen were less satisfactory [28]. Parachino et al. no longer perform a staging radionuclide bone scan in patients with PSA < 20 ng/ml and normal procollagen level, diminishing the number of radionuclide bone scans and increasing the overall net savings for the health care system [29]. Hosoya et al. concluded that serum PICP levels correlate well with the results of bone scans, while serum ICTP levels increase in patients with PC regardless of the presence of bone metastases [30]. In another study it was found that the areas under receiver operating characteristics curves were 0.86 for PlCP, 0.87 for alkaline phosphatase, 0.88 for PSA and 0.85 for PAP [31]. The best accuracy rates for PICP, alkaline phosphatase, PSA and PAP to predict bone lesions were 84, 87, 86 and 84%, respectively. PlCP provided a greater specificity and positive predictive value. These serum markers correlated significantly with the extent of disease on bone scan ($p < 0.05$). The incidence of positive serum PICP and alkaline phosphatase decreased significantly in response to endocrine therapy in patients with bone metastasis, and increased progressively in association with progression of the tumor ($p < 0.05$) parallel to PSA and PAP. Moreover, Kylmala et al. concluded that type I collagen degradation product (ICTP) gives information about the nature of bone metastases and has prognostic value in PC [32].As far as PICP is concerned, Jukkota et al. showed its value in the determination of the aggressiveness of BC [33] .Shimozuma et al. showed that in BC patients the best diagnostic efficiency by ROC analysis was provided by ICTP followed by Pyr or D-Pyr, BALP, PICP and OC and significance was observed between ICTP and OC. Multiple logistic regression analysis adjusted for age revealed that the only significant marker related to bone metastases was ICTP [34]. Blomqvist et al. concluded that, of all the biochemical parameters studied in BC patients, the changes in ICTP showed the best correlation with treatment response [35]. PICP and ICTP changes in patients with progressive disease differed significantly from those in patients with responding and stable metastases, whereas no difference was found between responders and stable patients. In our study, when PICP was compared with ICTP, there was a statistically significant difference ($p < 0.05$). ICTP levels were more increased in BC patients, as bone metastases in this type of cancer are of lytic and mixed type. In 2 of our patients without bone metastases increased ICTP levels were considered to be falsely positive.

There was no increase in sensitivity and specificity of CA 15-3 when combined with PICP. In BC patients the sensitivity and specificity of PICP was increased only when combined with CEA and ICTP (4% and 10%, respectively).

Regarding LC patients, Ebert et al. showed that the currently available bone markers cannot replace bone scintigraphy, either for screening or in the diagnosis of bone metastasis [36]. However, a panel consisting of total alkaline phosphatase (TAP), BALP, aminoterminal propeptide of type I collagen (PINP), pyridinoline (PYD) crosslinks, deoxypyridinoline (DPD) crosslinks and ICTP may be of some value as an adjunct tool to bone scintigraphy for this purpose. In the same cancer type Ylisirnio et al. found that in multivariate regression analysis, ICTP in contrast to PICP, was prognostic factor for poor survival in LC patients [37]. Another research team from Japan also showed that, especially, ICTP could be a good indicator of the progression and multiplicity of disease, and it could help in the follow-up and in the monitoring of therapy for bone metastasis from LC [38]. Our study, despite the small number of LC patients, is in accordance with the above studies, since we also found a significant dif-

ference in ICTP levels between patients with and without bone metastases.

In UBC tumor markers seem to be a good alternative to the routine urinary cytology [39]. In fact PICP and ICTP measurements have not yet been reported. However,an important significance between the number of bone metastases and the PICP levels has been reported, which is in accordance with our results, even with the limitation of the small study population [40].

In conclusion, ICTP and CA 15-3 are the most reliable markers for the early diagnosis of bone metastases in BC patients. PICP could be useful for diagnosing early bone metastases of PC and in combination with PSA and bone scan can be an additional tool in the follow-up of PC patients. For LC patients, ICTP showed a significant difference in the discrimination of patients with and without bone metastases. Regarding UBC patients PICP showed a significant difference in the discrimination of patients with and without bone metastases. However, newer and better organised studies are needed to establish the role of PICP and ICTP biomarkers in comparison to others, on the basis of the characterization of the nature of bone disease, as detected by bone scintigraphy in breast, prostate, lung and urinary bladder cancer patients.

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