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Comparison of Markers of Bone Formation and Resorption in Prostate Cancer Patients to Predict Bone Metastasis

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Abstract. We investigated the usefulness of two biochemical markers of bone formation (PICP, the carboxy-terminal propeptide of type I procollagen, and bone ALP, bone-derived alkaline phosphatase) and a marker of bone resorption (ICTP, the carboxy-terminal telopeptide of type I collagen), to determine whether the presence of bone metastasis in prostate cancer could be evaluated and the extent of bone metastasis could be stratified by the serum levels of these markers, compared to total alkaline phosphatase (T-ALP) and prostate-specific antigen (PSA). The serum levels of PICP, bone ALP, ICTP, T-ALP and PSA were significantly higher in patients with both prostate cancer and bone metastasis (n=49) than in patients with benign prostatic hyperplasia (n=35) and patients with prostate cancer without bone metastasis (n=70). The superiority of a marker in the rate of detection of bone metastasis was evaluated with receiver operating characteristic curves. The serum marker levels were compared as a function of metastatic burden in bone (i.e., the extent of disease, EOD grade). We found that bone ALP is the most suitable marker for evaluating bone metastasis, especially for stratifying the degree of bone metastasis. Both PICP and ICTP were useful in this respect, but rather inferior to bone ALP. T-ALP had the lowest ability for detecting bone metastasis, but its correlation with the EOD grade was excellent, second to that of bone ALP. PSA showed limited reliability for stratifying the extent of bone metastasis.

Key words: Carboxy-terminal propeptide of type I procollagen (PICP), Carboxy-terminal telopeptide of type I collagen (ICTP), Bone-derived alkaline phosphatase, Bone metastasis, Prostate cancer

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BIOCHEMICAL markers of bone formation have been developed [1]. During the formation of type I collagen, the carboxyterminal propeptide of type I procollagen (PICP) is cleaved from procollagen molecules. The circulation of this propeptide is released only during the synthesis of collagen [2, 3]. The bone isoenzyme of alkaline phosphatase (bone ALP), a tetrameric glycoprotein, is localized in the plasma membrane of osteoblasts and is released into the circulation as a dimer by phospholipase cleavage. An immunoradiometric (IRMA) assay with two monoclonal antibodies was developed [4, 5]. One of the two monoclonal antibodies used in this IRMA has been reported to exhibit less than 3% cross-reactivity with the liver isoenzyme of ALP purified from human liver [4].

Biochemical markers of bone resorption have also been developed [1]. At the time of bone resorption, pyridinoline (Pyr), deoxypyridinoline (D-pyr), and the carboxyterminal telopeptide of type I collagen (ICTP) are excreted through collagen degradation, and their excretion rates are considered parameters of the rate of bone degradation [6, 7].

The accurate evaluation of the metastatic lesions of prostate cancer is essential for tumor staging at diagnosis. The most common metastatic lesion of prostate cancer is bone, but the extent of bone metastasis is usually difficult to estimate accurately. The assessment of metastases to bone is based on

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bone X-ray-p (X-p) and radioisotopic bone imaging. It is known that bone X-p examination has low sensitivity, and sometimes substantial osseous destruction occurs before the bone X-p shows an obvious metastatic region. The radioisotope bone scan is a more reliable tool for the determination of the metastatic status. Several hot spot sites are commonly revealed by these bone scans, but they represent false-positive results in some cases, revealing the limitations of the bone scan [8]. Other means for the accurate detection and evaluation of bone metastases are therefore being investigated.

The present study was undertaken to examine the usefulness of these biochemical markers of bone formation (PICP, bone ALP) and bone resorption (ICTP) for the assessment of metastatic bone involvement in patients with prostate cancer, compared with total alkaline phosphatase (T-ALP) and prostate-specific antigen (PSA).

Subjects and Methods

Subjects

A total of 154 individuals were examined and stratified into the following three groups: prostate cancer patients with bone metastasis (n=49), prostate cancer patients without bone metastasis (n=70), and patients with benign prostatic hyperplasia (BPH) (n=35). All of the subjects were inpatients of Chiba University Hospital. The diagnosis of all patients was confirmed histologically. No therapeutic intervention, rectal examination or endoscopic procedure was performed in any of these patients immediately before the blood collection. None of the patients had impaired renal function, as evidenced by normal serum creatinine and urea nitrogen levels.

All serum samples were collected in the morning after an overnight fast and were stored at −60 °C until assayed.

Prostate cancer with bone metastasis: Forty-nine patients aged 56–85 yr with metastatic prostate cancer before treatment comprised this group. The assessment of metastases to bone was based on bone X-p and radioisotopic bone imaging. Bone scans were performed with ^{99m}Tc-methylene-diphosphonate. One patient did not receive a bone scan because of his poor general condition. Computed tomography (CT) and magnetic resonance imaging (MRI) were also performed in some patients. Clinical staging was done before the biochemical determination.

The extent of bone metastases (extent of disease, EOD grade) in each patient was classified by the method of Soloway *et al.* [9] as follows. EOD 1, the number of bony metastases is less than six, each of which is less than 50% of the size of a vertebral body (one lesion about the size of a vertebral body would be counted as two lesions); EOD 2, the number of bone metastases is between six and 20, with the size of lesions as described above; EOD 3, the number of metastases is more than 20 but less than a "super scan "and EOD 4, "super scan" or its equivalent, i.e., more than 75% of the ribs, vertebrae, and pelvic bones have lesions. The EOD grading of the 48 patients with bone metastasis as shown by bone scan was determined; 12 patients were EOD 1, 21 were EOD 2, 10 were EOD 3 and 5 were EOD4.

Prostate cancer without bone metastasis: This group consisted of 70 patients aged 56–90 yr, with untreated prostate cancer. When the bone scan of a patient was positive in only one region and the finding was equivocal, corresponding bone X-p, MRI and CT were performed. Some patients of this group were subsequently diagnosed as having osteoarthritis or Schmorl nodule.

Benign prostatic hyperplasia (BPH): Thirty-five BPH patients aged 52–87 yr without evidence of bone disease were studied. All sera of these patients were obtained before the operation (retropubic prostatectomy or transurethral resection). Subjects with a previous history of bone fracture, rheumatoid arthritis, or other painful bone disease were excluded from the study.

Markers of bone formation

The serum bone ALP levels were assayed by the Tandem-R Ostase assay (Hybritech Inc., San Diego, CA, USA), a two-site IRMA with two murine monoclonal antibodies directed against different epitopes on bone ALP [5, 6]. The intra- and inter-assay coefficients of variation are 3.7–6.7% and 7.0–8.1%, respectively. The detection limit and normal range are 0.2 and 2.3–19.9 ng/m*l*, respectively [5].

The serum PICP levels were measured by a radioimmunoassay (RIA) based on the two-antibody method, with a PICP RIA kit (Orion

Diagnostica, Espoo, Finland) [3]. The intra- and inter-assay coefficients of variation are 1.9–8.0% and 3.0–3.9%, respectively. The detection limit and normal range are 1.2 and 26–222 ng/ml, respectively [3].

Markers of bone resorption

The serum ICTP levels were assayed by an RIA (Telopeptide ICTP, Orion) based on the two-antibody method [7]. The intra- and inter-assay coefficients of variation are 3.6–5.0% and 4.0–8.7%, respectively. The detection limit and normal range are 0.34 and 1.8–5.0 ng/ml, respectively [7].

Markers of prostate cancer and total alkaline phosphatase

The serum PSA levels were determined with the Tandem-R PSA Assay (Hybritech) [10]. The intra- and inter-assay coefficients of variation are 1.4–5.8% and 3.3–6.3%, respectively. The detection limit and normal range are 0.13 and 0–4.0 ng/ml, respectively [10].

The serum activity of T-ALP was measured at 37 °C according to the optimized standard method of the German Society for Clinical Chemistry by an automated colorimetric assay and para-nitrophenyl phosphate as a substrate. The T-ALP values are expressed as IU/L. The normal range is 72–206 IU/L.

Statistical analysis

Differences in the PICP, bone ALP, ICTP, T-ALP, and PSA levels were tested by the Wilcoxon-Mann-Whitney test. The sensitivity, specificity and accuracy (the ratio of true positives plus true negatives to the total number of cases) of PICP, bone ALP, ICTP, T-ALP and PSA were calculated with several arbitrarily chosen cut-off levels. Receiver operating characteristic (ROC) curves, which are graphic presentations of the pairs of sensitivity and 100 minus the corresponding specificity, were constructed. The differences between the areas under the ROC curve were

Table 1. Serum PICP, bone ALP, ICTP, T-ALP, and PSA levels in patients with benign prostatic hyperplasia (BPH), patients with prostate cancer without bone metastases, and patients with prostate cancer with bone metastases

Disease	BPH (n=35) Mean ± SD (median)	prostate cancer without bone metastases (n=70)	prostate cancer with bone metastases (n=49)
Age (yrs)	69.5 ± 8.2 (70)	72.2 ± 8.5 (74)	72.2 ± 8.0 (73)
PICP	99.8 ± 35.3 (94)	100.7 ± 44.7 (88)	259.9 ± 228.2[a,b] (155)
bone ALP	11.9 ± 7.0 (11.0)	10.8 ± 6.6 (10.1)	65.3 ± 70.7[a,b] (26.4)
ICTP	3.5 ± 1.7 (3.2)	4.5 ± 2.7[c] (4.3)	10.2 ± 7.6[a,b] (7.6)
T-ALP	155.1 ± 42.5 (153)	155.5 ± 59.8 (141)	571.2 ± 750.2[a,b] (310)
PSA	6.4 ± 4.7 (5.6)	57.3 ± 111.5[d] (19.3)	625.6 ± 960.6[a,b] (219)

PICP, bone ALP, ICTP, and PSA (ng/ml) and T-ALP (IU/L). a, $P<0.0001$ *vs.* BPH patients; b, $P<0.0001$ *vs.* prostate cancer patients without bone metastasis; c, $P=0.0130$ *vs.* BPH patients; d, $P<0.0001$ *vs.* BPH patients. BPH, benign prostatic hyperplasia; PICP, carboxy-terminal propeptide of type I procollagen; bone ALP, bone-derived alkaline phosphatase; ICTP, carboxy-terminal telopeptide of type I collagen; T-ALP, total alkaline phosphatase; PSA, prostate-specific antigen.

estimated by a previously reported method [11]. The correlations of the EOD grade and PICP, bone ALP, ICTP, T-ALP and PSA were evaluated by Spearman's rank correlation test.

Results

PICP, bone ALP, ICTP, T-ALP and PSA in BPH and prostate cancer

The serum levels of PICP, bone ALP, ICTP, T-ALP and PSA were significantly higher in the patients with both prostate cancer and bone metastasis than in the patients with BPH and those with prostate cancer without bone metastasis. The PICP, bone ALP and T-ALP levels in the patients with prostate cancer without bone metastasis were similar to those in the BPH patients, whereas the ICTP and PSA levels were significantly higher in the former group than in the latter group (Table 1). The results of the ROC analysis are shown in Fig. 1. The best accuracy levels and corresponding marker levels were 79.9% (200 ng/ml) for PICP, 83.8% (25 ng/ml) for bone ALP, 83.8% (6.5 ng/ml) for ICTP, 83.1% (300 IU/L) for T-ALP and 85.7% (80 ng/ml) for PSA. The areas under the ROC curve and corresponding standard error of the mean (SEM) were 82.8%, 0.039 for PICP, 87.4%, 0.030 for bone ALP, 85.2%, 0.040 for ICTP, 77.2%, 0.044 for T-ALP, and 89.8%, 0.031 for PSA. PSA was therefore better than bone ALP for detecting bone metastasis, followed by ICTP, PICP and T-ALP. The areas under the ROC curve for bone ALP and ICTP were significantly larger than that for T-ALP (*P* value: bone ALP *vs.* T-ALP; <0.005, ICTP *vs.* T-ALP; <0.05). The coefficients of correlation between the ROC areas of T-ALP and those of PSA, bone ALP, ICTP, and PICP were 0.15, 0.61, 0.33 and 0.53, respectively. Since the coefficient of correlation between T-ALP and PSA was much lower than those for the other markers, the area under the ROC curve for PSA was not significantly different from that for T-ALP. There were no other significant differences among the ROC curves.

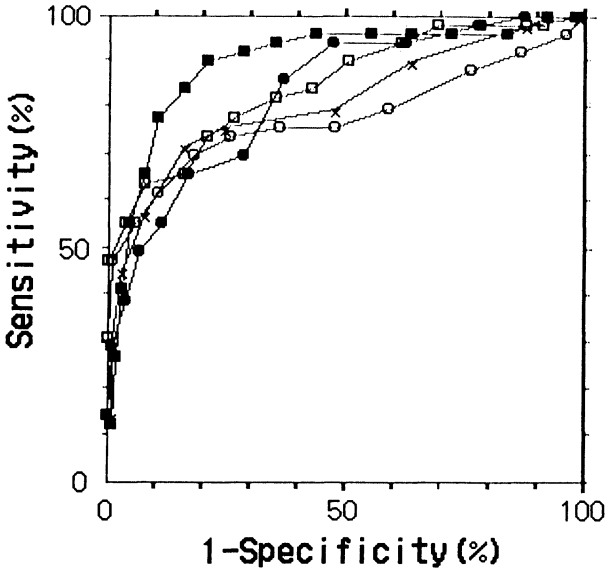

Fig. 1. Receiver operating characteristic (ROC) curves of PICP, bone ALP, ICTP, T-ALP, and PSA. Prostate cancer patients with metastatic spread to bone (n=49) showing abnormal levels were regarded as true-positive; patients with BPH (n=35) and prostate cancer patients without bone metastases (n=70) showing abnormal levels were regarded as false-positive. ●, PICP; □, bone ALP; X, ICTP; ○, T-ALP; ■, PSA. Units are ng/ml for PICP, bone ALP, ICTP, and PSA, IU/L for T-ALP.

Relationship of PICP, bone ALP, ICTP, T-ALP, and PSA to the extent of bone metastases

We compared the serum levels of PICP, bone ALP, ICTP, T-ALP and PSA as a function of metastatic burden in bone estimated by the method of Soloway (Fig. 2). The mean values and median values for PICP, bone ALP, ICTP and T-ALP increased with the extent of bone metastasis. In contrast, the mean and median values for PSA in the EOD 3 patients were lower than those in the EOD 2 patients. The PICP, bone ALP, ICTP, T-ALP and PSA levels in the EOD 4 patients were significantly higher than those in the EOD 1 patients. Similarly, the PICP, bone ALP, ICTP and T-ALP values in the EOD 2 and EOD 3 patients were significantly higher than those in the EOD 1 patients. The PSA levels in the EOD 1, EOD 2 and EOD 3 patients were not significantly different. The PICP, bone ALP and T-ALP levels in the EOD 3 and EOD 4 patients were significantly higher than those in the EOD 2 patients. In contrast, the PSA levels in the EOD 2, EOD 3, and EOD 4 patients were not significantly different. The correlations of EOD grade with the PICP, bone ALP, ICTP, T-

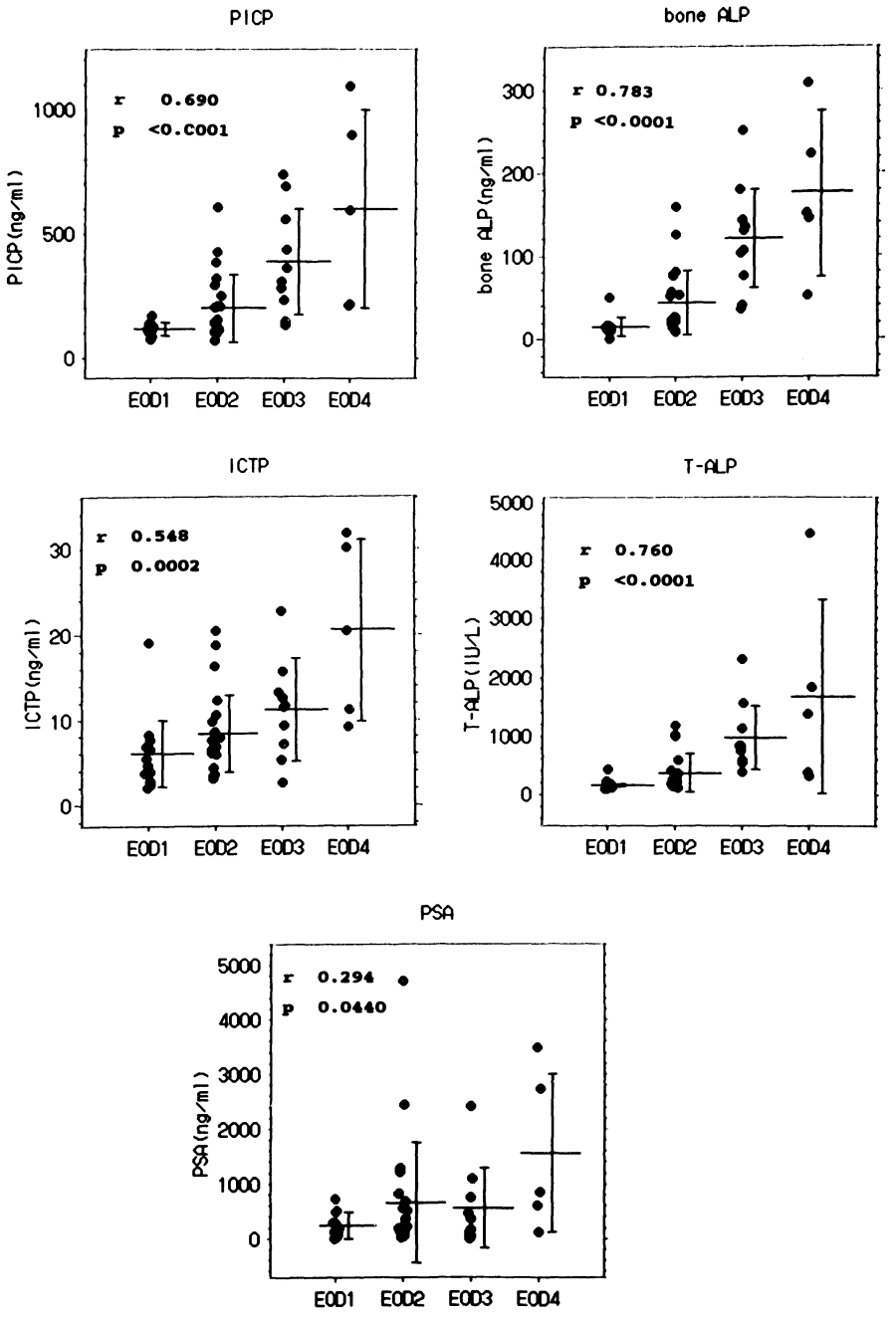

Fig. 2. Individual values for five biochemical markers of bone formation, resorption and prostate cancer in 48 prostate cancer patients with bone metastasis as a function of the extent of the disease (EOD) grade (EOD 1, n=12; EOD 2, n=21; EOD 3, n=10; EOD 4, n=5). For each marker, horizontal lines represents the mean ± SD for each group. The coefficient of correlation (r) and p value between EOD grade and each marker were obtained by Spearman's rank correlation test.

ALP and PSA levels were evaluated by Spearman's rank correlation test. The correlation of bone ALP with the EOD grade was the highest, followed by T-ALP, PICP and ICTP, and that of PSA was the lowest (Fig. 2).

Discussion

The usefulness of the biochemical markers of bone formation and bone resorption has been evaluated in several studies [12–15], but few such studies examining metastatic bone involvement have been done. In the present study, parameters of bone formation and bone resorption were determined in BPH and prostate cancer patients with and without evidence of metastatic bone involvement. The results demonstrated that the levels of both the bone formation and bone resorption markers were significantly higher in the prostate cancer patients with bone metastasis than in the BPH patients and prostate cancer patients without bone metastasis. This observation is in agreement with the findings in previous reports [16, 17] using the urinary excretions of Pyr and D-Pyr. Although an alternative enzyme immunoassay for Pyr and D-Pyr has been developed, it was reported that, in a study with the ROC curve, urinary Pyr and D-Pyr were not suitable for indicating metastatic bone involvement [18].

T-ALP is still the parameter most widely used for estimating bone metastasis, even though its clinical value in evaluating osteoblast activity is restricted, because T-ALP is composed of several isoenzymes whose most important fractions originate in the liver as well as bone. The area under the ROC curve for T-ALP was previously reported as 61% [18]. This rate is lower than that obtained in the present study (77%). The higher rate in the present study might be attributable to a difference between prostate cancer and other cancers in the tumor-induced enhancement of osteoblasts (approximately half of the previous report's patients had breast cancer). The percentage of the area under the ROC curve for T-ALP was the lowest among the markers examined in the present study, but T-ALP was superior to PICP, ICTP and PSA with respect to stratifying the metastatic burden of bone by EOD grade.

In the present study, the area under the ROC curve for bone ALP was significantly larger than that for T-ALP, and bone ALP was the most useful marker for stratifying the degree of bone metastasis, but we observed a substantial overlap in the distribution of bone ALP levels among the three groups; this observation coincides with the findings of previous reports [19, 20]. The ROC curves in the present study indicate that bone ALP and ICTP are superior to T-ALP as markers for evaluating bone metastasis. It has been reported that ICTP was a superior marker to bone ALP [21]; that finding differs from those of the present study. The cause of this discrepancy may be that osteolytic metastases were present in 80% of the patients in that study. The low discriminating power of PICP compared to bone ALP (both are bone formation markers) in the present study is in accordance with another previous report [22]. We observed that the area under the ROC curve for PSA was the largest, but was not significantly different from that for T-ALP. The reason is that the calculation of differences in the areas under the ROC curve included the correlation between the ROC areas of each marker [11], and the coefficients of correlation for PSA and T-ALP were rather low.

The present findings showed that PSA was inferior to PICP, bone ALP, ICTP and T-ALP with respect to stratifying the metastatic burden of bone. It has been reported that, since higher-grade tumors produce less PSA, advanced-stage tumors that contain high-grade tumor cells may actually release less PSA into the blood [23]. Another possible explanation for the low correlation between PSA and the degree of bone metastasis is based on the coexistence of neuroendocrine cell tumors. The incidence of neuroendocrine cells in prostate cancer has been debated, but at least 10% of prostate cancers have a marked proportion of this cell type [24]. A quantitative assay of serum chromogranin A, a neuroendocrine cell product, has recently been applied to studies of prostate cancer. It has been reported that 67% of the patients at stage D2 with high serum levels of chromogranin A had normal serum PSA [25]. PSA was therefore not a useful marker for stratifying the extent of bone metastasis.

Prostate cancer is the only cancer that consistently produces osteoblastic, rather than osteolytic, bone metastasis. More than 90% of bone lesions due to prostate cancer are osteoblastic. Breast cancer is the second most common cause of osteoblastic metastasis, but the rate is low (8%) [26]. In the present study, the serum levels of ICTP in even the prostate cancer patients with bone metastasis were increased in all EOD grades. This high level of ICTP might be due to the stimulation of osteoclasts by metastatic cancer cells or the coupling

of bone formation and resorption. Attention has been focused on the role of cytokines in mediating bone remodeling [27], but the relationship between cytokines and bone metastasis in prostate cancer is not clearly understood. Bone ALP is produced by osteoblasts. In the present study, the correlation of the extent of bone metastasis with ICTP, a bone resorption marker, was relatively low compared to PICP and bone ALP, which are bone formation markers. This is attributed to the imbalanced coupling accompanying bone metastasis.

The radioisotope bone scan has limitations in assessing metastatic burden accurately. Since benign disorders of the bones and joints may produce false positive findings, in our institution, when a bone scan of a patient is positive in only one region and equivocal findings are obtained, corresponding bone X-p, MRI and CT are performed. In the present study, the clinical staging was done before the biochemical determination. The levels of the bone formation and resorption markers and the degree of bone metastasis were not correlated in some cases. The determination of the levels of bone formation and resorption markers may play a helpful and complementary role in differentiating degenerative change from metastasis.

In conclusion, the serum levels of both bone formation and bone resorption markers were increased in prostate cancer patients with bone metastasis, and bone ALP was found to be the most suitable marker for evaluating bone metastasis, especially for stratifying the degree of bone metastasis. Both PICP and ICTP were useful in this respect, but rather inferior to bone ALP. T-ALP had the lowest ability for detecting bone metastasis, but its correlation with EOD grade was excellent, second to that of bone ALP. PSA showed limited reliability for stratifying the extent of bone metastasis.

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