Serum Osteocalcin Measurements in Prostate Carcinoma Patients with Skeletal Deposits Shown by Bone Scintigram: Comparison with Serum PSA/PAP Measurements

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The correlation of technetium-99m-HMDP bone scintigraphic findings, serum osteocalcin as a measure of bone turnover and prostate-specific antigen (PSA) and/or prostate acid phosphatase (PAP) was determined in 19 men with bone metatasis due to prostatic carcinoma. Six of the 19 patients with metastases on bone scan showed elevation of osteocalcin. These patients had extensive metastatic disease. All 19 men with positive bone scans had high serum PSA and/or PAP levels. Serum osteocalcin measurement is less sensitive to detection of bone deposits than PSA/PAP measurements ($p<0.0008$).

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Osteocalcin, a vitamin K-dependent protein, is a calcium-binding protein of bone and the most abundant noncollagenous bone protein (*1*). Osteocalcin is synthesized by osteoblasts and is believed to play a role in the mineralization process; the process may be under the influence of calcium-regulating hormones calcitonin and parathyroid hormone, and vitamin D (*1,2*).

Osteocalcin, alkaline phosphatase, and hydroxyproline have been considered to be biochemical markers for bone metabolism (*1*). Serum levels of osteocalcin are a reflection of bone turnover which is believed to be more specific than that of the biochemical markers alkaline phosphatase and hydroxyproline. Because the serum concentration of alkaline phosphatase is contributed to by other areas of the body, including the liver, gastrointestinal tract, placenta, and tumors, it is not specific for bone metabolism (*3,4*).

Osteocalcin has been found to be elevated in the following disorders: Paget's disease of the bone, primary hyperparathyroidism, renal osteodystrophy, and cancer with skeletal metastases. Osteocalcin has the important advantage of being a specific marker for bone disease (*5*).

Prostate carcinoma is one of the most common cancers in men (*6*), and most prostate-carcinoma patients eventually show skeletal involvement (*7*). Since scintigraphic or radiographic patterns of skeletal metastases originating from prostate carcinoma are characteristically osteoblastic, with the introduction of radioimmunoassay (RIA) for prostate-specific antigen (PSA) and osteocalcin it is hoped that such assays will provide additional information relating to the skeletal involvement of prostatic cancer. To assess the clinical utility of osteocalcin, PSA, and prostate acid phosphatase (PAP) in conjunction with technetium-99m (^{99m}Tc) bone scintigraphic findings, we recently measured osteocalcin, PSA, and PAP levels in bone scintigrams in 38 patients with prostate carcinoma.

MATERIALS AND METHODS

Thirty-six consecutive prostate-carcinoma patients, aged 45 to 78, who were referred for bone scintigrams to evaluate skeletal metastases were studied. Histopathologic confirmation with Gleason's grading was established. A 5-ml blood sample was obtained from each patient before i.v. injection of the bone-imaging agent ^{99m}Tc-hydroxy methylene disphonate (HMDP). Serum aliquots were stored at −20° to −27°C until assayed.

Assay Procedures

Serum osteocalcin value was assayed using a commercial kit (INCSTAR, Stillwater, MN), which is based on radioimmunoassay (*8*). The normal range of the osteocalcin is 2.3 to 6.3 ng/ml.

PSA was measured using a commercial kit (TANDEM-R PSA, Hybritech, Inc., San Diego, CA) assay, which is based on two-site immunoradiometric assay (*9,10,11*). The normal range of the PSA is 0-4 ng/ml.

PAP was measured using a kit (TANDEM-R PAP, Hybritech, Inc.) assay, which is based on immunoradiometric assay (*12*). The normal range of PAP is 0-3 ng/ml.

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Bone Scintigraphy

Each patient received 10–15 mCi ^{99m}Tc-HMDP intravenously. Two to three hours later, anterior views of total-body bone scan were obtained by Siemen's LFOV, ROTA, or orbiter camera; then multiple posterior "spot" images were obtained. All images were displayed on transparent film. Each bone image was compared with available radiographs, and the presence or absence of metastatic bone disease was determined. Bone images were interpreted as positive or negative for skeletal metastases. The criteria of positive bone scan included bone scan showing three or more focal areas of increased skeletal activity (*13–15*). Bone scan results were compared with osteocalcin, PSA, and/or PAP values for each patient.

RESULTS

Nineteen of the 36 patients had three or more sites of focal skeletal uptake, forming the basis of this report (Table 1). Thirteen patients had extensive bone metastases (>6 lesions) while six patients had more than three foci of abnormal radiotracer localization. Table 1 summarizes serum osteocalcin, PSA, and/or PAP, and CT of the abdomen and/or radiographic findings as compared with scintigraphic positive bone metastases. For comparison, the results in nine of the 36 patients with negative bone scan are summarized in Table 2. Patient 1 of Table II had a rectal digital examination on the day before the blood sample was obtained which resulted in a high serum PSA value. Subsequent serum PSA levels at 3 wk and 6 wk later measured 0.5 ng/ml and 0.1 ng/ml, respectively. Eight of the 36 patients' bone scans showing one or two bone lesions were excluded from this study because metastases of these lesions had not been confirmed by radiography.

Statistical Analysis

In the 13 patients with extensive skeletal metastases, the serum osteocalcin level was compared with the serum PAP and/or PSA level. All 13 of these patients had abnormally high serum PAP and/or PSA values

TABLE 1
Serum Osteocalcin, PSA, and PAP in Positive Bone Scintigrams

Patient	Age	Gleason grade‡	PSA (ng/ml)	PAP (ng/ml)	Osteocalcin (ng/ml)	Bone scan	* CT or †x-ray of abdomen or pelvis
1	70	3 + 3	4.9	4.9	8.7	Extensive metastases	*† Osteoblastic bone lesions
2	56	3 + 3	1871	442	7.1	Extensive metastases	*† Osteoblastic bone lesions
3	70	3 + 3	38.4	8.7	6.6	Extensive metastases	* Large adrenal gland, right
4	45	4 + 4	1684	120	6.6	Extensive metastases	† Multiple blastic lesions
5	71	3 + 4	14	9.5	6.3	Extensive metastases	No CT or x-ray available
6	63	3 + 3	1880	141	13.4	Extensive metastases	*† Osteoblastic lesions in lumbar vertebrae and pelvis
7	49	3 + 4	420	42	2.1	Extensive metastases	* Multiple osteoblastic lumbar vertebrae and pelvis
8	74	4 + 4	65	7.4	1.9	Extensive metastases	*† Liver lesions; osteoblastic lesions in skull and pelvis
9	71	3 + 2	1.5	26.4	3.2	Extensive metastases	*† Osteoblastic lesions in the lumbar vertebrae
10	62	4 + 5	>100	18.2	5.5	Extensive metastases	No CT or x-ray available
11	64	3 + 3	9.3	4.7	3.1	Extensive metastases	† Osteoblastic lesions in pelvis and vertebrae
12	61	3 + 4	34	44	3.7	Extensive metastases	No CT or x-ray available
13	76	3 + 3	17.2	1.4	0.8	Extensive metastases	*† A liver mass; osteoblastic lesions in pelvis
14	66	4 + 2	62.3	4.3	4.0	T-11 compression fracture T-12 cold lesion Left 3rd rib anteriorly Right 9th rib posteriorly	No CT or x-ray available
15	63	3 + 3	9.1	3.1	4.1	Skull, ribs, vertebrae, sternum	Osteoblastic lesions in vertebrae
16	71	3 + 4	8.6	6.9	6.0	Ribs; Right femur; T-vertebrae	
17	65	3 + 3	26	2.7	0.0	T3, sternum, 4th rib	† Destructive rib lesion
18	74	4 + 4	73	10.7	2.3	Sacrum, L5, Rt humerus	
19	78	5 + 4	>100	26	3.4	T-11, L-3, ribs	† Osteoblastic lesions in lumbar pelvic, vertebrae

‡ Gleason grading system is based on the degree of glandular differentiation and the growth pattern of the tumor in relation to the stroma as evaluated on lower power examination. Combined grade is obtained by adding predominant grade (primary grade, first digit) and other grade (secondary grade, second digit) (*26–27*).

PSA = <4 ng/ml.

PAP = <3 ng/ml.

Osteocalcin = 2.3–6.3 ng/ml.

TABLE 2
Serum Osteocalcin, PSA, and PAP Negative Bone Scintigrams

Patient	Age	Gleason grade‡	PSA (ng/ml)	PAP (ng/ml)	Osteocalcin (ng/ml)	Bone scan findings	Comments/* CT or † x-ray abdomen or pelvis
1	70	4 + 3	6.6§	2.1¶	3.9	Negative	Rectal exam the day before serum obtained; post radical prostectomy
2	67	3 + 2	45	25.9	2.0	Negative	Pelvic lymph nodes metastases; orchiectomy done later
3	66	3 + 3	2.6	—	0.9	Negative	No CT or x-ray available; post retropubic prostectomy
4	73	3 + 2	1.1	1.4	1.4	Negative	No CT or x-ray available; on ** DES therapy
5	72	4 + 4	0.4	1.5	3.6	Negative	† Negative; post radical prostectomy
6	60	3 + 2	0.4	1.0	1.9	Negative	† Negative; post retropubic prostectomy
7	65	3 + 2	1.4	1.8	2.0	Negative	* Negative; post irradiation to prostate
8	59	2 + 3	0.5	1.6	2.7	Negative	* Negative; post radical prostectomy
9	62	3 + 3	0.9	1.2	2.8	Negative	† Negative; post DES and leuprolide therapy and post orchiectomy

‡ Same as in Table I.
§ PSA level at 3 wk and 6 wk later measured 0.5 ng/ml and 0.1 ng/ml, respectively.
¶ PAP level at 3 and 6 wk later measured 1.3 and 0.9 ng/ml, respectively.
** DES = diethylstilbestrol.
Normal ranges: PSA = <4 ng/ml; PAP = <3 ng/ml; and Osteocalcin = 2.3–6.3 ng/ml.

while only 6 of 13 patients had abnormal high serum osteocalcin. Serum osteocalcin measurement is less sensitive in the detection of bone deposits than serum PSA/PAP measurement ($p<0.025$ by McNemar's test). Of 19 patients with bone scan positive for skeletal metastatic bone disease, only 6 patients had abnormally high serum osteocalcin. All 19 patients had abnormally high serum PSA/PAP levels. These results also confirm the greater sensitivity ($p<0.0008$ by McNemar's test) of serum PAP/PSA measurements over serum osteocalcin in the detection of bone deposits revealed by bone scans.

DISCUSSION

Bone scintigraphy has been well established for the detection and monitoring of skeletal metastases. Because an abnormal bone scan is nonspecific for metastatic deposits, correlative radiographs are routinely made to evaluate sites of increase in uptake on bone scintigraphy. Bone scans with multiple (three or more) areas of abnormal uptake are considered strongly suggestive of metastatic bone disease (*13–15*). In a recent study of cancer patients with one or two bone scan abnormalities, Jacobson et al. concluded that correlative radiographs showing a benign abnormality are reliable (*16*). Based on these findings, we excluded those patients with one or two bone scan abnormalities and included those patients with bone scans showing three or more focal lesions or extensive metastatic deposits.

PAP measurement has been used for clinical staging of prostate cancer patients (*17*); PSA is an organ-specific marker and has been reported to be more sensitive than PAP in the detection of prostate carcinoma (*18, 19*). Serum PSA/PAP levels in our study were positively correlated with positive bone scans, while osteocalcin was less sensitive in the detection of bone deposits than PSA/PAP measurements.

As observed by others (*19, 20*), the abnormally high PSA level in Patient 1 in Table 2 was explained by the fact that the patient had a rectal digital examination on the day before the blood sample was obtained. This was also evidenced by the return of the serum PSA level to normal in subsequent 3-wk and 6-wk follow-up. In this patient, serum PSA was more apt to give a false-positive as the result of a rectal examination than that of PAP measurement; all serum PAP levels were within normal range including those obtained immediately after rectal examination. The blood samples for PSA assay, therefore, should be obtained either before or two to three weeks after rectal examination or other procedures involving manipulation of the prostate. In Patient 2 of Table 2, the elevation of PSA and PAP may be explained by pelvic lymph nodes metastases. This patient subsequently underwent hormone therapy.

Serum osteocalcin derives largely from new cellular synthesis and is a marker for bone formation and a marker of osteoblastic activity (*1*). Osteocalcin correlates with histomorphometric indices of bone formation; changes in serum osteocalcin levels reflect the activity of the osteoblast (*1*). Increased serum osteocalcin may reflect osteoblastic activity with acceleration of bone turnover (*1*). Characteristically, positive skeletal metastases originating from prostate gland are usually osteoblastic. Our results indicate that only 6 of 19

positive bone metastatic disease had elevation of serum osteocalcin. Serum osteocalcin measurement is less sensitive than serum PSA/PAP measurements in the detection of bone metastasis. Low sensitivity of osteocalcin assay as for bone scan findings is unknown and subject to further study.

Multiple factors responsible for the accumulation of bone-imaging agent in the bone scintigrams include blood flow, extraction efficiency, capillary permeability, extracellular space hydrostatic pressure, electrical potential, and local changes in pH (*21–24*). Extraction efficiency depends on reactive new osteoblastic activity (*21–24*). Sympathetic tone also plays an important role in tracer localization; normally one-third to one-half of the arterioles in bone are closed (*23*). Localized inhibition of sympathetic control which produces neurovascular flush, results in vascular dilation and permeability (*21–25*) resulting in more bone-imaging agent localizing in that region of the bone. It is speculated that the disparity between serum osteocalcin and positive bone lesions may be explained as follows: instead of osteoblastic activity resulting in the synthesis and/or release of osteocalcin, area(s) of high activity in the bone scan may be predominantly attributed to increased regional blood flow and decreased sympathetic tone.

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