Can bone metabolism markers be adopted as an alternative to scintigraphic imaging in monitoring bone metastases from breast cancer?

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Abstract. Bone scintigraphy plays a major role in the diagnosis of bone metastases. The clinical utility of new biochemical markers of bone metabolism has recently been investigated in various bone diseases. This study evaluated the role of some bone metabolism markers in comparison with bone scan in the follow-up of breast cancer patients. We studied 149 patients with breast cancer, 33 (22%) of whom had bone metastases. IRMAs were used for the evaluation of blood levels of osteocalcin, bone alkaline phosphatase (BAP), the C-terminal propeptide of type I procollagen and the C-terminal cross-linked telopeptide of type I collagen (ICTP). Multivariate regression analysis showed that menopausal status (P=0.007) and metastatic bone lesions (P=0.001) affected bone marker levels. When considering postmenopausal women, the only subset in which bone metabolism marker behaviour could be reliably investigated, we found a high degree of overlap in marker distribution for scan-positive and scan-negative patients. Discrimination between scan-negative and scan-positive patients based on the above markers, taken singly or jointly, was assessed by means of logistic discriminant analysis. The best discrimination was achieved with BAP, closely followed by ICTP. BAP and ICTP together gave a slight improvement over the use of the two markers separately. However, even in this case the degree of discrimination was poor and its clinical utility was limited. In fact, to achieve a specificity of 95%, the sensitivity of the test was about 20%; conversely, with a sensitivity of 95%, the specificity was below 10%. In conclusion, based on our findings, we believe that blood levels of the investigated markers cannot replace bone scintigraphy in the follow-up of breast cancer patients for the early detection of bone metastases.

Key words: Bone scintigraphy – Bone metabolism – Osteocalcin – Bone alkaline phosphatase – C-terminal propeptide type I procollagen – C-terminal cross-linked telopeptide of type I collagen

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Introduction

Bone is the most frequent site of metastatic spread from breast cancer, bone metastases being demonstrated by autopsy in about 70% of cases [1]. Furthermore, as observed by different authors, about one-third of breast cancer patients have bone metastases at the time of their first recurrence [1, 2]. Skeletal metastases are often accompanied by life-threatening complications including spontaneous fractures, spinal cord compression and malignant hypercalcaemia, and they contribute significantly to the morbidity caused by advanced breast cancer.

The diagnosis of bone metastases usually requires a combination of nuclear medicine, radiographic and clinical laboratory methods. However, all these techniques have their methodological limitations in terms of specificity and sensitivity. Bone scintigraphy plays a major role in the diagnosis and follow-up of bone metastases [3]. In a 10-year follow-up study our group found that bone scan can reach a very high sensitivity and a good specificity, with a positive predictive value of 70% and a negative predictive value of 99.8%. Furthermore, this study underlined the importance of bone scans during follow-up because some patients may have asymptomatic lesions and a scintigraphic diagnosis can precede radiological evidence by several months [4]. Nevertheless, dosimetric and cost-effectiveness considerations could limit the use of repeated bone scans during the follow-up of asymptomatic patients [3, 5, 6].

Radiographs have limited sensitivity in the diagnosis of skeletal metastases, particularly if medullary rather than cortical metastases are present. Skeletal radiography of areas of bone pain may demonstrate metastases, but it has been estimated that 50% of cortical bone must

be focally destroyed before lytic metastases will become apparent [7]. Skeletal X-rays are often used in combination with bone scintigraphy to confirm focal uptake on bone scans, as they can demonstrate specific structural alterations due to the metastatic lesions. Computed tomographic (CT) scans and/or magnetic resonance imaging (MRI) has recently been adopted to better define these alterations [8–10].

Laboratory tests are non-invasive, inexpensive, easy to perform and well accepted by patients. Some of these tests have been proposed as possible substitutes for X-ray nuclear medicine methods in the follow-up of cancer patients. This has already happened with prostate-specific antigen in prostate cancer [11] and with thyroglobulin in differentiated thyroid cancer [12, 13]. Controversy exists over the clinical usefulness of laboratory tests (e.g. CA 15.3) versus other diagnostic tools (X-rays, ultrasonography, bone scintigraphy) in the follow-up of breast cancer, especially with regard to their ability to modify the clinical management of patients in a general context of cost-effectiveness [14]. Against this background, several oncological institutions maintain a strictly instrumental follow-up in those patients with the worst prognostic parameters and at high risk of relapse, so that intensive treatment protocols can be activated in cases of recurrence.

In this study we examined bone metabolism markers, including traditional markers (serum alkaline phosphatase and urinary excretion of calcium and hydroxyproline) on the one hand and new biochemical markers (bone generation and collagen pathways) on the other. Serum alkaline phosphatase and urinary excretion of calcium and hydroxyproline are the most widely utilized laboratory tests for the detection of bone involvement in breast cancer. However, these tests lack specificity and sensitivity [15–18]. New biochemical markers of bone metabolism (i.e. products of osteoblastic cells and products of collagen synthesis or degradation) have been introduced recently and their clinical utility has been investigated in various bone diseases including osteoporosis and Paget's disease. An interesting point is the potential use of these biochemical markers in the diagnosis and follow-up of neoplastic lesions metastasized to the skeleton. On the basis of these considerations we investigated four markers of bone metabolism, namely the C-terminal propeptide of type I procollagen (PICP), the C-cross-linked telopeptide of type I collagen (ICTP), osteocalcin (BGP) and bone alkaline phosphatase (BAP).

The aim of this study was to investigate whether the presence of bone lesions in breast cancer patients, as assessed by radionuclides, may be demonstrated by testing the levels of the aforementioned metabolism markers in blood. In other words, we wanted to establish whether the determination of these laboratory parameters can be reliably used as a substitute for bone scintigraphy.

Materials and methods

Case series. The case series considered was composed of 149 consecutive female breast cancer patients referred for bone scintigraphy; 37 were pre-menopausal (mean age, 38; range, 26–43) and 112 post-menopausal (mean age, 53; range, 41–63). All patients had had a diagnosis of cancer histologically confirmed postoperatively and underwent different treatments depending on pathological stage and prognostic factors, according to the protocols adopted at the National Cancer Institute in Milan. Bone scintigraphy represented one step in the clinical surveillance. Thirty-three patients (22%) had metastatic bone lesions, varying in number from one to eight. Patients were considered negative after negative scintigraphy and a negative follow-up for at least 10 months to rule out the presence of undetectable lesions at the time of the examination. Scintigraphically positive patients had subsequent X-ray confirmation and/or their clinical course indicated the presence of skeletal disease or progression. Blood samples were obtained at the time of bone scintigraphy. All patients had given their informed consent.

Bone scintigraphy. Bone scans were performed following intravenous injection of 555–740 MBq of technetium-99m medronate. Images were acquired with a dual-head tomographic gamma camera equipped with a low-energy, high-resolution (LEHR) collimator (Toshiba GCA 7200) 3 h after tracer administration. Total body scintigrams were collected by scanning with a speed of 12 cm min^{-1} on a matrix of 512×1024 pixels.

In selected cases, regional planar scintigrams were acquired with the same gamma camera on a matrix of 512×512 (duration=10 min) to better define suspected areas of increased activity. Images were evaluated in double-blind manner by two experienced nuclear medicine specialists.

Measurement of bone metabolism markers. Serum was separated by centrifugation at 2500 rpm for 15 min at 4°C and aliquots were immediately frozen at –20°C until assay.

Serum ICTP and PICP were measured by means of a radioimmunoassay (RIA) using the telopeptide ICTP [^{125}I] and procollagen PICP [^{125}I] kit from Orion Diagnostic (Espoo, Finland), purchased from Italiana Laboratory Bouty (Milan, Italy). The intra- and interassay CVs were 4.2% and 5.3% for ICTP (n=10) and 3.1% and 4.5% for PICP (n=10). The analytical sensitivity was 0.5 μg/l for ICTP and 1.2 μg/l for PICP. The normal value range was 1.8–5.0 μg/l for ICTP and 50–170 μg/l for PICP.

The ICTP assay uses an antiserum directed against ICTP released from the digestion of human bone collagen by bacterial collagenase or trypsin and purified by two successive reverse-phase separations on high-performance liquid chromatography [19], whereas the PICP assay is based on human PICP isolated from human skin fibroblast cultures [20].

The Osteocalcin MYRIA-C kit, purchased from Italiana Laboratory Bouty (Milan, Italy), was employed for the measurement of BGP serum levels. The kit is a one-step assay and uses an antibody which recognizes a central part of the native molecule of osteocalcin. The intra- and interassay coefficients of variation (CVs) were 3.0% and 5.4% (n=10). The detection limit was 0.6 μg/l. The normal value range was 8.0–15 μg/l.

Serum skeletal alkaline phosphatase was measured by means of the Tandem-R Ostase IRMA kit of Hybritech Europe (Liege, Belgium). The assay uses two monoclonal antibodies directed against the human bone isoenzyme and BAP purified from human SAOS-2 osteosarcoma cells as a standard. The intra- and interassay CVs were 7.9% and 8.0% (n=10), respectively. The detection limit was 2.0 μg/l. The normal value range was 4.0–20 μg/l.

Statistical methods. Bone marker distribution was described in terms of 5th percentile, 50th percentile (median) and 95th percentile. Correlation between markers was estimated by computing Spearman's correlation coefficients.

As a preliminary step in the analysis, possible associations between patient or disease characteristics that might affect bone metabolism and bone markers were investigated. For this purpose a multivariate linear regression model was adopted in which log-transformed marker measurements of each subject represented the response profile and the predictor variables considered were: menopausal status (pre- or post-menopausal), bone neoplastic lesions (none, present), neoplastic lesions at sites other than bone (none, breast primary or recurrent lesions, distant metastases), a-specific bone lesions (none, osteoarthrosis or fractures), and ongoing antineoplastic systemic treatments (none, chemotherapy, hormone therapy). The above variables were entered into the model by means of indicator (0–1) variables. Statistical significance was assessed at the conventional 5% level based on Wilk's lambda statistic.

Discriminant analyses were performed by using logistic regression models [21] in which bone scan outcome represented the dichotomous response and marker measurements, taken singly or jointly, were taken as the predictors. Preliminary exploratory analysis based on generalized additive models [22] suggested a linear relationship (on the logit scale) between the response and PICP or ICTP, whereas a non-linear relationship (roughly of sigmoid shape) was observed for BGP and BAP.

Coherently, PICP and ICTP were entered into the logistic models by means of linear terms, whereas two-knots restricted cubic splines were adopted for BGP and BAP [23, 24]. Optimal spline knots positions were at 18 and 30 μg/l for BGP and 20 μg/l for BAP.

The main criterion for assessing model discriminative ability was the *c* statistic, corresponding to the non-parametric estimate of the area under the receiver-operating characteristic (ROC) curve [25]. The *c* statistic may vary from 0.5, denoting the lack of discrimination between scan-negative and scan-positive patients, to a maximum of 1, denoting perfect discrimination. A jack-knife approach, as supported by SAS PROC LOGISTIC [26], was used when computing the *c* statistic to reduce the bias of classifying the same data from which the classification criterion is derived.

The sensitivity, specificity, positive predictive value and complementary negative predictive value were also computed, according to the following definitions. Sensitivity (Se) is the probability of a positive test (in our context probability of bone scan positivity, as computed from marker values, above a given threshold) in a "diseased" subject (a patient with bone metastases), whereas specificity (Sp) is the probability of a negative test (probability of bone scan positivity, as computed from marker values, below the threshold) in a non-diseased subject (negative bone scan). Both the sensitivity and the specificity depend on the threshold chosen: low threshold levels tend to yield a high sensitivity and a low specificity, whereas the opposite is true for high thresholds. Positive predictive value (PPV) and complementary negative predictive value (1–NPV) quantify the probability that the disease is present given that the test is positive or negative, respectively. Statistical comparison between different logistic regression models was based on likelihood ratio tests, where appropriate, or by considering the Akaike information criterion (AIC) for comparing non-hierarchical models [27].

Finally, to investigate the relationship between marker levels and the number of bone metastases, the former were regressed on the latter by means of a linear regression model.

Results

The relevant patient and disease characteristics are reported in Table 1. The sample of 149 subjects was quite heterogeneous. Multivariate regression analysis indicated that factors significantly affecting bone marker levels, apart from a neoplastic bone lesion (P=0.0010), were the presence of other metastases (P=0.0016) and menopause (P=0.0070). In particular, whilst patients with other metastases consistently showed increased marker levels, post-menopausal women, as compared to pre-menopausal women, showed increased levels of BGP and BAP, but reduced levels of PICP and ICTP.

The above findings would suggest separate discriminant analyses in different patient categories defined by the above factors. However, considering the limited information available on patients with other metastases (eight cases) and on pre-menopausal patients (36 women, only three of whom had bone metastases), we restricted our further investigations to post-menopausal women (105 women, 24 of whom had bone metastases), who represented the majority of patients in our sample as well as in the general population of breast cancer patients.

Table 2 summarizes the distribution of each marker in the two patient subgroups defined by the presence of a negative or a positive bone scan. The median levels of PICP, ICTP and BAP were higher in women with bone metastases, whereas the opposite was true for BGP. However, Table 2 also shows that there was a substantial overlap in the marker distribution in scan-positive and scan-negative patients; the 5th percentiles in particular did not meaningfully differ in the two patient subgroups, while a lesser degree of overlap was observed for 95th percentiles.

Table 1. Patient and disease characteristics.

	No. (%) of patients	
Menopausal status		
Pre menopause	37	(25)
Post menopause	112	(75)
Neoplastic bone lesions		
None	116	(78)
Present	33	(22)
Concomitant neoplastic lesions		
None	100	(67)
Primary tumour or local recurrence	41	(28)
Distant metastases	8	(5)
Aspecific bone lesions		
None	102	(68)
Osteoarthrosis or fractures	47	(32)
Systematic treatment		
None	62	(42)
Chemotherapy	9	(6)
Hormone therapy	78	(52)

Table 2. Bone metabolism marker distribution

	5th percentile	50th percentile (median)	95th percentile
PICP (µg/l)			
Scan –	72.7	101.9	165.1
Scan +	66.0	119.4	234.4
ICTP (µg/l)			
Scan –	1.3	2.2	4.4
Scan +	1.7	2.6	10.5
BGP (µg/l)			
Scan –	2.3	6.5	20.6
Scan +	0.8	6.0	38.5
BAP (µg/l)			
Scan -	5.1	9.7	17.1
Scan +	4.6	14.9	25.2

Except for ICTP versus PICP, Spearman correlation coefficients between bone metabolism markers were significantly greater than zero. However, the degree of correlation was relatively low, ranging from 0.09 to 0.39, well below the maximum achievable value of 1.

Results from logistic analyses are reported in Table 3, according to the markers included in the model. For each model the following statistics are reported: the likelihood ratio chi-square test for the fitted model vs the model containing only the intercept term, the corresponding degrees of freedom and *P* value, the AIC and the *c* statistic (area under the ROC curve). According to the number of variables included in the models, the latter are listed from top to bottom in order of descending *c*, that is, from best to worst. Ordering based on AIC was practically the same. When considered singly, all markers, except for PICP, showed a significant association with the presence of bone metastases. The best discrimination between scan-negative and scan-positive patients was achieved with BAP (*c*=0.689), closely followed by ICTP (*c*=0.659). When marker pairs were considered on-

Table 4. Statistics derived from the logistic model including ICTP and BAP (see text for explanation)

Threshold	Classified as negative	False-negative	Sensitivity (%)	Specificity (%)
0.060	0	0	100.0	0.0
0.080	2	1	95.8	1.2
0.100	17	4	83.3	16.0
0.120	33	4	83.3	35.8
0.140	50	6	75.0	54.3
0.160	57	7	70.8	61.7
0.180	63	8	66.7	67.9
0.200	66	8	66.7	71.6
0.220	71	10	58.3	75.3
0.240	73	10	58.3	77.8
0.260	74	10	58.3	79.0
0.280	75	11	54.2	79.0
0.300	77	11	54.2	81.5
0.320	78	12	50.0	81.5
0.340	83	15	37.5	84.0
0.360	86	16	33.3	86.4
0.380	87	17	29.2	86.4
0.400	89	19	20.8	86.4
0.420	91	19	20.8	88.9
0.440	92	19	20.8	90.1
0.460	94	19	20.8	92.6
0.480	94	19	20.8	92.6
0.500	97	20	16.7	95.1

Table 3. Results from logistic discriminant analyses in 105 post-menopausal patients

Logistic models	LR χ^2	*df*	*P*	AIC	*c*
1. BAP	13.345	2	0.0013	105.539	0.689
ICTP	9.775	1	0.0018	107.110	0.659
BGP	6.887	2	0.0320	111.997	0.568
PICP	0.593	1	0.4414	116.292	0.529
2. ICTP,BAP	16.980	3	0.0007	103.905	0.737
PICP,BAP	13.362	3	0.0039	107.522	0.700
BGP,BAP	13.985	4	0.0073	108.899	0.690
ICTP,BGP	12.341	3	0.0063	108.544	0.677
PICP,ICTP	9.828	2	0.0073	109.056	0.658
PICP,BGP	6.927	3	0.0378	113.957	0.568
3. ICTP,BGP,BAP	17.255	5	0.0040	107.630	0.740
PICP,ICTP,BAP	17.056	4	0.0019	105.828	0.737
PICP,BGP,BAP	14.047	5	0.0153	110.838	0.711
PICP,ICTP,BGP	12.342	4	0.0150	110.543	0.676
4. PICP,ICTP,BGP,BAP	17.365	6	0.0080	109.519	0.744

LR χ^2, Likelihood ratio test chi-square; *df*, corresponding degrees of freedom; *P*, *P* value; AIC, Akaike information criterion; *c*, *c* statistic

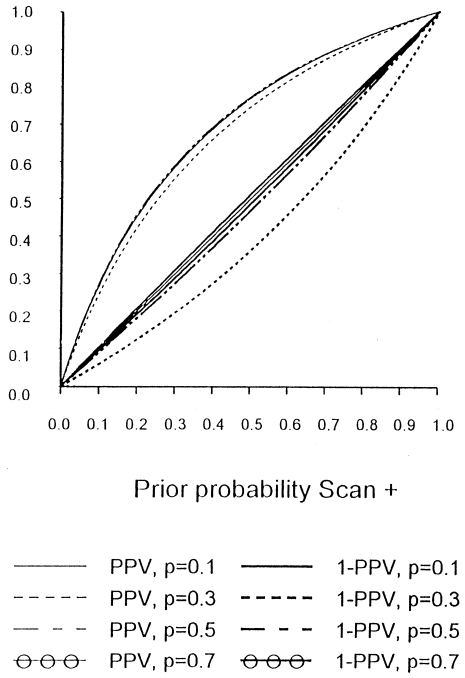

Fig. 1. Positive predictive value (*PPV*) and complementary negative predicted value (*1-NPV*) in the logistic model including ICTP and BAP for threshold values *P*=0.1, 0.3, 0.5 and 0.7

ly the joint modelling of BAP and ICTP (*c*=0.737) gave a non-negligible improvement over the use of the two markers separately. The improvement was, however, not significant according to the likelihood ratio test (*P*=0.0566). No further improvement of practical importance could be obtained when jointly considering three or all four markers.

Table 4 reports sensitivity and specificity estimates according to the probability of bone scan positivity (threshold) as predicted from the logistic model including BAP and ICTP. The second and third columns report the number of patients classified as scan-negative (predicted probability of bone scan positivity below or equal to the threshold) and, among these, the number of patients with bone lesions (false-negative). As can be observed in Table 4, the sensitivity tended to be remarkably low, even at very low threshold levels.

Figure 1 shows PPV and 1-NPV curves for various threshold choices (*P*), again considering the combined use of BAP and ICTP. The degree of displacement of the curves from the diagonal (line) indicates the extent to which the test can serve the purpose of ruling out (1-NPV curves) or confirming (PPV curves) the diagnosis. In all conditions tested, 1-NPV curves showed little displacement from the diagonal, whereas a more favourable picture emerged for PPV curves for suitably chosen thresholds.

Finally, when considering the 24 women with proven bone metastases, a linear relationship was observed between circulating levels of all markers tested after logarithmic transformation of the original measurements and the number of lesions detected with bone scan. The association was significant for BGP (*P*=0.0163) and BAP (*P*=0.0190); a low *P* value was obtained also for ICTP (0.0605).

Discussion

The organic part of the bone matrix consists of 90% type I collagen produced from an osteoblastically synthesized precursor (procollagen) with a molecular weight of about 450 kDa. During the extracellular processing of this precursor, cleavage of the N-terminal and C-terminal extension peptides by specific proteinases occurs before the collagen molecules are assembled into fibres [28]. These peptides are released into the blood with a stoichiometric ratio of 1:1 with the collagen molecules incorporated in the extracellular matrix [29]. This is why their levels can be considered an indicator of bone formation. In particular, the fragment cleaved off from the C-terminus, known as the C-terminal peptide of type I procollagen (PICP), is a glycoprotein with a molecular weight of about 100 kDa, consisting of three polypeptide chains connected to each other by disulphide bonds.

Alkaline phosphatase (AP) is the most commonly used serum marker to assess osteoblast function. The main drawback of AP measurement is its lack of specificity. In fact, several isoforms of AP may be produced by tissues other than bone and consequently the AP concentrations in blood come from different sources, in particular the liver and kidney. Bone alkaline phosphatase (BAP) is present on the surface of osteoblasts and is released during the process of matrix mineralization [30]. In an attempt to improve the specificity of BAP serum measurement, monoclonal antibody-based assays have been developed to differentiate bone from liver isoenzymes [31, 32].

Osteocalcin, also called bone gla-protein (BGP), is a small non-collagenous protein (molecular weight 5.8 kDa) produced by osteoblasts during the matrix mineralization phase [33]. Its function is not well known, but it is assumed to bind calcium by the γ-carboxyglutamate (gla) residues contained in the protein. A fraction of the synthesised protein does not accumulate in bone but is released directly into the circulation [34]. Some fragments of the protein may also be released during bone resorption, especially when there is a high turnover. Several different assays have been used for BGP determination, employing different standard preparations (bovine and human proteins) and different immunoreagents (monoclonal and polyclonal antibodies) [35]. The consequences of this methodological heterogeneity, together with the fact that the molecule easily loses its immunological activity at room temperature, are the discrepancies in the literature regarding the changes in serum osteocalcin in physiological or pathological conditions [36].

After collagen is incorporated into bone matrix, cross-links form a stable network of collagen fibrils.

Pyridinoline and deoxypyridinoline are two of these cross-links. During bone resorption, degradation of extracellular collagen occurs, and the various cross-linking components are released either in peptide-free or in peptide-bound form. ICTP, released through type I collagen degradation, includes those cross-links [37] and is found in an immunochemically intact form in blood [38]. PICP, BAP, BGP and ICTP have been tested in this study to evaluate their clinical utility in discriminating breast cancer patients with or without bone metastases. The marker results were compared to those of bone scintigraphy, which were considered as the gold standard.

As a preliminary step we investigated by multivariate regression analysis the factors influencing bone marker levels in our series of patients. The analysis shows that besides the presence of neoplastic bone lesions also the presence of visceral metastases and menopausal status can affect the serum levels of the marker. In particular post-menopausal patients (as compared to pre-menopausal patients) showed increased levels of BGP and BAP but reduced levels of PICP and ICTP. The apparently paradoxical increase in markers of osteoblastic activity such as BGP and BAP in a stage of life (post-menopause) characterized by bone loss can be explained by the fact that osteoblastic activity does not reflect a positive balance in bone formation. Conversely PICP, which is released only at the collagen formation phase, is a better index of bone formation [39]. The reasons for reduced levels of a bone resorption marker such as ICTP in post-menopausal women are not easy to identify. Probably the ICTP assay does not reflect such minimal changes as occur after-menopause, even though it may be a good marker for osteoporosis, as has been observed by several authors [40–42].

In view of the small number of pre-menopausal patients with skeletal metastases in our study we restricted our investigation of the accuracy of metabolic markers in detecting bone lesions to post-menopausal patients. In this group the median levels of bone metabolic markers were generally higher in patients with than in those without bone metastases. However, we found a substantial overlap in the marker distribution for scan-positive and scan-negative patients. When markers were considered singly the best discriminating marker between scan-negative and scan-positive patients was BAP, closely followed by ICTP. When we considered marker pairs, only the joint modelling of BAP and ICTP gave an appreciable improvement over the use of the two markers separately. No further improvement could be obtained with the association of three or all four markers. In our series the diagnostic accuracy of bone metabolic marker evaluation was poor and the clinical utility limited. In fact, to achieve high specificity (95%), the sensitivity of the test is below 20%; conversely, with a high sensitivity (95%) the specificity is below 10%.

The above findings allow us to draw the following conclusions. In spite of recent proposals published in the literature regarding the possibility of replacing bone scintigraphy with bone metabolism markers to detect the presence of skeletal metastases [43], our results show the poor diagnostic utility of these laboratory tests in post-menopausal patients, which is the group with the highest likelihood of skeletal metastases. In fact, the specificity and sensitivity of these determinations are unsatisfactory, and it is not realistic to propose their use in the diagnosis of skeletal localizations. Moreover, when there is a diagnostic suspicion of skeletal metastases, there is a need to confirm and evidence the site of the localization. Oncologists therefore need whole-body imaging to discover the topography of the metastatic site. Even if the laboratory tests had a better diagnostic efficacy, for therapeutic decision-making it would still be reasonable to carry out diagnostic imaging.

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