Radioimmune Imaging of Bone Marrow in Patients with Suspected Bone Metastases from Primary Breast Cancer

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Radioimmune imaging of bone marrow was performed by technetium-99m- (^{99m}Tc) labeled antigranulocyte monoclonal antibody BW 250/183 (AGMoAb) scans in 32 patients with suspected bone metastases from primary breast cancer. AGMoAb scans showed bone marrow defects in 25/32 (78%) patients; bone invasion was subsequently confirmed in 23 (72%) patients. Conventional bone scans performed within the same week detected bone metastases in 17/32 (53%) patients ($p < 0.001$). AGMoAb scans detected more sites indicating metastatic disease than bone scans in 12 of these 17 patients (71%). All patients with bone metastases in the axial skeleton had bone marrow defects at least at the sites of bone metastases. Of 15 patients with normal, or indicative of, benign disease bone scans, 8 patients (53%) presented with bone marrow defects in the AGMoAb scans. Bone invasion was confirmed in six of them. AGMoAb bone marrow scans provide a method for the early detection of bone metastatic invasion in patients with breast cancer and suspected bone metastases.

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Bone involvement from breast cancer is currently detected by bone scintigraphy and X-ray examinations. Both methods detect invasion of the bone tissue, but do not allow assessment of the bone marrow. Assessment of bone marrow in patients with breast cancer is of interest since bone marrow is the primary soil of metastatic bone disease (*1,2*). If bone marrow invasion precedes bone tissue invasion, therefore, detection of bone marrow involvement would prompt systemic rather than localized treatment at an earlier stage.

Posterior iliac crest bone marrow aspiration and bone biopsy are invasive methods, which can provide histologic evidence of bone marrow carcinosis. If needle aspiration or bone marrow biopsy are performed at the time of first recurrence, as many as 27% of the patients present with bone marrow carcinosis (*2*).

However, needle aspiration and bone marrow biopsy are limited because of the minimal tissue volume examined in comparison with the whole bone marrow mass (*3*), and because they do not give information regarding the localization and extent of metastatic bone disease (*2*). Moreover, performing the biopsy without knowing if the chosen regions are involved gives low positive findings. Despite these inherent limitations, bone marrow biopsy has proven to have prognostic value (*3,4*), as a positive bone marrow biopsy is a predictor of early relapse in bone and involves a higher death risk.

Noninvasive assessment of bone marrow in breast cancer by means of radiocolloid studies is only occasionally performed (*5*). The disadvantages are that only the reticuloendothelial component is visualized and there is high liver and spleen uptake. It has been shown, however, that these studies can detect more lesions than conventional bone scans in various carcinomas and lymphomas (*6*).

AGMoAb scans allow excellent whole-body bone marrow visualization (*7*). It has been shown that bone marrow metastases present in these scans as cold spots (*8*). This study was undertaken to assess the usefulness of radioimmune imaging of bone marrow in patients with primary breast cancer suspected of metastatic bone involvement.

METHODS

Patients

We studied 32 consecutive female patients (mean age 50 yr, range 25–79) with histologic evidence of infiltrant ductal carcinoma of the breast. Bone metastases were suspected in all patients because of bone pain and/or elevated serum alkaline phosphatase. The stage of the disease (American Joint Committee in Cancer Staging) when patients were referred to our laboratory was: I, 4 patients; II, 12 patients; III, 8 patients; and IV, 8 patients (Table 1). Patients underwent conventional bone scans to rule out bone metastases and bone marrow radioimmune imaging with AGMoAb (Behringwerke, Mar-

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TABLE 1
Descriptive Data and Results in all Patients

No.	Age	Stage	Extension	Previous treatments	Bone scan*	Bone-marrow		Diagnosis§
						Scan†	Expansion‡	
1	64	II	Local	S.	N	(2)	0	+ Biopsy
2	40	III	LN	S. Ch. Rt. Hr.	N	N	2	− Follow-up
3	46	II	Local	S. Ch. Rt.	N	N	0	− Follow-up
4	63	IV	Lung	Ch. Rt. Hr.	M (6)	(>10)	2	+ X-ray
5	64	IV	LN	S. Ch. Rt. Hr.	M (2)	(3)	2	+ MRI
6	79	II	LN	S. Ch. Hr.	B (1)	N	0	− Follow-up
7	54	III	Lung	Ch. Rt.	M (1)	(1)	2	+ CAT
8	34	II	Local	S. Ch. Rt. Hr.	M (1)	(1)	2	+ Biopsy
9	57	III	LN	S. Ch. Rt.	N	N	2	− Follow-up
10	39	III	LN, Liver	S. Ch. Rt.	B (1)	(2)	0	+ Evolution
11	45	I	Local	S.	N	N	0	− Follow-up
12	43	II	LN	S. Ch. Rt.	M (5)	(7)	2	+ X-ray
13	38	IV	LN	Ch.	M (>10)	(>10)	2	+ Concordance
14	52	III	LN	S. Ch.	M (1)	(1)	1	+ Evolution
15	71	I	Local	S. Rt.	M (1)	(3)	1	+ CAT
16	65	III	Peritoneum	S.	N	(1)	2	+ Evolution
17	42	I	Local	S.	N	(1)	0	− Follow-up
18	32	IV	Local	Ch.	M (>10)	(>10)	2	+ Concordance
19	31	II	LN	S. Ch.	M (1)	(3)	1	+ CAT
20	65	I	Local	S.	N	N	2	− Follow-up
21	56	III	LN	S.	M (2)	(2)	2	+ CAT
22	58	IV	Local	—	M (4)	(6)	1	+ X-ray
23	40	II	LN	S. Ch.	B (1)	(4)	2	+ CAT
24	67	II	Local	S. Hr.	N	(2)	1	− Follow-up
25	27	II	Local	S. Hr.	M (1)	(1)	2	+ MRI
26	49	IV	LN	S. Ch. Hr.	M (>10)	(>10)	0	+ Concordance
27	43	II	Local	S. Rt.	N	N	2	− Follow-up
28	58	IV	Local	Ch.	M (>10)	(>10)	0	+ Concordance
29	53	II	LN	S. Rt.	M (1)	(2)	0	+ Evolution
30	67	III	LN	S. Ch. Hr.	N	(7)	2	+ Evolution
31	25	IV	LN	S. Ch. Rt.	M (3)	(4)	2	+ X-ray
32	60	II	LN	S. Ch.	N	(>10)	2	+ Biopsy

* Bone scan as: N = normal; M = metastatic (number of sites); and B = benign.
† Bone marrow scan as: N = normal (number of sites).
‡ Grades of bone marrow expansion.
§ Diagnosis as positive (+) or negative (−) for bone invasion and means of confirmation.
S = surgery; LN = lymphatic nodes; Ch. = chemotherapy; Rt. = radiotherapy; and Hr. = hormonotherapy.

burg, Federal Republic of Germany) scans for bone marrow assessment. All procedures were done in accordance with the ethical regulations of our institution. Informed consent was obtained from all patients.

Antibody Description and Preparation

The AGMoAb is of murine origin, it belongs to the IgG_1 isotype and detects a 180-kdalton glycoprotein. The AGMoAb is an anti-CEA that crossreacts with NCA-95 (a nonspecific cross reacting antigen) (*9*). NCA-95 is present on the cellular membrane of virtually all human granulocytes. The number of epitopes is 2×10^5 per granulocyte and the affinity constant is 2×10^9 1/mol (*10*). After intravenous injection of the AGMoAb, >90% of circulating granulocytes carry the AGMoAb (*9,11*). It has been shown that labeling of granulocytes with the AGMoAb does not produce impairment of granulocyte function nor cytotoxic effects (*7,10*). The peripheral leukocyte count after injection of the AGMoAb is stable (*11*). Plasma free activity at 10 min and 1 hr postinjection is 85% and 20%, respectively (*11*). At 5 hr postinjection 18%–21% of whole-body radioactivity is localized in the liver, and 8.5% in the spleen (*12*). Labeled granulocytes in the bone marrow (>90% of total-body granulocytes) provide excellent bone marrow visualization in the scans.

Following sterile procedure, 5 ml of physiologic saline solution was added to a vial of dried substance containing 2.7 mg of 1,1,3,3-propane tetraphosphonic acid, tetrasodium salt 2 H_2O and 0.12 mg of tin-(II)-chloride 2 H_2O. One milliliter of the obtained solution was then added to one vial of dried substance containing 1 mg of the AGMoAb and it was carefully and gently shaken for a few seconds. The vial was then put into a shielded container and 4 ml containing 1,480 MBq of ^{99m}Tc was added to it. After an incubation period of 10 min, the labeling efficiency was >95% (thin-layer chromatography), and the labeled compound was ready for administration.

Procedure

The AGMoAb was injected slowly intravenously. The antibody dose was 0.5 mg with 740 MBq of ^{99m}Tc in a volume of 2.5 ml. Images were obtained in all cases between 5-6 hr postinjection. Whole-body scans consisting of multiple views were done using a conventional large field of view camera (Siemens Orbiter) with a high resolution, low-energy collimator. An average of 600,000 counts per view were obtained. Images were produced and interpreted on radiographic film.

Whole-body bone scans consisting of multiple views were obtained after the intravenous injection of 740 MBq of ^{99m}Tc-MDP within the same week using the same instrumentation.

Interpretation of Scans

Scans were interpreted by consensus among three experienced observers. Normal bone marrow distribution was considered if homogeneous and symmetrical AGMoAb uptake was present in the axial skeleton and proximal one-third of the femoral and humeral shafts. A focal decrease in uptake or focal defects in bone marrow scans were interpreted as bone marrow invasion (Fig. 1). Femoral bone marrow expansion was defined as the presence of bone marrow distal to the first one-third of the femoral shaft and was graded as: 0, no expansion; 1, activity present in the second third of the shaft; 2, activity present in the last third of the shaft (Fig. 2). Bone scans were interpreted as normal, as indicative of primarily benign disease, or as indicative of bone metastases.

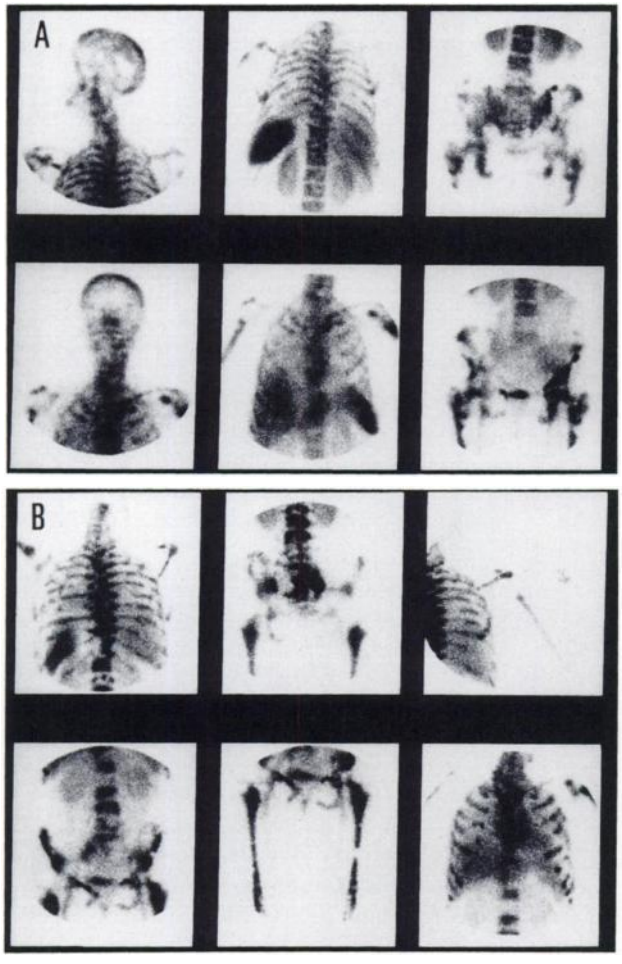

FIGURE 1
(A) Bone marrow scan of Patient 18 and (B) bone marrow scan of Patient 26. Multiple defects indicating widespread metastatic bone marrow involvement.

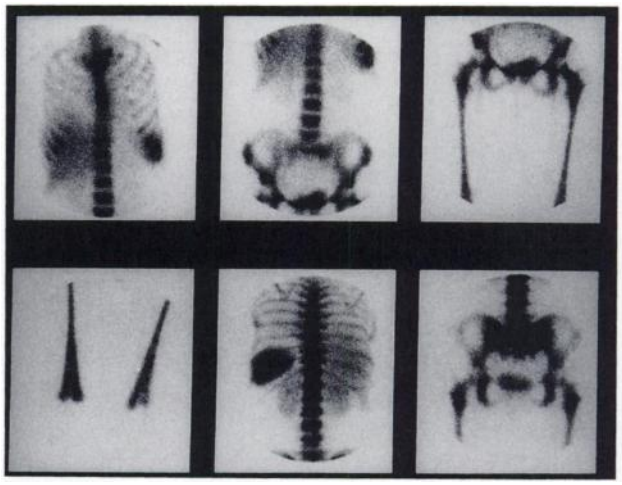

FIGURE 2
Bone marrow scan of Patient 9 showing only bone marrow expansion grade 2 in femoral shafts.

Follow-up after bone marrow scans was 6 mo. When both procedures (bone and bone marrow scans) were concordant indicating widespread metastatic disease (>10 lesions), further diagnostic methods were not considered necessary. Confirmation by evolution was obtained when subsequent bone scans performed within the follow-up demonstrated bone metastases in previous sites interpreted as bone marrow invasion in the AGMoAb scans. Confirmation was also obtained when bone invasion was detected by other imaging techniques such as X-ray, CAT, or magnetic resonance imaging. Bone marrow biopsy was performed when clinically indicated.

The Chi-square test with Yates correction was used to compare proportions.

RESULTS

AGMoAb scans showed focal defects in the bone marrow of 25/32 (78%) patients, bone invasion was confirmed in 23 of these patients (Table 1). Seventeen of the 23 patients (74%) had bone tissue invasion detected by the bone scans at the time of the AGMoAb study. AGMoAb scans detected more patients with metastatic bone disease (23 (72%)) than bone scans (17 (53%)) ($x^2 = 14.19$ $p < 0.001$) (Table 1). AGMoAb scans detected more metastatic sites than bone scans in 20 patients, including the 4 patients in whom both studies showed widespread metastatic disease (>10 sites) (Table 1). All patients with metastatic bone tissue invasion detected by bone scans presented with bone marrow defects in the AGMoAb scans. In the 17 patients with metastatic bone tissue invasion detected by the bone scans, AGMoAb scans detected more sites than bone scans in 12 patients (71%) (Table 1). Patients with bone tissue invasion in the axial skeleton as seen

in the bone scans presented with bone marrow defects in the AGMoAb scans at least at the same sites.

Of 15 patients with normal or indicative of benign disease bone scans, 8 patients (53%) presented with bone marrow defects in the AGMoAb scans. Bone marrow metastatic involvement was confirmed in 6 of the 8 patients (Table 1). The presence of bone marrow defects in the AGMoAb scans was in relation with the stage of the disease: 17/24 (71%) patients with Stages I–III, and 8/8 (100%) patients with Stage IV had bone marrow defects in the AGMoAb scan (Table 1).

Bone marrow expansion (grade 1, in 5 patients and grade 2, in 18) was observed in 23/32 (72%) patients. It was seen more frequently in patients with previous chemotherapy and/or radiotherapy ($x^2 = 6$, $p < 0.05$) (Table 2). Patients with Stages III–IV or with >3 defects in the bone marrow scan seldom presented without bone marrow expansion ($x^2 = 6.25$, $p < 0.05$ and $x^2 = 4.45$, $p < 0.05$, respectively) (Table 2).

DISCUSSION

Retrograde blood flow through the paravertebral venous system communicating with the sinusoids of the bone marrow and the enhanced susceptibility of bone marrow, because of the special microanatomical aspects of its vasculature, determine the seeding of tumor cells in the bone marrow (*13*). This would be followed by invasion of bone tissue matrix and finally by invasion of the cortical bone (*14*).

Our study shows that in patients with primary breast cancer and suspected bone metastases, radioimmune imaging of bone marrow detects more patients with metastatic bone disease than conventional bone scans. In patients with metastatic bone tissue involvement, AGMoAb scans detect more metastatic sites than bone scans. This has been reported before in various carcinomas and lymphomas (*6,8*).

In breast cancer, the high prevalence of positive bone marrow biopsies at the time of first recurrence and the fact that the presence of bone marrow micrometastases is a predictor of subsequent development of overt bone metastases (*2*), indicates that bone marrow is the primary soil for bone metastases. Kamby et al. (*2*) reported that only 78% of patients with histologic evidence of bone marrow carcinosis had demonstrable bone metastases by conventional methods. Mansi et al. (*4*) reported that 53% of patients who developed bone metastases at first relapse had bone marrow micrometastases at presentation. Redding et al. (*15*) demonstrated bone marrow involvement in 24% of breast cancer patients with no lymph node involvement using an immunocytochemical method. Cote et al. (*16*), using monoclonal antibodies to examine bone marrow specimens, found bone marrow involvement in 35% of patients with Stages I–III. In our study, 71% of patients with Stages I–III had bone marrow involvement. Our higher incidence is probably due to a more selected population with clinical suspicion of bone metastases, and to the fact that bone marrow scans allow whole-body bone marrow examination.

In our series, eight patients with normal or benign bone scans showed bone marrow defects in the AGMoAb scan. Confirmation of bone invasion was obtained in six of them. In the remaining two patients, there was no evidence of bone invasion after 6 mo of follow-up. Patient 17 presented with a single defect and Patient 24 presented with two defects in the AGMoAb scans, which we could not relate to metastatic bone disease. Conversely, Patient 6 had a solitary abnormality in the bone scan interpreted as benign degenerative disease, but no bone marrow defect at the site of the lesion was seen. Although it is known that benign diseases can produce a local decrease in bone marrow tissue (*17*), it seems possible that sometimes bone marrow is not affected by these diseases.

Seven patients with normal bone marrow scans (clinical Stages I–III) in our series, were found free of bone metastatic disease with conventional methods at 6 mo follow-up. However, we do not know if any of these patients could have micrometastases demonstrable by means of needle aspiration or biopsy at the time of the AGMoAb scan.

All metastatic sites seen in the bone scans corresponded to focal defects in the AGMoAb scans, except in one patient (No. 14), who had a metacarpal metastasis seen in the bone scan but missed in the bone marrow scan due to normal lack of bone marrow in the distal bones. At that time, the AGMoAb scan detected sternal bone marrow invasion which appeared in the bone scan 6 mo later. In our series, in those patients in whom confirmation was obtained by follow-up bone scans, conversion time to a definitively metastatic bone scan was 3–6 mo.

Bone marrow scans provided valuable additional information. Patient 32 is a good example. The bone scan was considered normal despite some concern about the

TABLE 2
Bone Marrow Expansion Versus Previous Treatments, Stage of the Disease, and Number of Defects in the Bone Marrow Scan

		Chemotherapy and/or radiotherapy		Stage of disease		Number of defects	
		No	Yes*	I–II	III–IV†	0–3	>3‡
Bone marrow Expansion	No	3	6	6	3	7	2
	Yes§	5	18	10	13	14	9

* $x^2 = 6$ ($p < 0.05$).
† $x^2 = 6.25$ ($p < 0.05$).
‡ $x^2 = 4.45$ ($p < 0.05$).
§ Including grades 1 and 2 of bone marrow expansion.

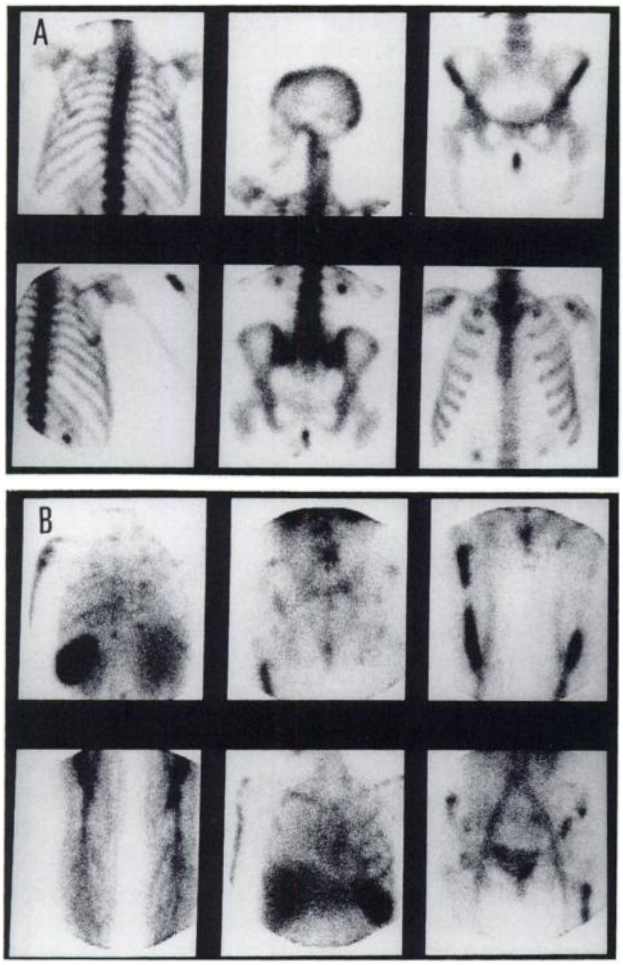

FIGURE 3
Patient 32. (A) Bone scan. (B) Bone marrow scan showing diffuse bone marrow invasion with distal presence of bone marrow in femoral shafts.

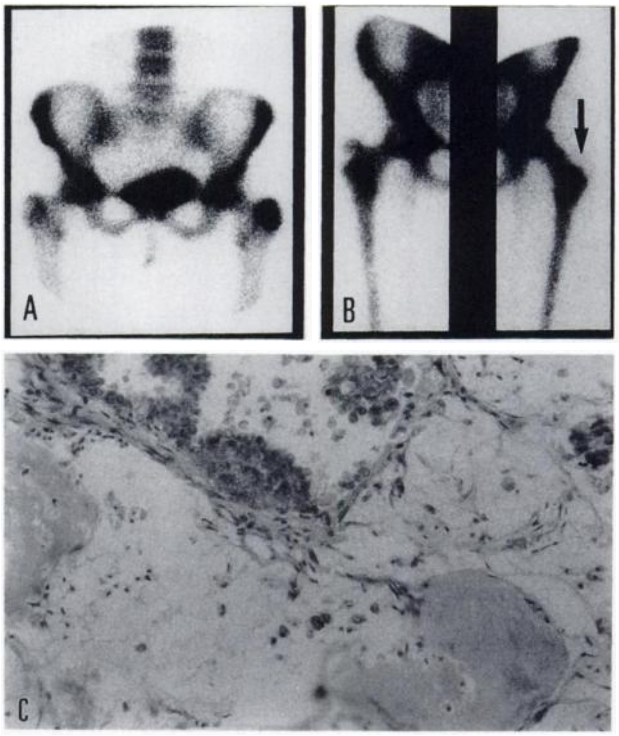

FIGURE 4
Patient 8. (A) Bone scan showing left great trochanter involvement. (B) Bone marrow defect at the same site. (C) Hystologic evidence of invasion by tumor cells (Hematoxylin eosin ×200).

intensity of uptake in the axial skeleton, but bone marrow scan showed dramatically diffuse bone marrow invasion in the axial skeleton with bone marrow expansion (Fig. 3). Patient 8 presented with a solitary abnormality in the bone scan and absence of bone marrow at the same site; metastatic invasion was confirmed at biopsy (Fig. 4). The latter example also indicated the potential ability of bone marrow scans to guide biopsy or needle aspiration in selected patients (*18*).

From a clinical point of view, assessment of bone marrow status is important for treatment strategy (*2*), as patients with bone marrow invasion can benefit from aggressive systemic treatment (*2–4*). Treatment strategy was changed in 6 of our 32 patients because of the AGMoAb scan results. Patients 23, 30, and 32 presented with normal or benign bone scans and AGMoAb scans showing multiple defects indicative of metastatic disease. In these patients chemotherapy was prompted. In three patients (Nos. 15, 19, 29) in whom localized treatment would have been considered because of single metastatic lesions seen in the bone scan, AGMoAb scans indicated widespread metastatic disease and chemotherapy was also prompted.

As AGMoAb scans provide a method to readily visualize whole-body bone marrow, information regarding distribution and viability of bone marrow can be obtained. Bone marrow expansion was seen in 23 patients, in relation to previous treatments and the extension of the disease. However, bone marrow expansion was also seen in four patients without evidence of bone involvement. In breast cancer, it remains to be ascertained whether bone marrow expansion is a consequence of bone marrow invasion, or if it is a normal response of bone marrow to the disease itself or to treatment. Bone marrow expansion has been found in 50% of patients 1 yr after large-field irradiation to the trunk in Hodgkin's disease (*19*), and it has also been observed in various other malignancies (*6*). Increased metabolic requirements, such as chronic demand for increased erythropoiesis can also result in expansion of the active bone marrow into the extremities (*20*). This has been shown in sickle cell anemia (*21*). Our data indicates that treatment plays a role in the development of bone marrow expansion. It is possible that chemotherapy alone, or in combination with radiation, can induce or accelerate the process of bone marrow expansion (*22*).

Theoretical drawbacks of AGMoAb scans are radiation burden to bone marrow and immunogenicity. The absorbed dose from AGMoAb in bone marrow is 0.01 mGy/MBq (*23*); this is similar to the absorbed dose in bone marrow from conventional bone imaging agents (*24*). Since AGMoAb is an intact murine antibody, an important proportion of patients develop human anti-mouse antibodies (HAMA). Joseph et al. (*7*) detected transient HAMA response in about 40% of patients after immunoscintigraphy with AGMoAb. This raises the possibility of future allergic reactions despite the fact that until now these have not been observed in patients injected up to three times (*7*). When previous radiotherapy had included regions of bone marrow within the radiation field, a decrease or defect in bone marrow uptake corresponding to the radiation field was observed. This pattern is easily distinguished from that of metastatic invasion, but assessment of focal bone marrow disease within the radiation field is not possible.

In conclusion, bone marrow scans detect more patients with metastatic bone disease than bone scans in primary breast cancer with suspected bone metastases. In patients with metastatic bone tissue invasion, bone marrow scans detect more metastatic sites than bone scans. Our data supports the concept that in the time course of bone invasion bone marrow is first invaded. Patients with risk of bone metastases due to stage of disease or clinical suspicion of bone metastases who present with normal or benign bone scans may benefit by a bone marrow scan.

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