Technetium-99m-Labeled Antigranulocyte Antibody Bone Marrow Scintigraphy

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Although bone scintigraphy is a sensitive method for detecting skeletal metastases, it is often equivocal for metastases due to poor specificity. This study evaluates ^{99m}Tc-antigranulocyte antibody (AGA) bone marrow scintigraphy in differentiating malignant from benign lesions, in 42 patients with skeletal tumors who had equivocal bone scans. **Methods:** AGA scans performed approximately 1 wk after ^{99m}Tc-MDP bone imaging were visually assessed for the presence of concordant marrow defects. Final diagnoses were made from radiological results, follow-up bone scans or clinical evaluation for 12 mo or longer. **Results:** The final diagnoses were: skeletal metastasis (19 patients), no metastasis (20 patients) and unconfirmed (3 patients). AGA scans could not determine the presence of a concordant defect in three patients because of overlying liver activity or previous irradiation of the region. Seventeen patients had bone marrow defects concordant with bone scan lesions, whereas 15/19 patients without metastasis had normal AGA scans. The sensitivity and specificity of AGA for detecting skeletal metastases were 100% and 79%, respectively. **Conclusion:** AGA scans had a low incidence of skeletal metastases in patients who had equivocal bone scans. Although a concordant marrow defect increases the possibility of metastasis, further radiological investigation to exclude benign disease is warranted.

Key Words: technetium-99m-antigranulocyte antibody; bone marrow scintigraphy; bone scintigraphy; bone metastasis

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Technetium-99m-methylene diphosphonate (MDP) bone imaging is the standard procedure to investigate suspected skeletal metastasis in patients with cancer. Because of poor lesional specificity, however, differentiation between benign disease and metastasis can be difficult when lesions are few and atypical for either etiology (*1–3*). Interpretating a bone scan as equivocal for metastasis can cause problems when determining the patient's therapeutic course.

A number of methods may be used to clarify the problem of equivocal bone scans. Plain radiographs are helpful if they demonstrate benign disease as the cause for the bone scan lesion, but they are not sufficiently sensitive in visualizing lesions (*4,5*). Computerized tomography (CT) and magnetic resonance imaging (MRI) yield high-resolution images with increased accuracy, but high costs, limited availability and their failure to screen the entire skeletal system restrict their use for selected cases only. Moreover, CT has a lower sensitivity for detecting marrow lesions (*6*), whereas false-positive results have been reported for MRI (*7,8*).

Bone marrow scintigraphy may be a useful alternative since it images the whole bone marrow where most skeletal metastases are known to originate. Radioactive iron, ^{111}In-chloride or ^{99m}Tc-labeled colloid are no longer used due to poor contrast and excessive hepatosplenic activity. Interest in bone marrow imaging, however, has been revived recently with the emergence of new radiopharmaceuticals with improved imaging qualities (*9*). Immunoscintigraphy using ^{99m}Tc-labeled antigranulocytic monoclonal antibody is one of the more promising methods. Recent results suggest that it provides a sensitive approach for establishing the presence and extent of malignant bone marrow infiltration (*10–12*). Its usefulness, however, in clarifying the nature of bone scans equivocal for metastasis has not been clearly defined.

This study was conducted to determine whether ^{99m}Tc-antigranulocytic antibody bone marrow (AGA) scintigraphy can be used to improve the specificity of skeletal bone imaging lesions.

MATERIALS AND METHODS

Patients

We studied 42 patients (18 men, 24 women; aged 18–79 yr; mean 52 ± 12 yr) with known malignant solid tumors. Fourteen patients had breast cancer, eight lung cancer, six bladder cancer, five uterine cancer, three renal cell carcinoma, one gastric cancer, one esophageal cancer, one rectal cancer, one pancreatic cancer, one liver cancer and one malignant thymoma. All patients had equivocal bone scans, i.e., one or two extracostal bone lesions and/or rib lesions of any number. Patients whose final diagnoses were not confirmed by radiological examinations, including CT and MRI, follow-up bone scans or pathology, were clinically evaluated for a minimum of 12 mo. All subjects gave informed consent prior to bone marrow imaging.

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TABLE 1
Scintigraphic Findings in Patients with Metastasis

Patient no.	Sex	Age	Cancer	MDP bone scan	Bone marrow scan	Confirmation	Result
1	F	52	Uterine	1 spine	1 spine	CT	TP
2	M	44	Liver	1 rib	1 rib and 3 spine	Clinical f/u	TP
3	F	50	Breast	Sacrum	Sacrum	f/u bone scan	TP
4	F	54	Breast	1 rib	1 rib	f/u bone scan	TP
5	M	63	Bladder	2 spine	2 spine	Clinical f/u	TP
6	M	35	Renal	2 rib	1 rib	Clinical f/u	TP
7	M	55	Lung	Pelvis and 1 rib	Pelvis and 1 rib	CT	TP
8	F	36	Breast	Sternum	Sternum	MRI	TP
9	F	55	Lung	1 spine	1 spine	MRI	TP
10	F	64	Pancreas	1 pelvis and 1 spine	1 pelvis	Clinical f/u	TP
11	F	56	Lung	1 pelvis and 1 spine	1 pelvis and 1 spine	Clinical f/u	TP
12	M	59	Stomach	1 spine	3 spine	Clinical f/u	TP
13	M	18	Thymus	2 ribs and sternum	1 rib and sternum	MRI	TP
14	F	30	Lung	Scapula and 3 ribs	Scapula	MRI	TP
15	F	46	Breast	1 rib and sternum	Sternum	f/u bone scan	TP
16	M	63	Esophageal	1 rib and 1 spine	1 spine	CT	TP
17	M	40	Lung	1 spine and ulna	1 spine	Biopsy	TP
18	F	42	Uterine	1 pelvis	Radiation field	CT	ND
19	F	40	Breast	1 spine	Liver overlap	Radiograph	ND

f/u = follow up; ND = not determined; TP = true-positive.

Preparation of Technetium-99m-Labeled AGA

CEA-79.4, an IgG2a type monoclonal antibody directed against nonspecific cross-reacting antigen-95 (NCA-95) was produced at Seoul National University, College of Medicine (Seoul, Korea). The antibody was purified by immunoaffinity chromatography from LS174T colon cell supernatants (*13*). Western blotting of extracts from human granulocytes and colon cancer cells (SNU-C4) with the antibody disclosed a single band with a molecular weight of 95,000 daltons. Immunohistochemical staining of peripheral blood smear and bone marrow aspirate using the antibody and the alkaline phosphatase anti-alkaline phosphatase method showed specific uptake of the antibody to human granulocytes and granulopoietic cells. The antibody was labeled with ^{99m}Tc by a transchelation method using 2-mercaptoethanol as a reducing agent and glucarate as a ligand, as described by Schwarz and Steinstrasser (*14*). The optimal labeling condition was a 1:3000 molar ratio of antibody to 2-mercaptoethanol, pH 5 (*15*). The labeling efficiency was 60% to 85%, and the labeled antibody was separated using a PD-10 column. Scatchard analysis revealed an affinity constant of 2 to 9 $\times$ 10^9 liters/mole; the number of binding sites per granulocyte was 0.4 to 1.9 $\times$ 10^5. After cell binding assay, the labeled antibody retained an immunoreactivity of 60%–65% (*15*).

Several safety tests, including the mouse antibody production (MAP) test and bacterial, mycoplasma and viral cultures, were negative.

Image Acquisition and Data Analysis

Bone scans were acquired 4 hr after injection of 740 MBq ^{99m}Tc-methylene diphosphonate (MDP). Multiple regional views of 400k counts were acquired by a large field of view gamma camera with a low-energy, general-purpose collimator. The AGA scan was obtained approximately 5 hr after intravenous injection of 370 MBq ^{99m}Tc-AGA (0.5 mg antibody). A Sepharose column was used to separate free technetium before injection. Multiple regional views were acquired with the same parameters used for MDP imaging. AGA immunoscintigraphy was performed within 1 wk of ^{99m}Tc-MDP imaging.

The MDP and AGA scans were blindly interpreted by two independent observers. Special attention was paid to the presence or absence of bone marrow defects concordant to bone scan lesion sites. AGA scans demonstrating at least one marrow defect concordant with a bone scan lesion were classified as positive, while those with normal findings were classified as negative. AGA scans in which concordance could not be evaluated due to obscured regions of interest (ROIs) from previous irradiation or overlying hepatosplenic activity were classified as indeterminate.

A final decision as to the presence or absence of skeletal metastasis was made at least 1 yr after the initial MDP and AGA scans had been obtained and all available data had been analyzed: plain radiographs, CT, MRI, follow-up bone scans and serial clinical evaluations. Radiological demonstration of a benign lesion to explain the bone scan lesion was interpreted as negative, as was clinical follow-up of over 12 mo without evidence of malignant disease. Patients were classified as having metastasis when MRI or CT revealed metastatic lesions, serial bone scans demonstrated aggravation, biopsy provided histologic evidence of malignancy or there was clinical progression in agreement with skeletal involvement. The sensitivity, specificity and diagnostic accuracy for AGA scans were calculated excluding those patients with an unconfirmed final diagnosis or those who had indeterminate AGA scans.

RESULTS

Tables 1 and 2 summarize the MDP and AGA scan findings. Of the 19 patients with metastasis (Table 1), Patients 1–17 had matched bone marrow defects concordant to the site of bone scan lesions, while the ROIs in Patients

TABLE 2
Scintigraphic Findings in Patients without Metastasis

Patient no.	Sex	Age	Cancer	Bone scan	Bone marrow scan	Confirmation	Result
1	M	68	Lung	1 spine	Normal	Radiography	TN
2	F	44	Uterus	2 spine	Normal	Clinical f/u	TN
3	M	79	Rectal	1 spine	Normal	CT	TN
4	F	59	Breast	1 rib	Normal	Clinical f/u	TN
5	M	62	Bladder	9 ribs	Normal	Radiography	TN
6	M	58	Bladder	2 ribs	Normal	Clinical f/u	TN
7	M	59	Bladder	2 ribs	Normal	Clinical f/u	TN
8	M	57	Renal	1 rib and 1 spine	Normal	Clinical f/u	TN
9	M	55	Renal	2 spine	Normal	Clinical f/u	TN
10	F	53	Breast	1 spine	Normal	Radiography	TN
11	F	55	Breast	2 spine	Normal	Radiography	TN
12	F	54	Breast	1 spine	Normal	Clinical f/u	TN
13	F	55	Uterine	1 spine	Normal	Clinical f/u	TN
14	F	56	Lung	1 spine	Normal	Clinical f/u	TN
15	M	63	Lung	6 ribs	Normal	Clinical f/u	TN
16	F	62	Breast	2 spine	2 spine	Radiography	FP
17	F	57	Breast	1 spine	1 spine	Radiography	FP
18	F	46	Breast	1 rib	1 rib	Radiography	FP
19	M	30	Bladder	2 spine	2 spine	Radiography	FP
20	F	35	Breast	1 rib	Radiation	Clinical f/u	ND

FP = false-positive; f/u = follow-up; ND = not determined; TN = true-negative.

18 and 19 were not interpretable. Patient 18's AGA scan showed diffuse hypoactivity in the pelvis due to previous radiation therapy, and, in Patient 19, the lower thoracic spine was obscured by overlying liver activity. Figures 1 and 2 show examples of true-positive results. There were no false-negative results.

Of the 20 patients without metastasis (Table 2), Patients 1–15 had normal AGA scans, Patients 16–19 had concordant marrow defects and Patient 20 had concordance of a rib lesion that was indeterminate due to previous radiation therapy of the thorax. Figures 3 and 4 demonstrate true-negative and false-positive results, respectively. Interestingly, four patients had bone marrow defects that matched the bone scan lesion sites, although these proved to be benign. False-positive lesions were found in the lower lumbar spine in three patients and in a single rib in another patient. In the three patients with lumbar spine lesions, radiographs showed that degenerative change was the cause for the lesions in two patients. For the lesion detected in the single rib, trauma was determined to be the cause since the patient had a history of an earlier chest wall injury, radiograph showed previous fracture in another rib and there had been no evidence of metastasis for over 1 yr.

The AGA scan results and final diagnoses are summarized in Figure 5. Of the 42 patients with equivocal bone scans, 23 had positive AGA scans (17 patients had metastasis, 4 no metastasis, 2 unconfirmed), 16 had negative scans (15 patients had no metastasis, 1 unconfirmed) and 3 had indeterminate scans due to ROI obscuration (these patients were lost during follow-up and did not have confirmatory tests).

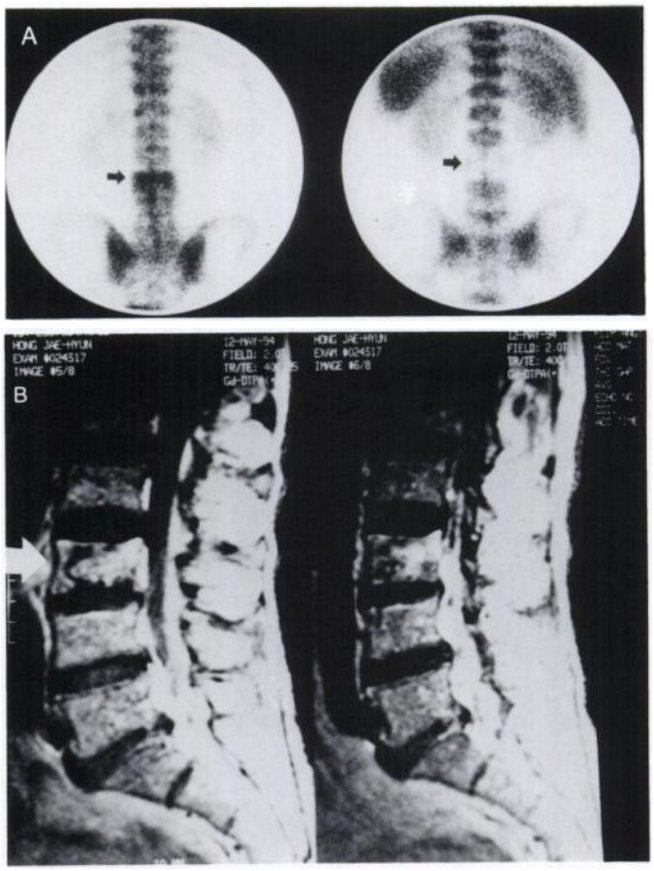

FIGURE 1. (A) Bone scan of a breast cancer patient shows a lesion in the third lumbar spine (arrow, left image). AGA scan clearly shows a focal marrow defect concordant with the bone scan lesion site (arrow, right image). (B) Metastatic nature of the lesion is confirmed by MRI (arrow).

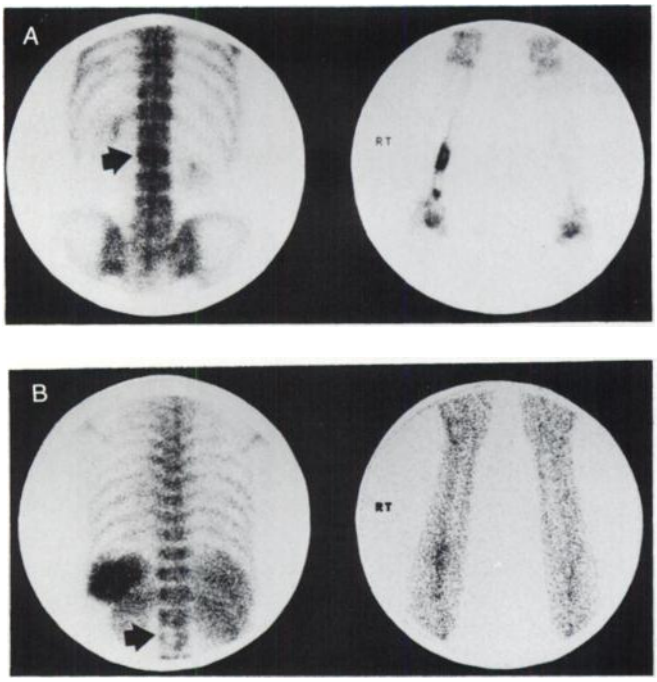

FIGURE 2. (A) Bone scan of a lung cancer patient shows moderate increased uptake in the second lumbar spine and an active ulnar bone lesion (arrow). (B) AGA scan did not demonstrate the ulnar lesion because there is no bone marrow at this site but did identify the lumbar spine lesion as a cold defect (arrow). Malignant cells were confirmed by bone biopsy.

The sensitivity and specificity of AGA scans to determine the presence or absence of skeletal metastases in cancer patients with equivocal bone scans is 100% and 79%, respectively. When indeterminate AGA scans are included, the sensitivity and specificity is 89% and 75%, respectively (Table 3).

DISCUSSION

Although bone imaging is the most effective whole-body screening procedure for detecting skeletal metastases, the procedure suffers from poor specificity because many benign diseases appear as scintigraphic lesions. Kamby et al. (*1*) reported a sensitivity of 96% in bone scan detection of skeletal metastasis in breast cancer patients, but the specificity was only 66%. Additionally, Michel et al. (*2*) found a low specificity of 24% in bone imaging of patients with non-small-cell lung cancer.

Where bone scan lesions have certain patterns that can lead to the correct diagnosis of metastasis, frequently only a few lesions are visualized, making a differential diagnosis

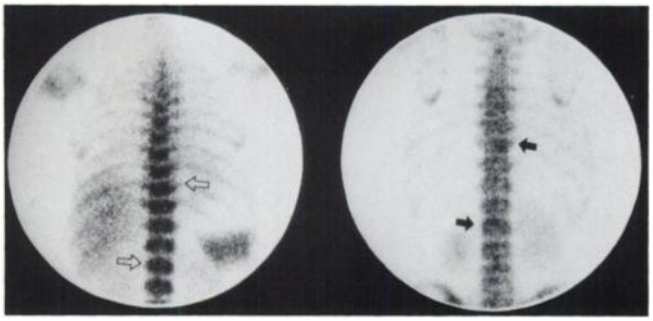

FIGURE 3. (Left) Normal AGA scan. (Right) Bone scan in a patient with breast cancer shows two equivocal spine lesions.

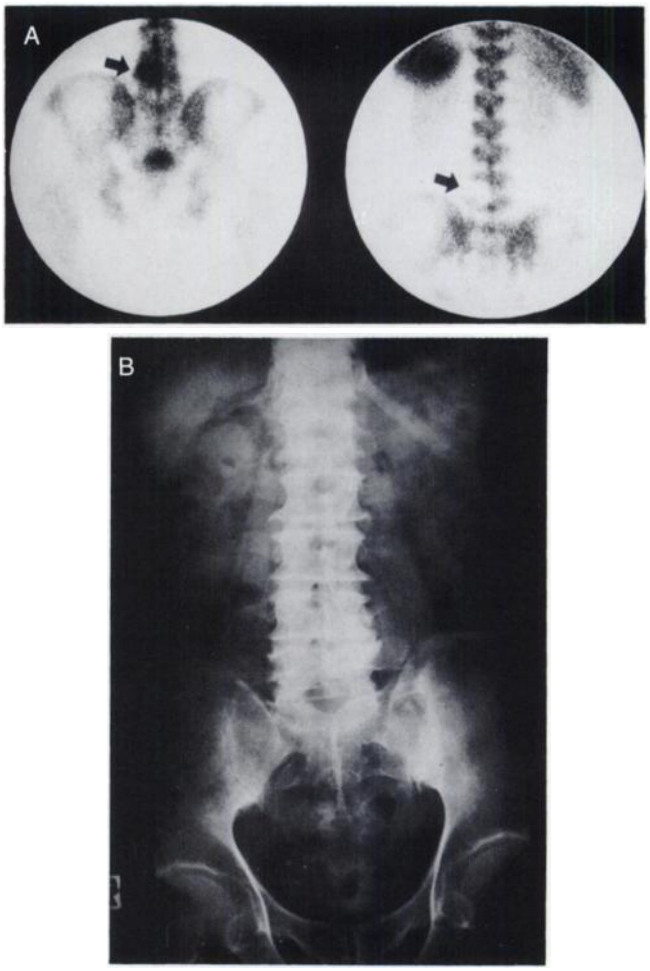

FIGURE 4. (A) AGA scan in a patient with bladder cancer shows a focal marrow defect in the fifth lumbar spine (right) concordant with the bone scan lesion (left). (B) Simple radiograph shows that the lesion was caused by degenerative disease of the spine.

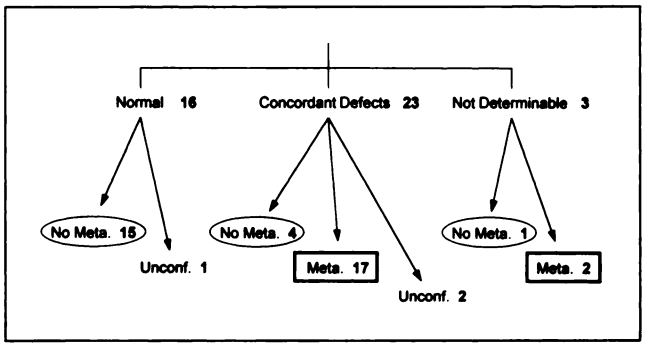

FIGURE 5. AGA scan result and final diagnosis in patients with equivocal bone scans. Meta. = metastasis; Unconf. = unconfirmed final diagnosis.

TABLE 3
Sensitivity and Specificity of AGA Scans

	Indeterminate AGA scans	
	Included	Excluded
Sensitivity	89%	100%
Specificity	75%	79%

difficult. Jacobson et al. (*16*) found a proportion of metastasis in 11% and 35% of patients with breast cancer who had one or two bone scan lesions, respectively. Thus, detecting solitary lesions on bone scan, particularly in the axial bone or ribs, of patients with known primary malignancy may be a diagnostic dilemma.

Although in such instances, correlative radiographs may reduce false-positive scan interpretations (*17*), the problem cannot be adequately resolved by this method alone. Radiographs require a loss of some 50% of mineral content before a lesion can be visualized (*4*). Thus, they are relatively insensitive in earlier disease states (*5*). CT or MRI can be useful in evaluating radiographically unexplained bone scan lesions (*17–18*). Limited availability, high costs and difficulty in whole-body evaluation limit their routine application. In addition, CT has low sensitivity for detecting marrow metastases (*6*), while MRI may show false-positive results in certain situations (*7,8*).

There has been interest in the use of bone marrow scintigraphy to evaluate skeletal metastasis since the majority of skeletal metastases seed the bone marrow through hematogenous spread. When they metastasize, cancer cells reach the bone marrow through the vasculature and attach and implant. The medullary arteries facilitate tumor extravasation due to slow blood flow in the medullary sinus, endothelial gaps and the lack of basement membrane (*19*). Bone marrow metastases have been shown to occur in breast cancer patients without cortical bone involvement (*20,21*).

Earlier trials of bone marrow scintigraphy using ^{99m}Tc-labeled colloids were less than satisfactory due to poor resolution and excessive hepatosplenic uptake. Previous studies with ^{99m}Tc-AGA bone marrow imaging, however, have shown a higher sensitivity for detecting skeletal metastasis than bone imaging (*10–12*).

In this study, we used AGA scans as a method to increase the specificity of bone scans equivocal for metastasis and not as a screening test. The sensitivity of 100% (89% if indeterminate scans are classified as false-negative) is higher than that reported in other studies (*11*), but our attention was directed to diagnostic accuracy rather than comparing the nature of each bone scan lesion. High sensitivity may partly explain why there were no patients whose bone scan demonstrated only peripheral lesions. Inclusion of such patients would lower the sensitivity score, since metastases to peripheral sites without erythropoietic marrow are not likely to be detected by bone marrow imaging. The only peripheral lesion included in this study (ulna) was not detected on the AGA scan, but an accompanying spinal lesion was detected which resulted in a classification as positive. This finding suggests that AGA scintigraphy may have limited or no value in evaluating equivocal bone scan lesions when they are peripherally located. Another reason for the high sensitivity may be due to the high contrast in the AGA scans, even in areas with relatively small amounts of erythropoietic marrow such as the ribs.

Three patients had indeterminate lesions. The reason for the indeterminate results in two patients was previous irradiation to the region, which decreased marrow uptake in the area. In the remaining patient, there was high liver activity obscuring the ROI and relatively poor bone marrow uptake of the whole bone marrow system. Since this patient eventually developed disseminated skeletal metastasis, confirmed by follow-up bone imaging 5 mo later, we believe we were actually observing diffuse bone marrow metastasis with extramedullary erythropoietic activity of the liver rather than an indeterminate AGA scan.

There was a false-positive rate of 21% (4/19 patients). In three patients, the false-positive lesion was caused by degenerative changes in the lumbar spine, and by rib fractures in another. Bone marrow scan defects are not totally specific for marrow and metastasis; a variety of benign diseases may also cause defects, including: vertebral marrow degenerations (*22*), focal necrosis (*23*), Paget's disease (*24*) and bone infarction (*25*).

CONCLUSION

Technetium-99m-antigranulocyte bone marrow immunoscintigraphy provides an efficient method for clarifying the etiology of bone scan lesions equivocal for metastasis. A normal AGA scan makes metastasis very unlikely, whereas the presence of marrow defects concordant with bone scan lesions increases the likelihood of metastasis. A matched marrow defect, however, is not completely specific for metastasis and correlative radiography is required to exclude benign causes. If there is no correlation between scintigraphy and radiography, further investigation by CT, MRI or biopsy may be warranted.

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